Light responsive Gold NPs-polymer hybrid LBL capsules for the Lysosomal Storage Disorder

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Abstract: *GM1 gangliosidosis is a rare lysosomal storage disorder caused due to the malfunctioning of lysosomal β-galactosidase that maintains the distribution of GM1 ganglioside within the body. Delivery of β -galactosidase inside the cells is the only potential approach to cure such disease. However, trafficking of this therapeutic protein into a cell is highly challenging. In this regard, stimuli-responsive nanocarriers have gained a lot of attention as they precisely control the delivery of payload under the influence of a particular stimulus. To this end, we report the formulation of light-responsive gold NPs-polymer hybrid LBL capsule for the intracellular delivery of β-gal enzyme and showed light-responsive breaking of gold NPs-polymer hybrid LBL capsule by using NIR laser exposure technique (980nm, a continuous laser beam of 1W power). The light-responsive breaking of gold NPs-polymer hybrid LBL capsules was verified by fluorescent microscopy and scanning electron microscope (SEM). We observed 1.5, 1.8- and 2.27-fold reduction in the capsules number after10 min, 15min and 20min laser exposure respectively. Further, β-gal enzyme release from gold NPs-polymer hybrid LBL capsule was verified after capsule lysis using assay buffer (ONPG). We observed 1835mU±113 β-gal entrapment within gold NPs-polymer hybrid LBL capsules (~500 nm). Gold NPs-polymer hybrid LBL capsules showed no cytotoxicity up to 150 capsules per cell concentration and were well internalized by the HeLa as well as L929 cells.*

Keywords: Gangliosidosis, beta-galactosidase, gold nanoparticles, LBL capsules, light responsive, stimuli responsive nanocarriers

1. Introduction

Gangliosides are complex glycolipids present in cell membrane all over the body, that are essential for maintaining membrane structure and organization of the cell.[1, 2] Intense variation of these vital constituents in the body can cause fatal effect including cell and tissue damage.[3, 4]The lysosomal hydrolase, an acid β -galactosidase is an enzyme that maintains the level of GM1 ganglioside within the body.[5] As enzymes are highly specific, deficient activity of this enzyme stops the breakdown of GM1 gangliosides(β-galactosidase substrate). This unusual storage of substrate, further leads to the condition named as GM1 gangliosidosis. The undue storage of GM1 ganglioside within cells [6] triggers long-lasting cellular and tissue damage, markedly in the brain, liver, spleen & bone marrow that results to severe retardation and death. Delivery of β -galactosidase inside the cells is the only potential approach to cure

such disease.[7] However, trafficking and controlled delivery of this therapeutic protein into intracellular targets are still highly challenging due to its inherent character including large molecular size, high water solubility, quick elimination, physical and chemical instability.[8]Nanocarriers such as liposomes, niosomes, micelles, nanoparticles, etc. can be the right choice for addressing these challenges. Nanocarriers facilitate the navigation of payload across biological barriers like cell membrane accompanied by stabilizing them.[9, 10] In this regard, stimuli-responsive nanocarriers are the more advanced approach that precisely control the delivery of payload under the influence of a particular stimulus.[11, 12] Release of the drug from these cargoes is controlled either by body's internal stimuli like enzyme, pH, temperature, other biochemical agents or can also be activated by external stimuli including light, ultrasound rays, magnetic field, electric field etc.[13] Recently, we reported internal stimuli-responsive nanocarriers for the intracellular delivery of β-gal enzyme *via* dextran sulphate/poly-l-arginine LBL polymeric capsules (DS/PA capsules) that shows the biodegradability due to the endogenous arginase enzyme present in cells.[14]Alternatively, light-responsive nanocarriers operate under the influence of external stimuli (light) and release the payload upon light exposure.[15, 16]Light-responsive nanocarriers triggers the release of cargo via photothermal effect using near-infrared radiation(NIR)with 700nm ~1100 nm wavelength.[17]Irradiation of NIR light on nanocarriers having strong NIR absorbance window such as gold-NPs,[18] Pd-NPs, graphene,[19] carbon-nanotubes[20] etc. that results in conversion of photo energy to thermal energy and that leads to an increase in temperature. This potential approach has been reported for photothermal therapy. [21]

Herein, we formulated light-responsive gold NPs-polymer hybrid LBL capsules polymeric nanocapsules for the delivery of beta-galactosidase enzyme. Gold NPs were used as photo absorber. To generate the photothermal process, layer-by-layer capsules shell is surface modified with metallic nanoparticles. Gold nanoparticles that have a characteristic feature like decent biocompatibility, high NIR absorbance, ease of large-scale synthesis and photostability, make best suitable as the photosensitizer.[22] External NIR excitation of gold NP hybrid LBL polymeric capsules converts light into heat through the photothermal process and result in softening, melting and breaking of polymeric nanocapsules. The best part of NIR- responsive nanocarriers is the drug release profile which can be controlled remotely by tuning several light-related parameters such as intensity, wavelength, power density, exposure time, etc.[23] Other factors include the concentration of gold NPs, glass transition temperature of the polymer. Near-Infrared radiation (NIR) is considered to be the best option as it shows deeper tissue penetration without much absorption and minimum harm to body cells.

Synthesis of enzyme loaded gold NPs-polymer hybrid LBL capsules was done by successive layer-by-layer fabrication of poly (sodium 4-styrene-sulfonate) (PSS) and poly (allylamine hydrochloride) (PAH) on β-gal-loaded silica templates. Gold nanoparticles were embedded just before the final layer of LBL assembly and silica template is removed to get β-gal loaded gold NPs-polymer hybrid LBL capsules. Gold NPs-polymer hybrid LBL capsules showed no cytotoxicity and were well internalized in HeLa and L929 cell lines. The light-responsive breaking of LBL capsules was confirmed by fluorescent microscopy and scanning electron microscope (SEM) using NIR (980nm, continuous laser beam of 1W power). Further, β-gal enzyme release from gold NPs-polymer hybrid LBL capsules was verified after capsule lysis using assay buffer (ONPG) (using ß-Galactosidase detection kit (GAL-A)).

2. Materials and Methods

2.1 Materials

Poly(sodium4-styrene-sulfonate)(PSS)Mw70000, poly(allylamine hydrochloride) (PAH) Mw 56 000, β-Gal enzyme, ß-Galactosidase reporter gene activity detection kit(GAL-A), rhodamine isothiocyanate (RITC), trypsin-EDTA, medium to high glucose Dulbecco's-modified eagle's medium (DMEM), 2-(4-Amidinophenyl)-6-indolecarbamidinedihydrochloride(DAPI), penicillin-streptomycin antibiotic, 3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide (MTT), gelatin, sodium citrate,3-(amino proply) triethoxysilane)(APTES),toluene were purchased from Sigma-Aldrich (India). Fetal bovine serum (FBS) was acquired from Gibco. Formaldehyde, liquor ammonia (24%), ammonium fluoride, ethanol, toluene, dimethyl sulfoxide (DMSO) were purchased from Merck Chemicals, (India). Chloroauric acid ($HAuCl_4.H_2O$) was obtained from Loba Chem. Hydrofluoric acid (40%w/v) was procured from Sd-fine chem. and tetraethylorthosilicate from Fluka. HeLa and L929 (wild type mouse fibroblast) cell lines were acquired from National Centre for Cell Science, Pune, India.

