Paper presentation -

Targeted Photothermal Lysis of the Pathogenic Bacteria, Pseudomonas aeruginosa, with Gold Nanorods

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Background -

- MDR Bacteria

  - Pseudomonas aeruginosa

- Metallic NPs can be used to fight against bacteria,

  - *N*-Acetyllactosamine Conjugated to Gold Nanoparticles Inhibits Enteropathogenic *Escherichia coli* Colonization of the Epithelium in Human Intestinal Biopsy Specimens

  - Laser-induced explosion of gold nanoparticles : potential role for nanophotothermolysis of cancer

  - Antibiotic-Conjugated Polyacrylate Nanoparticles: New Opportunities for Development of Anti-MRSA Agents

Gold NPs Vs Silver NPs -

- Silver nanoparticles have nonspecific biological toxicity.

- Gold nanoparticles are more photostable, nontoxic, and amenable to surface modification.

  - EG-coated gold resists protein adsorption

  - It is shown that CTAB bound to small gold spheres is not cytotoxic to human cells.

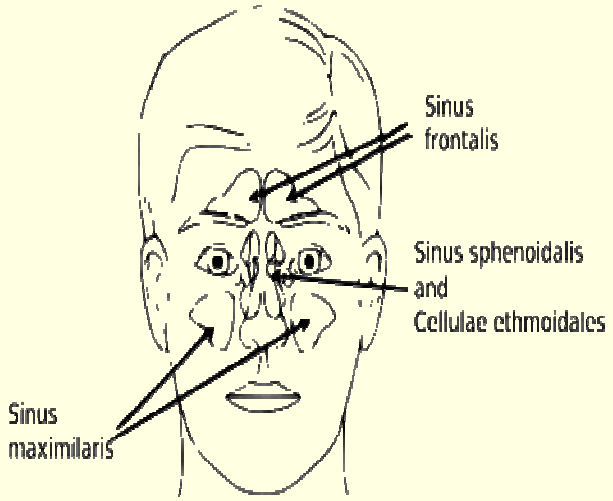
  - gold nanoparticles can be tuned to strongly absorb near-infrared(NIR) radiation depending on their shape and can ultimately transfer this energy into the surrounding environment as heat.

Approach -

- Step 1 :- Generation of primary antibodies,
- Step 2 :- Synthesis of gold nanorods,
- Step 3 :- Surface functionalization of gold nanorods,
- Step 4 :- Bioconjugation of gold nanorods,
- Step 5 :- Studying effect of NIR on gold nanorod bounded PA3 bacteria.

Generation of Primary antibodies -

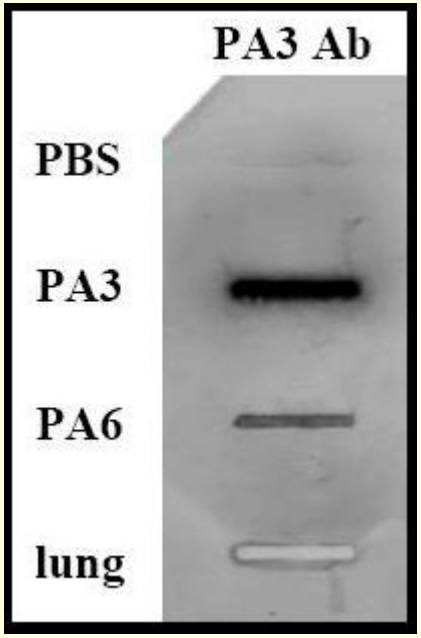
- PA3 Obtained from upper respiratory tract of sinusitis patient.
- PA3 were inoculated into rabbits followed by serum collection.
- Polyclonal antibody purification was performed using a commercially available Protein G agarose affinity column method.



Generation of Primary antibodies -

- Immunoblot analysis for checking specificity of polyclonal antibody.