2.2 Methods

2.2.1 Synthesis of β-gal loaded Gold NPs-Polymer hybrid LBL capsules

Synthesis of enzyme loaded gold NPs-polymer hybrid LBL capsules was done by consecutive layer-by-layer fabrication of poly (sodium 4-styrene-sulfonate) (PSS) and poly (allylamine hydrochloride) (PAH) on enzyme loaded silica templates. Gold nanoparticles were infiltrated just before the final layer of LBL assembly. Subsequently, the silica template was removed to get β-gal loaded gold NPs-polymer hybrid LBL capsules. Synthesis of gold NPs-polymer hybrid LBL capsules involves the following steps.

2.2.1.1 Synthesis of Silica Nanoparticles

For synthesizing silica templates, Stober's method was chosen.[24] For this, 4.7 ml of TEOS (tetraethyl orthosilicate) was hydrolysed using 54.4 ml of ammonia in 2ml of water and 50 ml of ethanol. The blend was gently stirred for 4hr at room temperature. The particles were separated by centrifugation. Further, they were rinsed with DI and ethanol and finally dried at 80ºC overnight.

2.2.1.2 APTES-Functionalization of Silica Template

To impart positive potential on silica template, APTES-functionalization was done. [25]For this firstly,600mg of silica nanoparticles were sonicated for 20 min in 106.5ml toluene. Then silica template was reflux in 460µl of APTES(3-(amino proply)triethoxysilane) for 24 h at 130ºC. Finally, APTES functionalized silica templates were collected washed and dried at 80ºC for 16 h.

2.2.1.3 Beta-Galactosidase loading on APTES-Functionalization of Silica Nanoparticles

For β-gal enzyme loading, APTES-functionalized silica templates (10 mg) were incubated with β-gal enzyme solution (8U in Z-buffer) at 4ºC for overnight. Further, enzyme loaded silica templates are separated at 3900 rpm and the supernatant was analyzed for β-gal content by using a standard protocol.

2.2.1.4 Synthesis of Gold NP

For the synthesis of gold NP, 10mg Chloroauric acid (HAuCl4. H2O) was dissolved in 100ml DI water in a round bottom flask and stirred for 15-20 min with the magnetic stirrer. Sodium Citrate (0.96ml,114 mg in 10ml of water) was added at 40-45ºC. The mixture was allowed to stir along with heating up to 80-85ºC. Heating was terminated as soon as the red colour was observed. The round bottom flask was allowed to stir until it comes to room temperature.

2.2.1.5 Synthesis of Gold NP hybrid LBL assembly

For the fabrication of gold nanoparticle hybrid LBL assembly, first layer was formed by adding negatively charged polymer, poly (sodium 4-styrene-sulfonate) (PSS) (1mg/mL in 0.5M NaCl) to β-gal loaded APTES-functionalized silica template (10 mg) and mixed on roto spin for 15 min followed by three-time washing with 0.5M NaCl to carry away unabsorbed polymer. After each step, the particles were collected at 3900 rpm for 3 min. Further, the next layer was formed by adding positively charged polymer, poly (allylamine hydrochloride) (PAH) (1mg/mL in 0.5M NaCl) and the layer was formed by the same processing steps. Subsequently, up to seven alternating polymeric layers of PSS and PAH were further synthesized similarly. After fabricating 7th layers and before incorporating gold NP, capsules were washed with DI water instead of 0.5 M NaCl solution as it can cause agglomeration of Gold NP. Next, 1ml of gold NP suspension was added into LBL capsule (7 layered) and allowed to mix on roto spin for 3hr.Thereafter by separating LBL capsules, excess gold NP was removed. Next, final layer (8th) of PAH polymer was formed. Then silica template was removed using HF buffer (0.75 M HF: 4 M NHF4). (Caution: Hydrofluoric acid is very harmful and must be deal with all safeguards as stated in Material Safety Data Sheet). Finally, enzyme loaded gold NPs-polymer hybrid LBL capsules were washed, collected (at 4500 rcf for 5 min) and dispersed DI water.

2.2.2 Estimation of β-gal loading in Gold NPs-polymer hybrid LBL capsules

To estimate loading of the beta-galactosidase enzyme on gold NPs-polymer hybrid LBL capsules, firstly β-gal enzyme retained on APTES-functionalized silica nanoparticles was assessed before forming LBL assembly. For this 10 mg of APTES-functionalized silica nanoparticles were incubated with β-gal enzyme solution (8U in Z-buffer) at 4ºC for overnight. Further enzyme loaded templates were removed at 3900 rpm for 3 min, the supernatant was separated and analysed by adding an equivalent volume of twofold strength ONPG assay buffer for an hour. Further, to evaluate β-gal enzyme entrapment within gold NPs-polymer hybrid LBL capsules, the enzyme loaded LBL capsules were mixed with an equal volume of twofold strength ONPG assay buffer for an hour and further admixture was ultrasonicated for 15 min, centrifuged at 27000 g for 10 min at 4ºC. Estimation of β-gal enzyme was done by measuring absorbance at 420 nm in microtiter spectrophotometer as per the procedure provided by the supplier.

2.2.3 Characterization of Gold NPs-Polymer hybrid LBL capsules

The size and morphology of silica template, gold NP and β-gal loaded gold NPs-polymer hybrid LBL capsules were acquired by Scanning electron microscope (SEM). APTES-functionalized silica NP was characterized by zeta potential and FTIR. The particle size of silica NP and gold NPs-polymer hybrid LBL capsules were assessed using dynamic light

scattering by Malvern zeta sizer. Estimation of enzyme loading in gold NPs-polymer hybrid LBL capsules was done by using ONPG a synthetic β-gal substrate (GAL-A) provided by Sigma. For enzyme release studies, laser exposure technique (980nm, a continuous laser beam of 1W power) was used to trigger capsule lysis. For LBL capsule lysis studies, images were obtained by Fluorescent Microscopy and Scanning electron microscope (SEM). Thermo Scientific Multiskan UV-vis spectrophotometer was used for measuring absorbance at 570 nm for MTT assay. The cell imaging was carried out by confocal laser scanning microscope (Carl Zeiss LSM 710) for capsule uptake studies.

2.2.4 Enzyme Release Studies

To trigger breaking of the capsules for β-gal enzyme release, NIR (Near Infrared) continuous laser exposure experiment was done on β gal loaded gold NPs-polymer hybrid LBL capsules at different time intervals (0 min,10 min, 15 min and 20 min) and β-gal enzyme release study was carried out. For capsule lysis laser exposure experiment, β-gal loaded PSS/PAH LBL polymeric capsules were used as a control sample. The sample was taken in eppendrof tubes and the bottom tip of the tube was subjected to irradiation by 980nm, NIR continuous laser beam (1W power) for 0min,10 min,15 min & 20min respectively. Samples were evaluated qualitative (Fluorescence microscopy and SEM) as well as quantitatively (UV Spectroscopy). Fluorescence microscopy and SEM Images were acquired to characterize the capsule lysis and UV Spectroscopy was used for quantitative estimation of the beta-galactosidase enzyme in the sample supernatant to characterizing enzyme release.

2.2.5 *In-Vitro* cell culture studies

Cell culture studies were carried out on HeLa and L929 cell lines. Cells were grown in DMEM medium along with FBS (10% v/v) and penicillin-streptomycin (1% v/v). All the cells were cultivated in a CO2 (5 %) incubator at 37ºC.

2.2.5.1 Cytotoxicity of Gold NPs-Polymer hybrid LBL capsules

For doing cytotoxicity studies of gold NPs-polymer hybrid LBL capsules, two cell lines HeLa and L929 were used and samples were studied by MTT assay. For MTT assay, nearly 10^4 cells were seeded in each well of a 96 well plate (containing DMEM) and kept for 6 h. Then after, altered concentrations of gold NPs-polymer hybrid LBL capsules (50,100 and 150 capsules/cell) were added to wells in triplicates and further 96 well plate was incubated for 18 h in an incubator (5% CO2 and at 37ºC). Next, MTT dye (0.5mg / mL) was added after removing media. Cells were further incubated for 4 h. Then MTT dye was taken away and DMSO was added. The samples were analyzed at 570 nm using UV-Vis spectrophotometer.

2.2.5.2 Capsule uptake studies of Gold NPs-Polymer hybrid LBL capsules

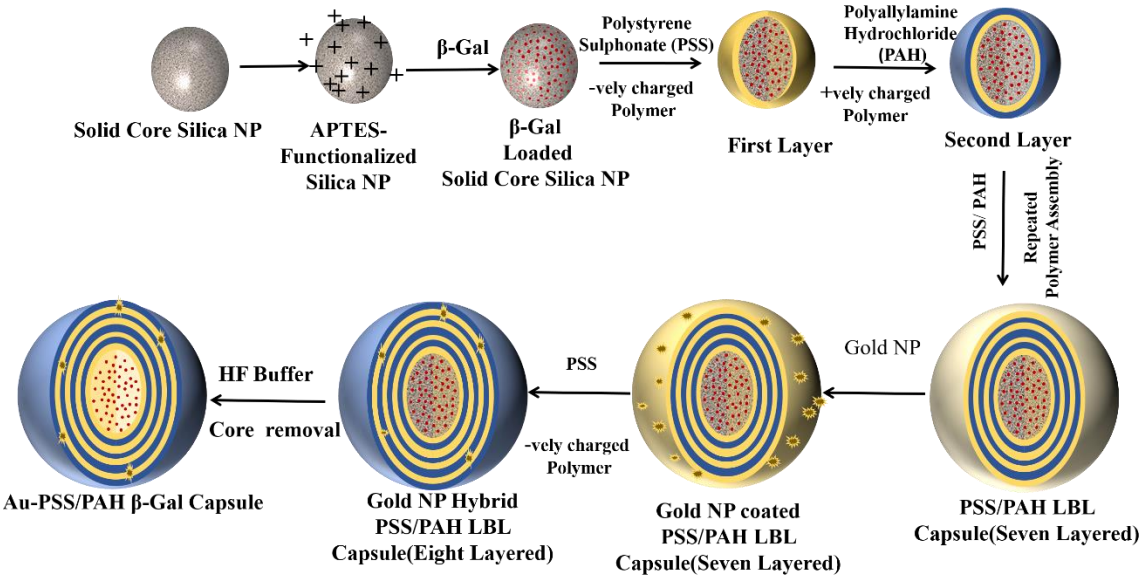
To validate the intake of capsules and to observe the localization of capsules inside the cells, capsules uptake studies were done. For capsule uptake studies, RITC labelled Gold NPs-Polymer hybrid LBL capsules were synthesized using RITC labelled PAH for LBL assembly. Approximately, 104 cells were seeded in a 24 well plate holding 13 mm gelatin (0.2% w/v) covered coverslips. Cells were rested for 6 h, to allow to adhere well. Cells were incubated (5% CO2 and at 37ºC) for 12 h with RITC labelled Gold NPs-Polymer hybrid LBL capsules (50capsules/cell). Then after, media was removed and cells were rinsed for thrice with PBS to

remove free capsules. Next 4% formaldehyde solution was used for fixing the cells and nuclei were marked using DAPI (10 µg/mL) for 10 min. Staining technique was used as per the protocols mentioned by the manufacturer. Finally, samples were fixed on glass slides and viewed under a confocal laser scanning microscope (Zeiss LSM 710).

3. Result and Discussion

3.1 Synthesis of enzyme loaded Gold NPs-Polymer hybrid LBL capsules

Scheme 1 shows the synthesis of enzyme loaded gold NPs-polymer hybrid LBL capsules prepared by template-based synthesis method that involves following steps: (a) Synthesis of silica nanoparticles (b) APTES-Functionalization of silica nanoparticles (c)β-gal loading on silica NP(d) Gold NP- Hybrid- Layer by Layer assembly of PSS and PAH polymer on silica NP (e) removal of silica core by hydrofluoric acid.



Scheme1. Schematic illustration of enzyme loaded Gold NPs-polymer hybrid LBL capsules

3.2 Characterization of Au-PSS/PAH β-gal loaded LBL polymeric capsules

The size and morphology of silica template, gold NPs and enzyme loaded gold NPs-polymer hybrid LBL capsules were acquired by Scanning electron microscope (SEM). Figure 1 shows the SEM images of solid-core silica NPs, gold NPs and β-gal loaded gold NPs-polymer hybrid LBL capsules NPs. We noted that the silica NPs were sphere-shaped monodispersed particles with ~500 nm size whereas gold NPs were polydisperse (15-20 nm) and non-spherical in shape. SEM images of gold NPs-polymer hybrid LBL capsules distinctly shows the presence of gold NPs that are embedded within a polymeric shell. The particle size of silica NPs and gold NPs-polymer hybrid LBL capsules (in range of ~500 nm size) also supported by dynamic light scattering by Malvern zeta sizer as shown in Figure 2. APTES-functionalized silica NPs were characterized for surface functionalization. As shown in Figure 3 A, FTIR spectroscopy of APTES functionalized silica NPs showed a peak at 3300 nm that demonstrate surface amine

modification. Further to confirm APTES functionalization of silica NPs, the surface potential was measurement by Malvern zeta sizer. Figure 3 B, demonstrate + 24.76 mV surface charges on APTES functionalized Silica Nanoparticles. This data also supports the APTES functionalization of silica NPs. The positive surface charges of APTES-functionalized Silica NPs (+24 mV, at pH 7) assisted the electrostatic accumulation of the first layer as PSS (negatively charged polymer) and followed by deposition of PAH (positively charged polymer).