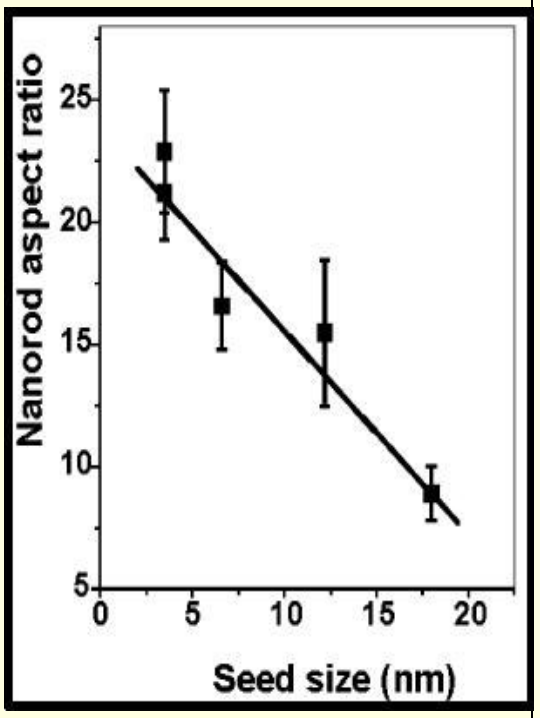
  - Pelleted cells(PA3,PA6,Small airway Epithelial cells) were spotted on nitro-cellulose membrane.
  - Membrane was incubated with PA3 antibody
  - Anti-rabbit IgG secondary antibody added for detection and scanned on a Licor Odyssey NIR scanner.



Synthesis of Gold nanorods -

- Seed mediated surfactant directed approach

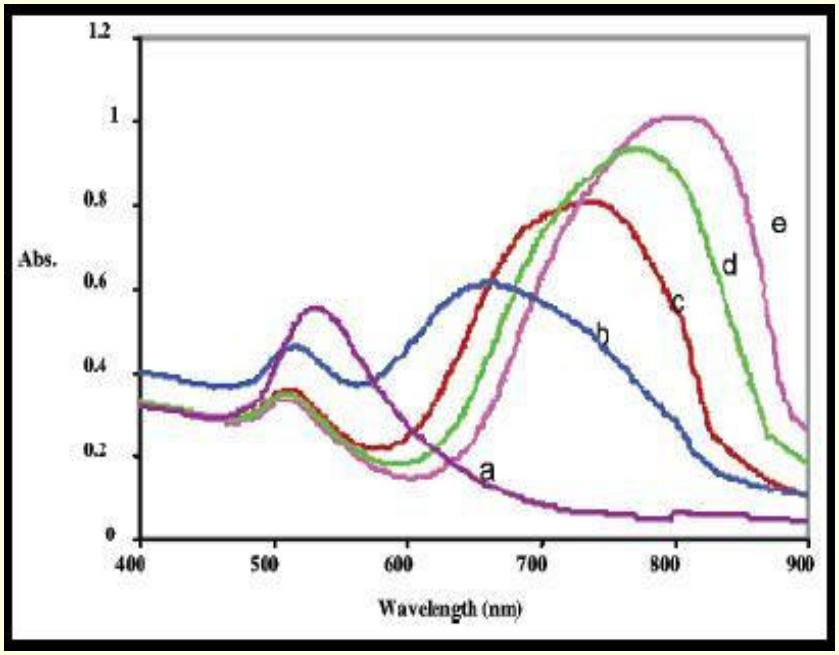
  - Aspect ratio is controlled by the relative concentrations of reagent and seed size.
  - The concentration of CTAB (0.1 M)is critical for nanorod growth.
  - presence of 5% Ag^+ raises the yield of gold nanorods to nearly 100%,compared to 20-40% in the absence of Ag^+ But aspect ratio possible is ~ 6
    and in absence of Ag^+ it can be ~ 25.



Synthesis of Gold nanorods -

- Aspect ratio and UV-vis spectrum

a – 1.35 ± 0.32
b – 1.95 ± 0.34
c – 3.06 ± 0.28
d – 3.50 ± 0.29
e – 4.42 ± 0.23



Synthesis of Gold nanorods -

10 mL of 2.5 x 10^{-4}M $HAuCl_4$ in 0.1M CTAB (under vigorous stirring) + 0.6 mL of 10 mM $NaBH_4$ (ice cold)

↓

0.12 mL Seed solution(brown)

+ ⟶ gold nanorods(brown)