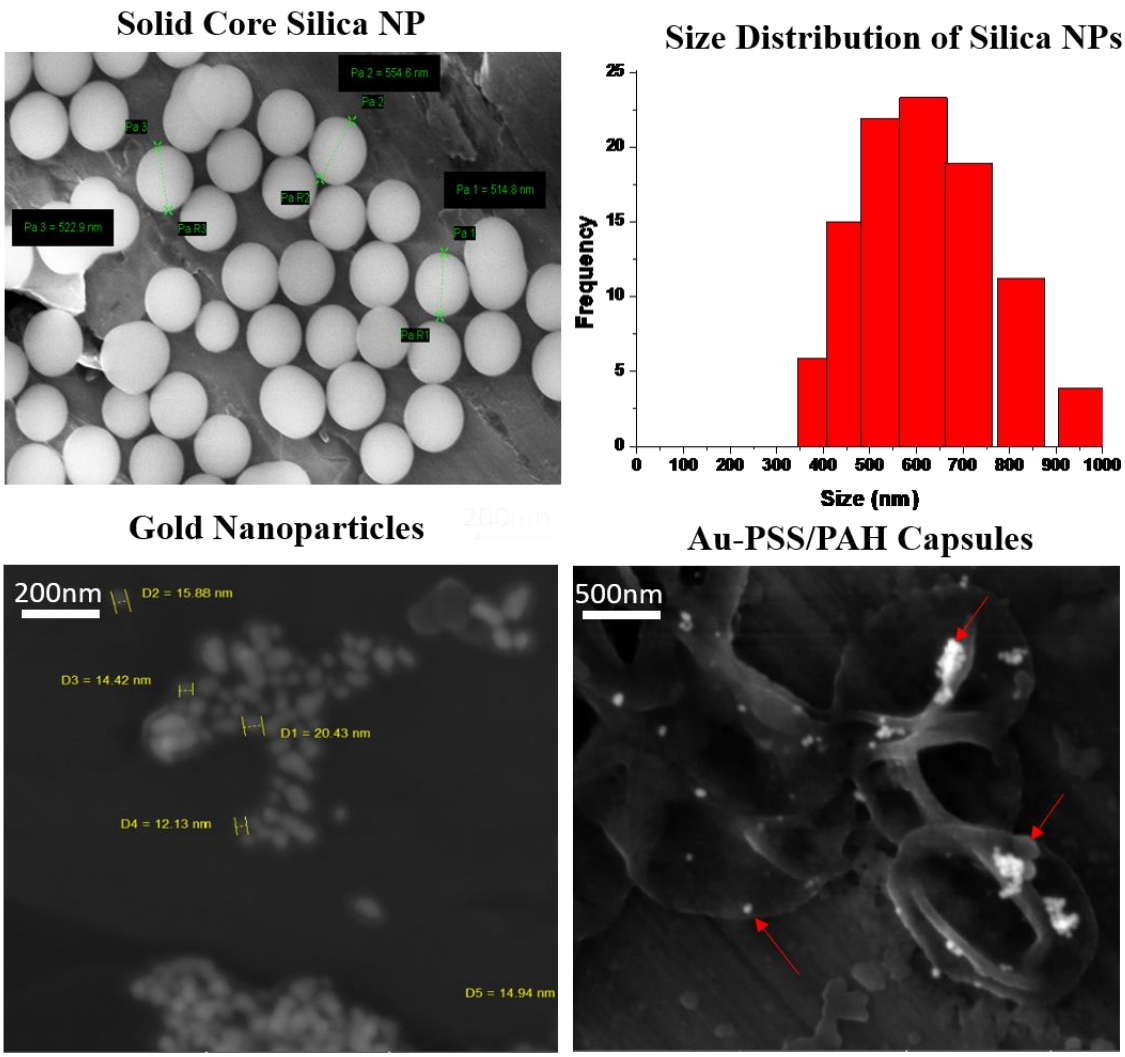


Figure 1. SEM image of solid core silica nanoparticles along with size distribution, Gold Nanoparticles and Gold NPs-Polymer hybrid LBL capsules

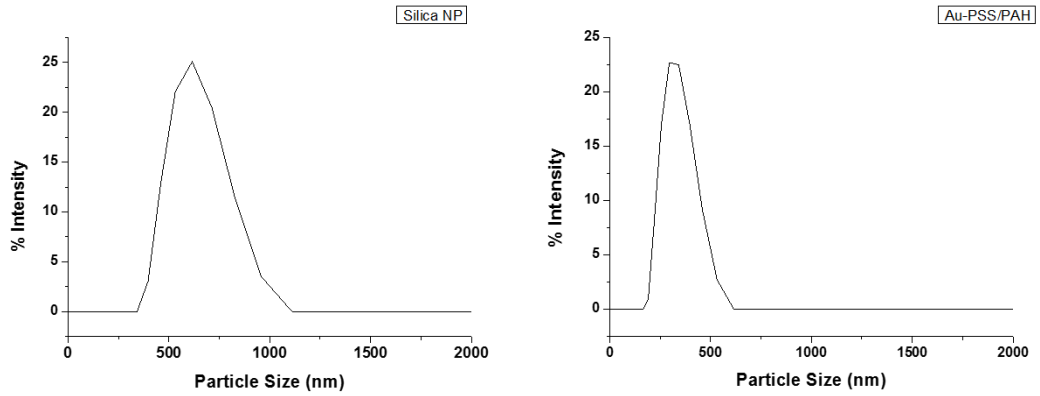


Figure 2. Particle size of Silica NP and Gold NPs-polymer hybrid LBL capsules by dynamic light scattering

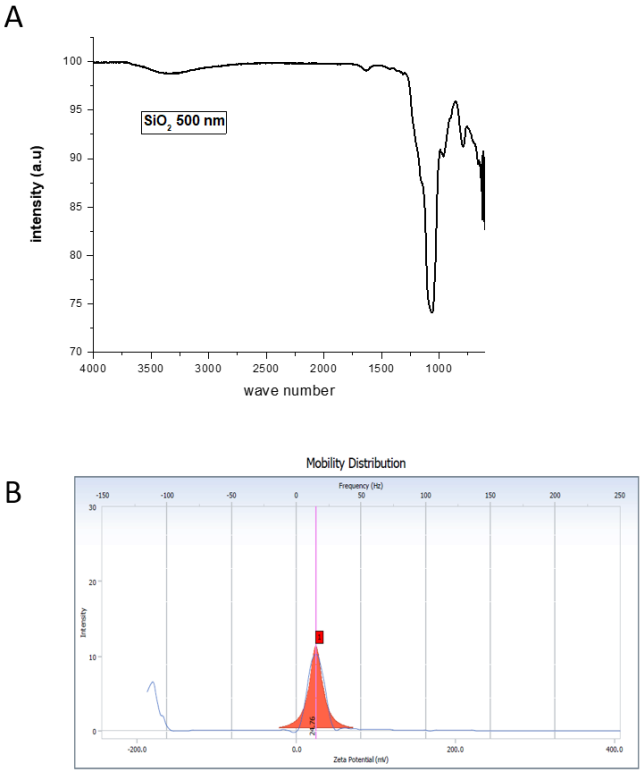


Figure 3. (A)FTIR spectroscopy of APTES-Functionalized silica NPs (B) Zeta potential of APTES-Functionalized silica NPs