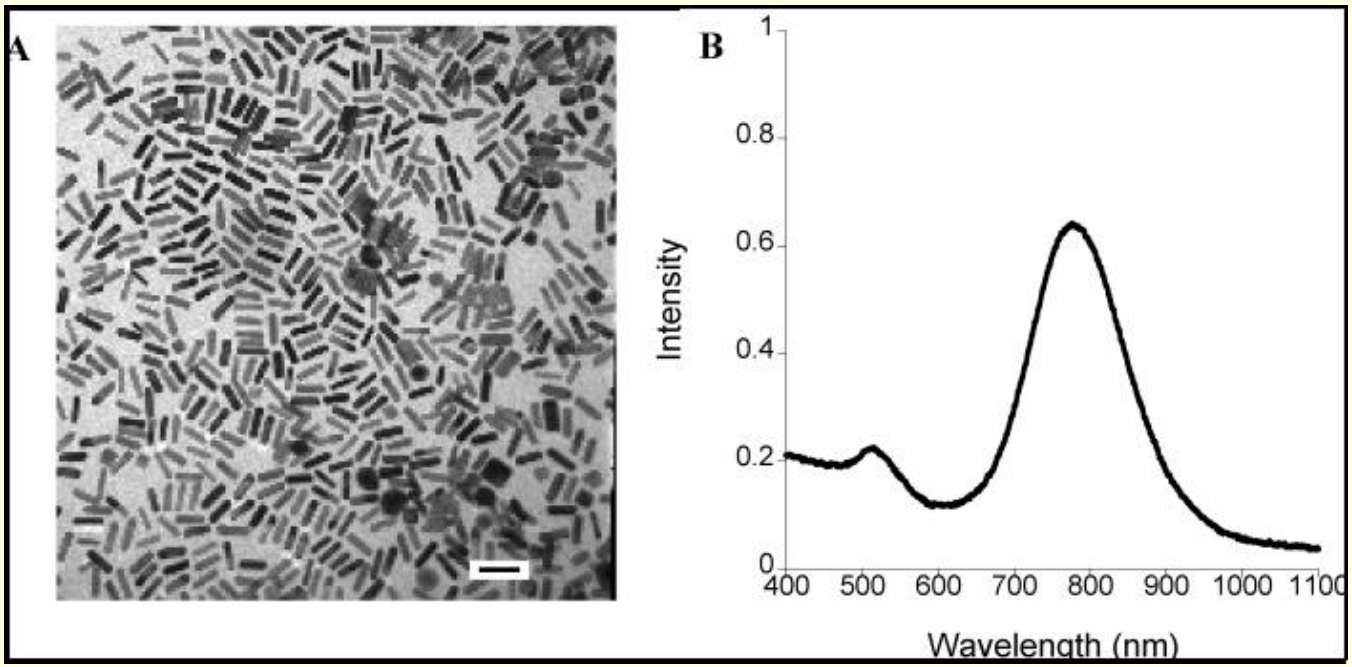
Stock solution(colourless)

↑

95 mL 0.1M CTAB solution + 1 mL 10mM $AgNO_3$ solution + 5 mL 10mM $HAuCl_4$ + 0.5 mL 0.1M ascorbic acid

Synthesis of Gold nanorods -

- **A** – TEM of 68nm x 18nm nanorods, scalebar – 100nm
- **B** – UV-vis spectrum, absorbance peak ~ 785nm



Surface functionalization -

- Step 1 :- poly(acrylicacid) (PAA) coating

PAA stock solution – 10mg/mL in 10mM NaCl solution,

1mL		200µL		100 µL
4x gold nanorods	+	PAA stock solution	+	10mM NaCl

↓

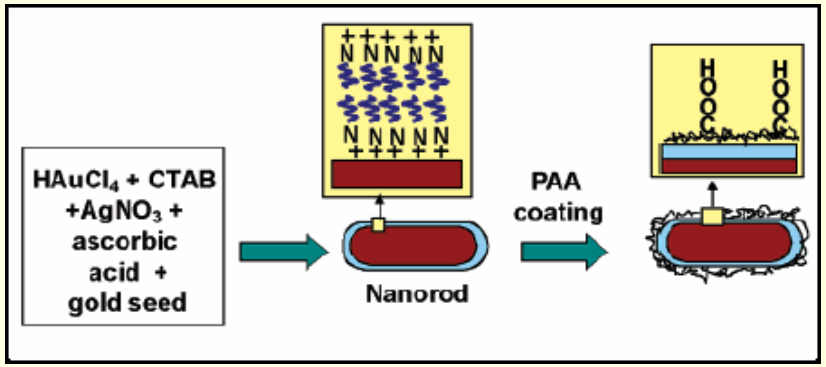
vortex

(2 min)

↓ after 30 min

Centrifuge

(14000rpm, 8 min)



- Redisperse pellet in 1mL DI water

Surface functionalization -

- Step 2 :- polyallylamine hydrochloride(PAH) coating

PAH stock solution – 10mg/mL in 10mM NaCl solution,

1mL PAA coated gold nanorods + 200µL PAH stock solution + 100 µL 10mM NaCl

↓

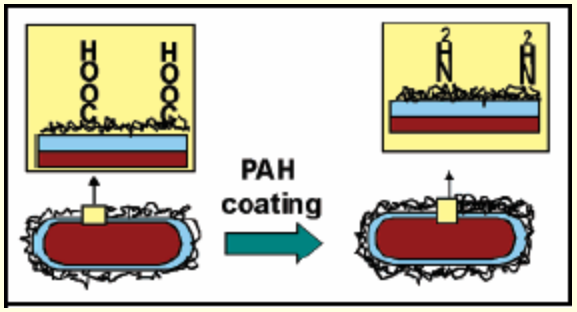
vortex

(2 min)

↓ after 30 min

Centrifuge

(14000rpm, 8 min)



- Redisperse pellet in 1mL DI water

Bioconjugation -

- Step 1:-

  A = 1mL of PAH coated gold nanorods were dispersed in MES ($C_6H_{13}NO_4S$) buffer (10mM, pH 5.5).

  B = IgG stock solution 1mg/mL in(10mM MES buffer,pH 5.5)

- Electrostatic attachment of antibody:-

  A (1mL) + B (100 µL) —vortex (2 min)→ store at 4° C (12-16h)

Bioconjugation -

- Covalent attachment of antibody :-

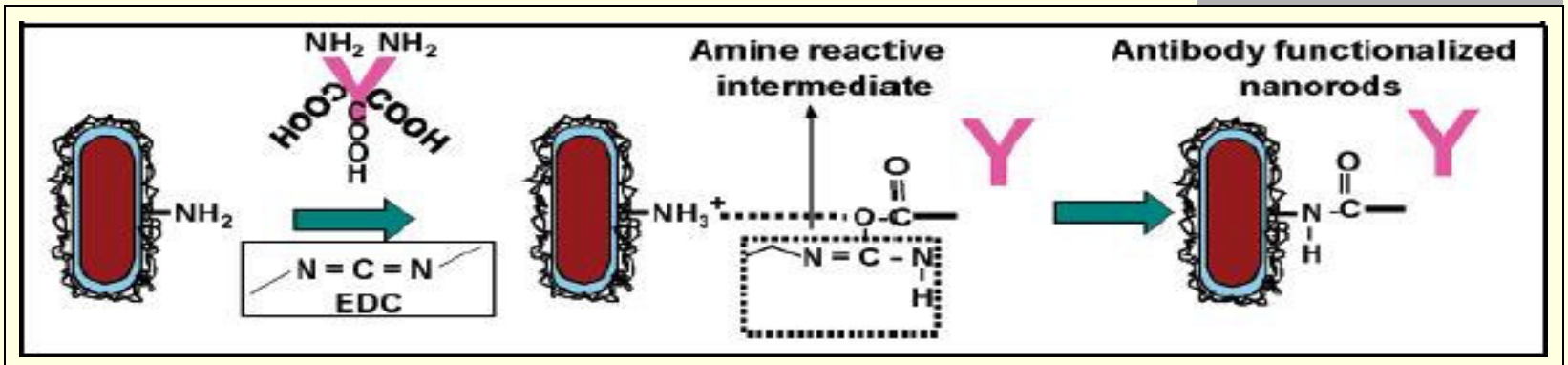
$$A\ (1mL) + B\ (100\ \mu L) \xrightarrow[(2\ min)]{vortex} C$$

After 15 min,

$$C + EDC\ (1.2\ mg) \xrightarrow[(2\ min)]{vortex} \text{store at } 4^{o}\ C\ (12\text{-}16h)$$