3.3 Estimation of β-gal loading in Gold NPs-polymer hybrid LBL capsules

Firstly, for the confirmation ofβ-gal enzyme loading, Gold NPs-Polymer hybrid LBL capsules were incubated with double strength ONPG (o- nitro phenyl ß -D-galactopyranoside) assay buffer and Confocal images (Figure 4) of treated capsules were examined. Formation of yellow colour compound (ortho nitrophenyl) formed due to the action of the β-gal enzyme on an synthetic substrate ONPG, as shown in Figure 4 confirms the enzyme loading in Gold NPs-Polymer hybrid LBL capsules. To quantify beta-galactosidase enzyme entrapment in gold NPs-

polymer hybrid LBL capsules, firstly β-gal enzyme retained on APTES-functionalized silica nanoparticles was assessed by removing enzyme loaded templates (at 3900 rpm for 3 min) and analyzing the supernatant using GAL-A double strength ONPG buffer. Further, to evaluate β-gal enzyme entrapment within gold NPs-polymer hybrid LBL capsules, the enzyme loaded LBL capsules were analyzed using double strength assay buffer (ONPG) (using ß-Galactosidase detection kit (GAL-A)). Quantification of β-gal was done as per the protocol provided by the manufacturer. We spotted 92-93% of β-gal enzyme retention on silica nanoparticles template (SGX) and 23-26% β-gal entrapment was observed on gold NPs-polymer hybrid LBL capsules.

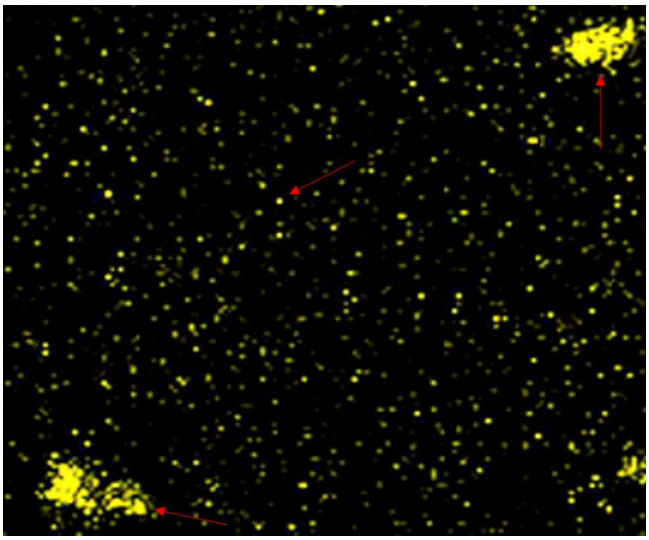


Figure 4. Confirmation of β-gal enzyme loading inside gold NPs-polymer hybrid LBL capsules via β-gal enzyme–artificial marker (ONPG) reaction inside the capsules.

3.4 *In-Vitro* cell culture studies

To analyze the delivery proficiency of gold NPs-polymer hybrid LBL capsules, the polymeric capsules were investigated for cytotoxicity studies and capsule uptake studies. HeLa and L929 cell lines were selected for doing cell culture studies. Cells were cultivated in DMEM culture medium added with 10% v/v FBS and 1% v/v penicillin-streptomycin. The cells were grow in a CO2 (5 %) incubator at 37ºC.

3.4.1 Cytotoxicity Studies of Gold NPs-Polymer hybrid LBL capsules

To analyze the effect of gold NPs-polymer hybrid LBL capsules on cell viability, cytotoxicity studies were done by using MTT assay. HeLa and L929 cell lines were seeded in 96 well plate and were incubated (5% CO2 and at 37ºC) for the period of 18 h with gold NPs-polymer hybrid LBL capsules up to 150 capsules per cell concentration. The cell viability was measured by adding MTT dye and further studied at 570 nm using UV-Vis spectrophotometer. Figure 5 demonstrates that the % cell proliferation of cells at all three concentration (50,100 and 150 capsules/cell) was above 80% in both cell lines (HeLa and L929). Therefore, gold NPs-polymer hybrid LBL capsules possess no cytotoxicity up to 150 capsules per cell concentration in HeLa as well as L929.

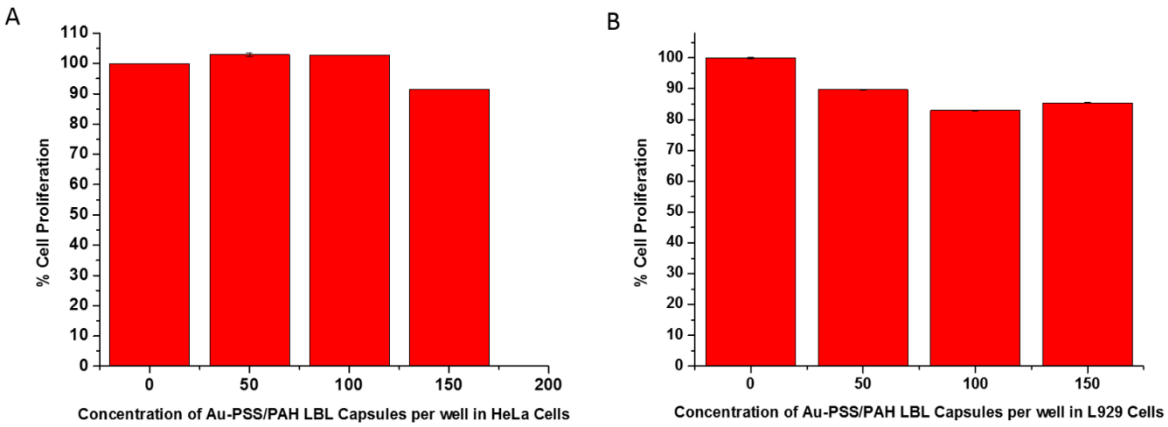


Figure 5. Cytotoxicity studies of gold NPs-polymer hybrid LBL capsules with (A) HeLa cells and (B) L929 cells

3.4.2 Capsule uptake studies of Gold NPs-polymer hybrid LBL capsules

To ascertain that of capsules are taken up by the cell and localized inside the cells, capsules uptake studies were done by using RITC labelled Gold NPs-Polymer hybrid LBL capsules. HeLa and L929 cells were incubated (5% CO2 and at 37ºC) for 12 h with RITC labelled Gold NPs-Polymer hybrid LBL capsules (50capsules/cell). Then after, following standard staining protocol, samples were spotted under a confocal laser scanning microscope. Figure 6 demonstrates confocal images that depict red fluorescence within cells due to the RITC labelled Gold NPs-Polymer hybrid LBL capsules after capsule uptake studies in HeLa (A) and L929 (B) cell. Noticeably, it can be substantiated that Gold NPs-Polymer hybrid LBL capsules are internalized by the cells. Further, the same experiment was repeated by using β-gal loaded Gold NPs-Polymer hybrid LBL capsules on HeLa cells to a localized β-gal enzyme with cells after capsule uptake experiment. Figure 7 represents confocal images of HeLa cells after the uptake of enzyme loaded Gold NPs-polymer hybrid LBL capsules. The yellow fluorescence as depicted in Figure 7, also confirms that β-gal loaded Gold NPs-Polymer hybrid LBL capsules are well internalized.