- EDC = 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide

Bioconjugation –

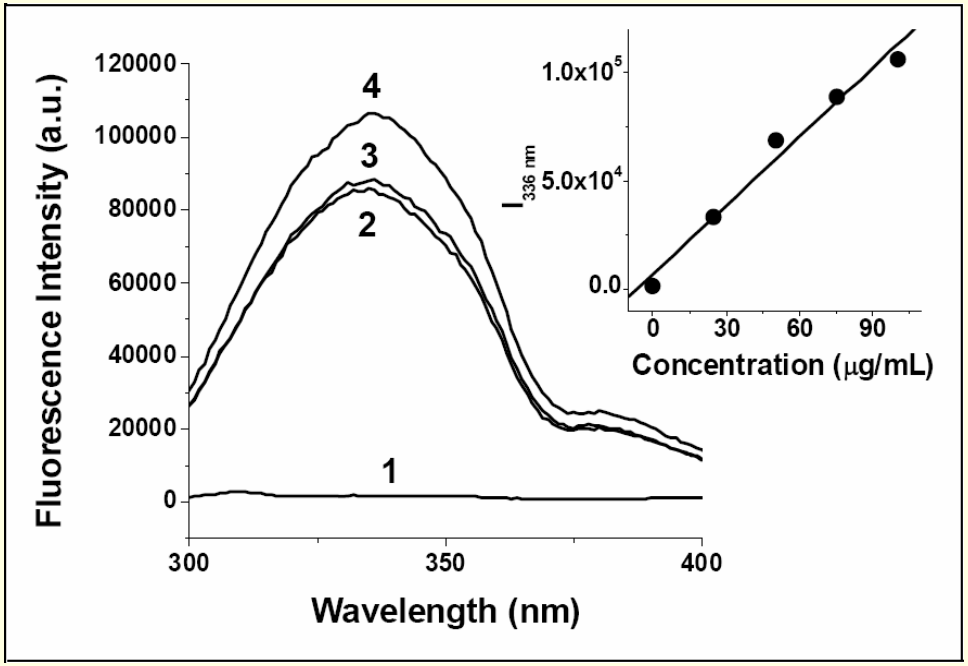


Quantification of bioconjugation by studying fluorescence of tyrosin, tryptophan at 280nm.

1 - MES buffer,2 –EDC linked conjugates supernatant

3 – electrostatically linked conjugates supernatant

4 - 100µg/mL IgG in MES



Bioconjugation -

- FTIR measurement to detect formation of nanorod - antibody complex.

Amide bands in FTIR,

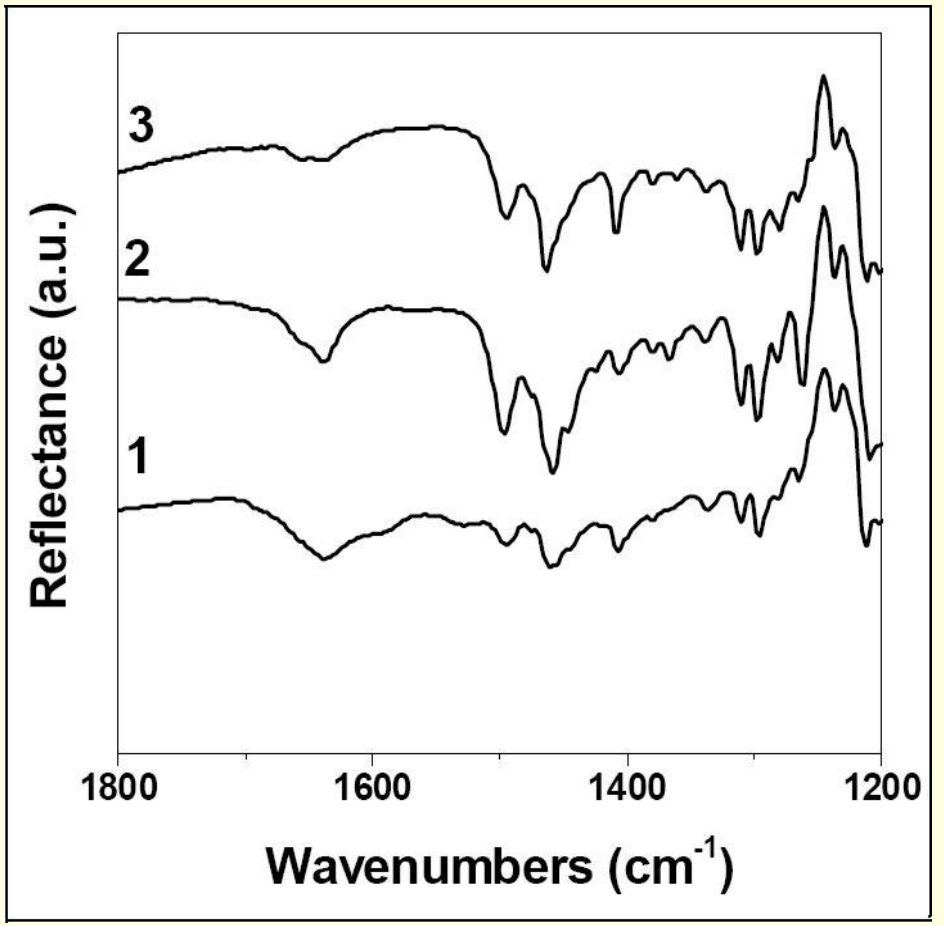
I – 1650 cm^{-1}

III – 1300-1200 cm^{-1}

1 – Native IgG

2 – EDC coupled IgG – gold nanorod conjugates

3 – Electrostatically coupled IgG – gold nanorod conjugates



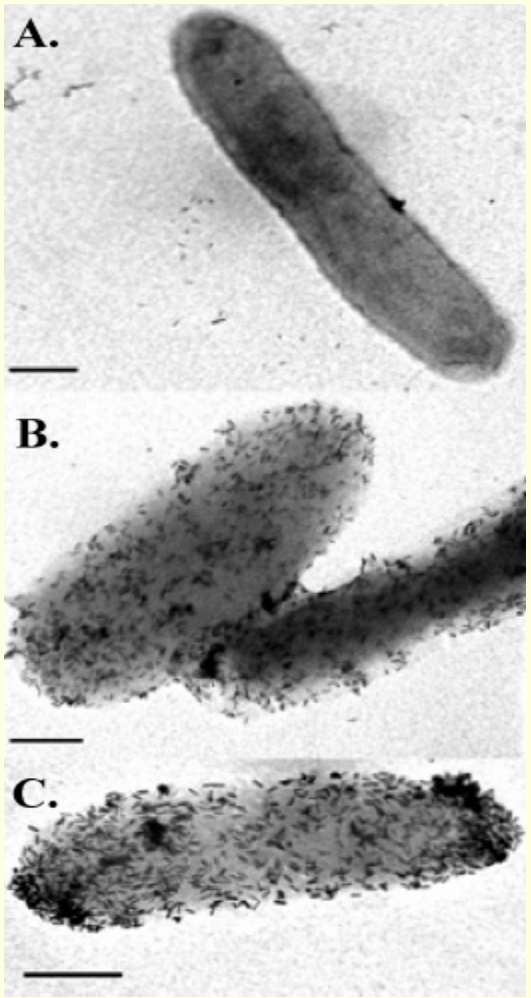
Targetting & Photothermal lysis -

- TEM image (30000 x) of bacteria incubated with appropriate - antibody-nanorod complex (20 min) Scale bar = 500 nm.

A - PA3 with nonconjugated nanorods

B - PA3 bound with electrostatically conjugated antibody-nanorod complexes

C - PA3 bound with covalently linked antibody-nanorod complexes using the EDC method.



Targetting & Photothermal lysis -

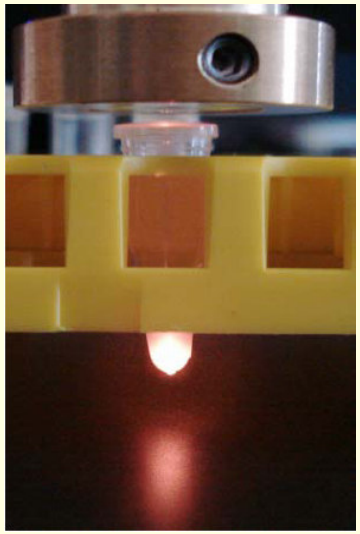
- To study the effect of NIR on PA3 bounded with antibody nanorod complex, procedure was –

  Step 1 :- Incubation of PA3(OD_{600}= 1.0), then pelleted and resuspended in 1 mL 0.85% NaCl.

  Step 2 :- Incubation of PA3 with antibody nanorod complex (20 min)and then washing with 0.85% NaCl and resuspending it in 500µL of 0.85% NaCl.

  Step 3 :- Exposure of prepared suspension to NIR(785nm,50mW) for 10 min.

  Step 4 :- Staining of cells for 15 min by live/dead stain (SYTO 9 and propidium iodide).