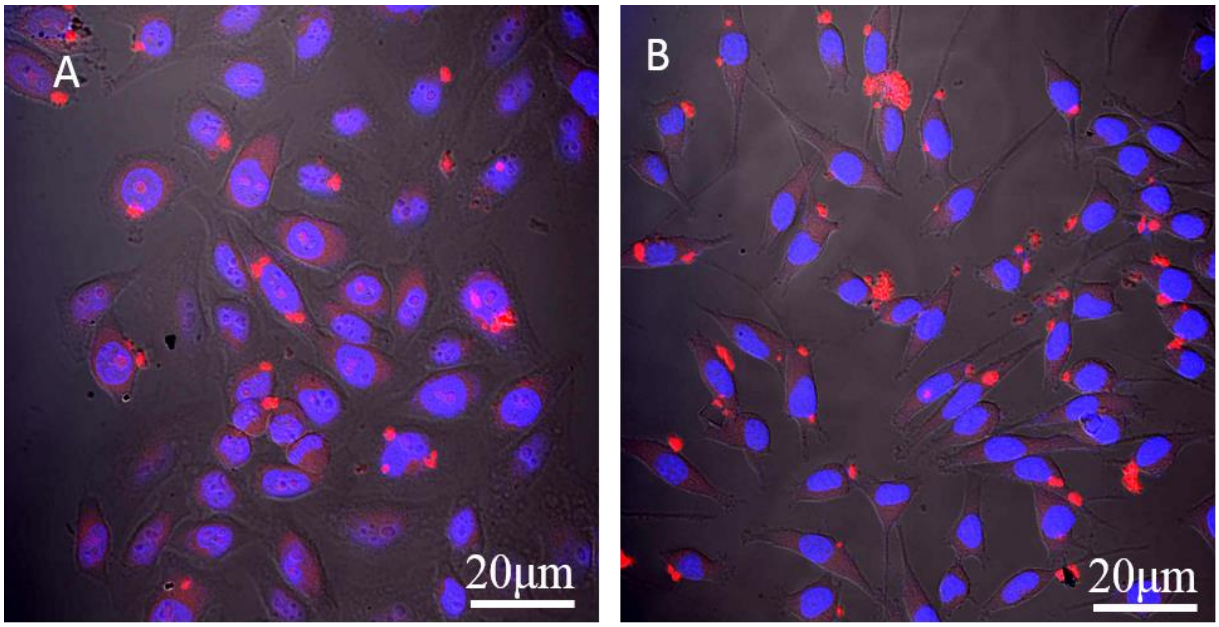


Figure 6. Confocal Images of capsule uptake studies of Gold NPs-polymer hybrid LBL capsules(A) HeLa cells (B) L929 cells

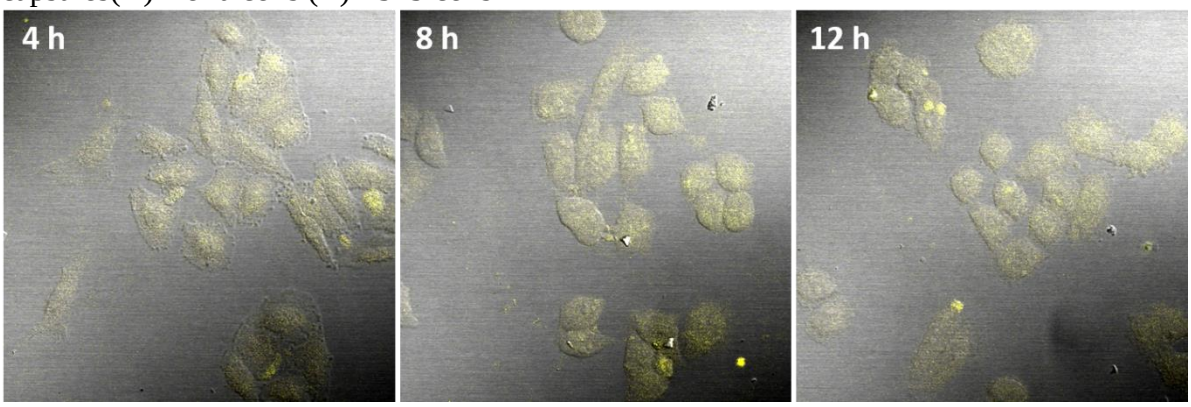


Figure 7. Confocal Images of HeLa cells after internalizingβ-gal loaded Gold NPs-polymer hybrid LBL capsules showing yellow fluorescence of ortho nitro phenol produced due to the reaction between β-gal enzyme and ONPG assay buffer after different time intervals.

3.5 Enzyme Release Studies

For enzyme release studies, NIR laser exposure technique (980nm, continuous laser beam of 1W power) was used to trigger capsule lysis. To ascertain breaking of Gold NPs-Polymer hybrid LBL capsules, images were acquired by Fluorescent Microscopy and Scanning electron microscope (SEM) and estimation of β-gal enzyme released after capsule lysis were done by UV-vis spectrophotometer by quantifying absorbance at 420 nm.

3.5.1 Capsule Lysis Studies of Gold NPs-polymer hybrid LBL capsules

To trigger breaking of the capsules for β-gal enzyme release, NIR (Near Infrared) continuous laser exposure experiment was done on β gal loaded gold NPs-polymer hybrid LBL capsules at different time intervals. The sample was exposed to 980nm, NIR continuous laser beam (1W power) for 0min, 10 min,15 min & 20min. Breaking of polymeric capsules was analyzed by Fluorescence microscopy and SEM. Figure 7 A demonstrate Fluorescence Microscopy Images

of laser exposure experiment on β gal loaded gold NPs-polymer hybrid LBL capsules at 0min, 10 min,15 min & 20min. The approximate number of RITC labelled gold NPs-polymer hybrid LBL capsules remaining after NIR laser exposure experiment was measured using Image J software (Figure 7 B). We observed 1.5, 1.8 and 2.27 fold reduction in the number of capsules after10 min,15min and 20min laser exposure respectively. Further, to substantiate breaking of the capsules, SEM images were acquired after similar laser exposure experiment after different time intervals. Figure 8 reveals the SEM images of β gal loaded gold NPs-polymer hybrid LBL capsules after 0min, 10 min,15 min & 20minNIR continuous laser exposure. SEM images also support the breaking of capsules by NIR laser exposure.

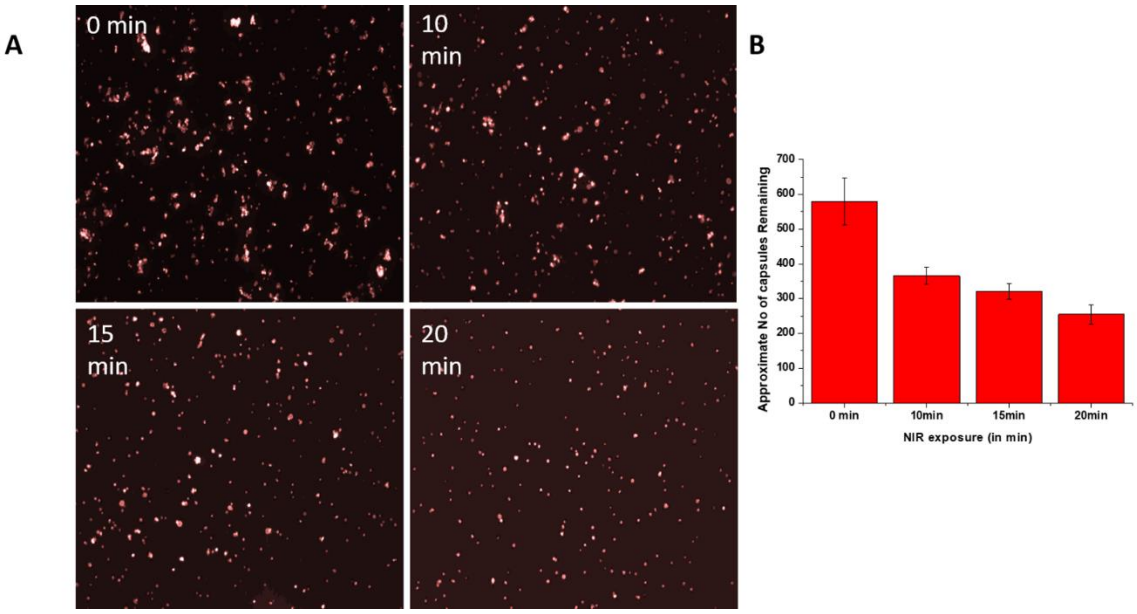


Figure 8. A. Fluorescent Microscopy Images of Au-PSS/PAH polymeric capsules (SGX as a template) after laser exposure experiment (0 min,10 min,15 min and 20 min time intervals) B. Approximate number of RITC labelled gold NPs-polymer hybrid LBL capsules remaining after NIR Laser Exposure experiment.

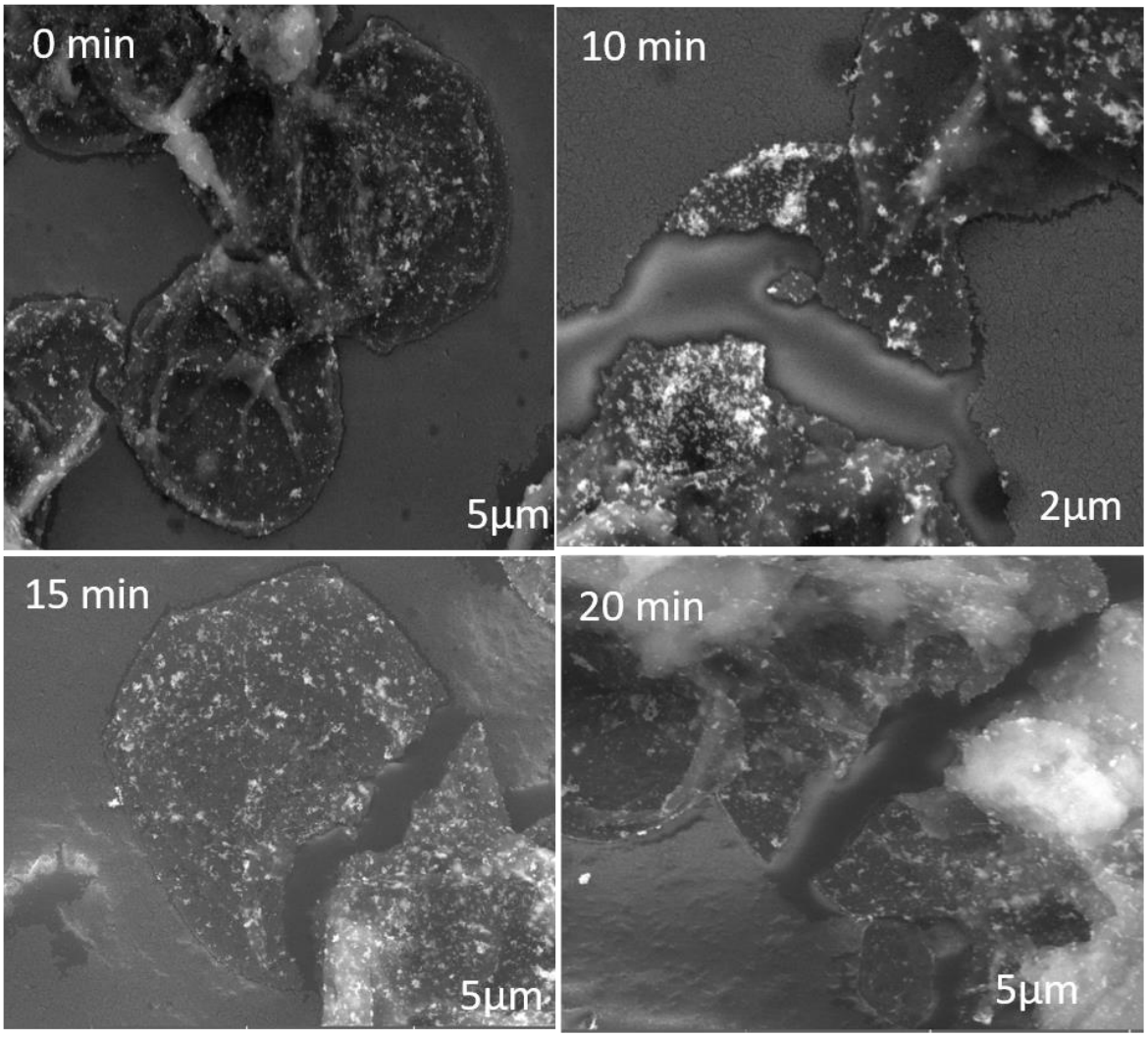


Figure 9. SEM Images of gold NPs-polymer hybrid LBL capsules (SGX as a template) after Laser Exposure at 0 min,10 min,15 min and 20 min time intervals

3.5.2 β –gal Release study of Gold NPs-polymer hybrid LBL capsules

For β-gal enzyme release studies, NIR (Near Infrared) continuous laser exposure experiment was done on β gal loaded Gold NPs-Polymer hybrid LBL capsules at different time intervals. The sample was exposed to 980nm, NIR continuous laser beam (1W/cm^2 power) for 10 min, 15 min & 20min and after breaking of polymeric capsules, β-gal enzyme release from the samples was analysed by UV-vis spectrophotometer by assessing absorbance at 420 nm. Figure 9 depicts the cumulative % enzyme released after 10 min, 15 min & 20min of NIR exposure. We observed 2.7, 3.6 and 6.6 % enzyme release after 10 min, 15 min & 20min of NIR exposure respectively. Here, it can be substantiated that β-gal loaded Gold NPs-Polymer hybrid LBL capsules show enzyme release on NIR exposure. The reason for the release of smaller amount can be the use of less power. The amount of enzyme release can be improved by increasing the NIR continuous laser beam power.