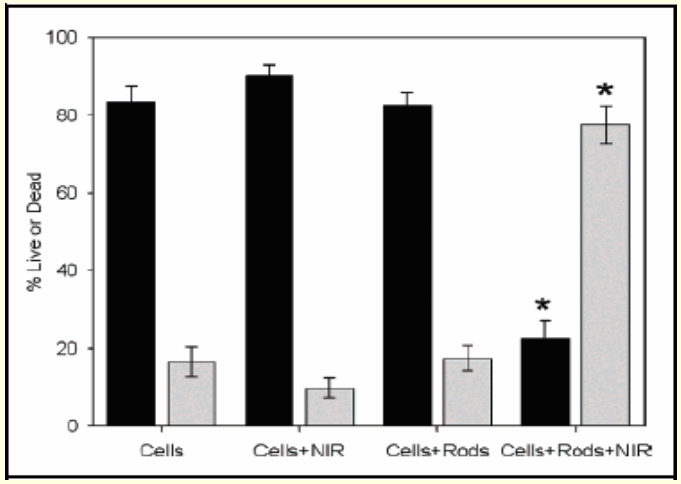
Results -

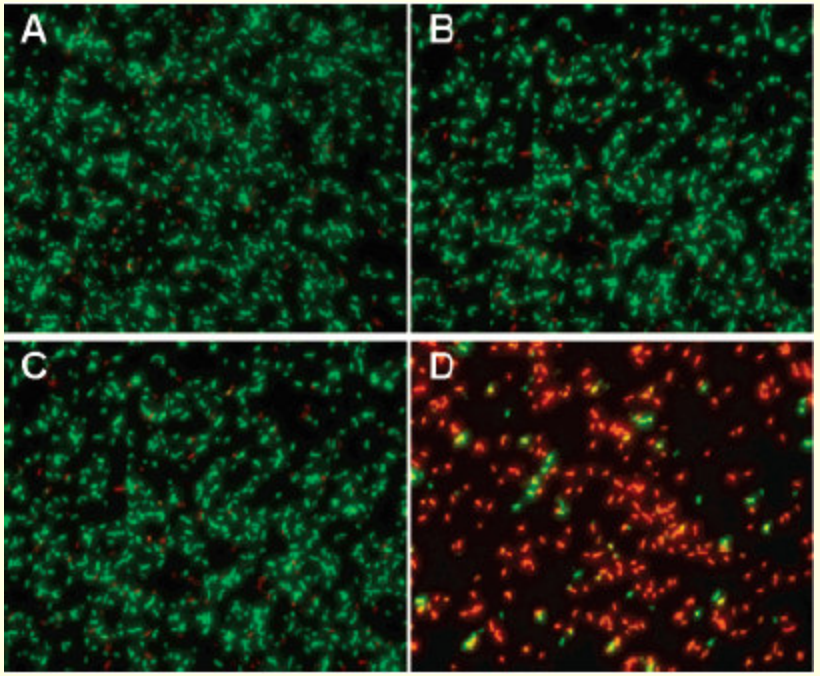
- Fluorescence images of live/dead stained cells,

A – Without nanorod/NIR exposure ;B – Without nanorod exposed to NIR

C – With nanorods and no exposure to NIR

D - With nanorods and exposed to NIR (10 min)



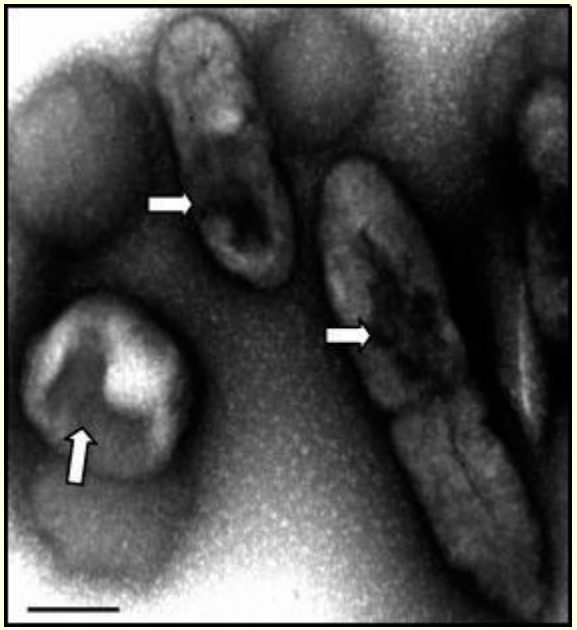


Reasoning -

- TEM image (30,000 x) of PA3 cells with attached antibody-conjugated nanorods following 10 min exposure to NIR.

  Scale bar = 500 nm.
- Arrows are indicating irreparable

  damage of cell membrane.



Thank you