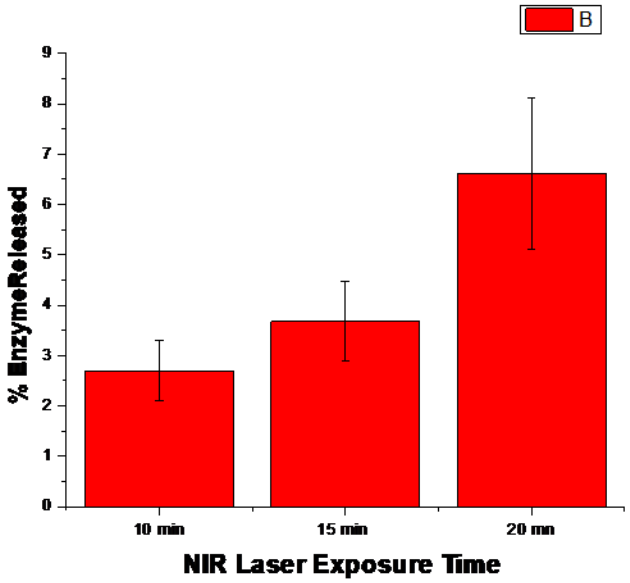


Figure 10. Beta-galactosidase release from Gold NPs-polymer hybrid LBL capsules after NIR laser exposure at different time interval (10,15 and 20min).

Conclusion

We report the formulation of light-responsive gold NPs-polymer hybrid LBL capsules capsule for the intracellular conveyance of β-gal enzyme and showed light-responsive breaking of LBL capsules capsule by using NIR laser exposure technique (980nm, continuous laser beam of 1W power). We confirmed the light-responsive breaking of gold NPs-polymer hybrid LBL capsules by fluorescent microscopy and scanning electron microscope (SEM). We observed 1.5, 1.8- and 2.27-fold reduction in the number of capsules after10 min, 15min and 20min laser exposure respectively. We observed 23-26% β-gal entrapment within gold NPs-polymer hybrid LBL capsules (~500 nm). Gold NPs-polymer hybrid LBL capsules showed no cytotoxicity up to 150 capsules per cell concentration and were well internalized by the HeLa as well as L929 cells. Thus, gold NPs-polymer hybrid LBL nanocapsules have the sound potential for delivery of protein drug as cargoes.

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References

1. *Kolter, T., Ganglioside biochemistry. ISRN biochemistry, 2012. 2012.*
2. *Robert K, Y., et al., Structures, biosynthesis, and functions of gangliosides-an overview. Journal of oleo science, 2011.* ***60****(10): p. 537-544.*
3. *Sandhoff, K. and K. Harzer, Gangliosides and gangliosidoses: principles of molecular and metabolic pathogenesis. Journal of Neuroscience, 2013.* ***33****(25): p. 10195-10208.*
4. *Lopez, P.H. and R.L. Schnaar, Gangliosides in cell recognition and membrane protein regulation. Current opinion in structural biology, 2009.* ***19****(5): p. 549-557.*

5. *Przybilla, M.J., et al., Comprehensive behavioral and biochemical outcomes of novel murine models of GM1-gangliosidosis and Morquio syndrome type B. Molecular genetics and metabolism, 2019.* ***126****(2): p. 139-150.*
6. *Brunetti-Pierri, N. and F. Scaglia, GM1 gangliosidosis: review of clinical, molecular, and therapeutic aspects. Molecular genetics and metabolism, 2008.* ***94****(4): p. 391-396.*
7. *Marques, A.R. and P. Saftig, Lysosomal storage disorders–challenges, concepts and avenues for therapy: beyond rare diseases. Journal of cell science, 2019.* ***132****(2).*
8. *Kristensen, M., D. Birch, and H. Mørck Nielsen, Applications and challenges for use of cell-penetrating peptides as delivery vectors for peptide and protein cargos. International journal of molecular sciences, 2016.* ***17****(2): p. 185.*
9. *Donida, B., et al., Monoolein-based nanoparticles for drug delivery to the central nervous system: A platform for lysosomal storage disorder treatment. European Journal of Pharmaceutics and Biopharmaceutics, 2018.* ***133****: p. 96-103.*
10. *Gigliobianco, M.R., et al., New Advanced Strategies for the Treatment of Lysosomal Diseases Affecting the Central Nervous System. Current Pharmaceutical Design, 2019.* ***25****(17): p. 1933-1950.*
11. *Mura, S., J. Nicolas, and P. Couvreur, Stimuli-responsive nanocarriers for drug delivery. Nature materials, 2013.* ***12****(11): p. 991-1003.*
12. *Tayo, L.L., Stimuli-responsive nanocarriers for intracellular delivery. Biophysical reviews, 2017.* ***9****(6): p. 931-940.*
13. *Rao, N.V., et al., Recent progress and advances in stimuli-responsive polymers for cancer therapy. Frontiers in bioengineering and biotechnology, 2018.* ***6****: p. 110.*
14. *Gupta, M., H. Pandey, and S. Sivakumar, Intracellular Delivery of β-Galactosidase Enzyme Using Arginase-Responsive Dextran Sulfate/Poly-l-arginine Capsule for Lysosomal Storage Disorder. ACS omega, 2017.* ***2****(12): p. 9002-9012.*
15. *Zhao, W., et al., Remote Light-Responsive Nanocarriers for Controlled Drug Delivery: Advances and Perspectives. Small, 2019.* ***15****(45): p. 1903060.*
16. *Linsley, C.S. and B.M. Wu, Recent advances in light-responsive on-demand drug-delivery systems. Therapeutic delivery, 2017.* ***8****(2): p. 89-107.*
17. *Sagar, V. and M. Nair, Near-infrared biophotonics-based nanodrug release systems and their potential application for neuro-disorders. Expert opinion on drug delivery, 2018.* ***15****(2): p. 137-152.*
18. *Ahmad, R., et al., Advanced gold nanomaterials for photothermal therapy of cancer. Journal of nanoscience and nanotechnology, 2016.* ***16****(1): p. 67-80.*
19. *Patel, D.K., Y.-R. Seo, and K.-T. Lim, Stimuli-responsive graphene nanohybrids for biomedical applications. Stem cells international, 2019.* ***2019****.*
20. *Maiti, D., et al., Carbon-based nanomaterials for biomedical applications: a recent study. Frontiers in pharmacology, 2019.* ***9****: p. 1401.*
21. *Park, H., et al., Gold nanoparticles for photothermal cancer therapy. Frontiers in chemistry, 2019.* ***7****: p. 167.*
22. *Kohout, C., C. Santi, and L. Polito, Anisotropic gold nanoparticles in biomedical applications. International journal of molecular sciences, 2018.* ***19****(11): p. 3385.*
23. *Lin, M., et al., Near-infrared light activated delivery platform for cancer therapy. Advances in colloid and interface science, 2015.* ***226****: p. 123-137.*
24. *Green, D., et al., Size, volume fraction, and nucleation of Stober silica nanoparticles. Journal of colloid and interface science, 2003.* ***266****(2): p. 346-358.*
25. *Vansant, E.F., P. Van Der Voort, and K.C. Vrancken, Characterization and chemical modification of the silica surface. 1995: Elsevier.*