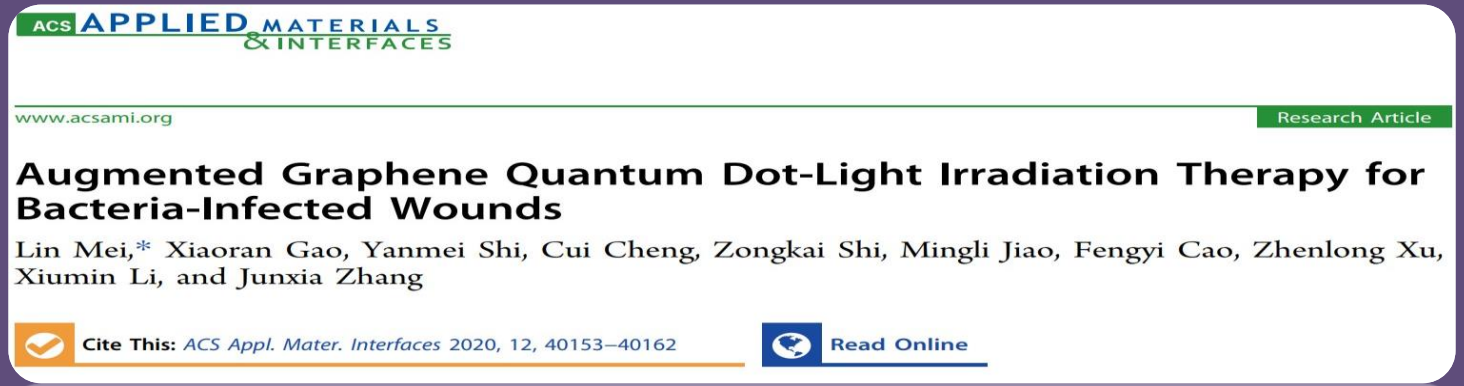
Shiraz University of Medical Sciences

In the Name of God



ACS APPLIED MATERIALS & INTERFACES

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Research Article

Augmented Graphene Quantum Dot-Light Irradiation Therapy for Bacteria-Infected Wounds

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Outline

Introduction	• Why phototherapy is attracted
Experimental	• Explain study design
Results & Discussion	• Interpretation of experiments
Conclusion	• Reviewing highlighted points

Introduction

- Infectious disease induced by pathogenic bacteria is one of the world's greatest health challenges
- The widespread use of antibiotics and anti-inflammatory agents

Increasing numbers of **drug-resistant bacterial** strains

So, designing new antimicrobial materials are crucial

Introduction

Recently, **phototherapy** methods have attracted wide attention

- ✓ High therapeutic efficiency
- ✓ Controllability
- ✓ Target selectivity
- ✓ Noninvasive nature

- ❑ Photothermal therapy (PTT)
- ❑ Photodynamic therapy (PDT)

❖ PTT & PDT use light irradiation to kill bacteria without bacterial resistance

Introduction

- ❑ The heat-induced by **PTT** can increase
  - ✓ The temperature of ambient tissue
  - ✓ Enhance antimicrobial efficacy
  - ✓ Increasing blood flow
  - ✓ Increasing oxygen supply
  - ✓ Enhancing singlet oxygen ($^1O_2$) generation

- ❑ Reactive oxygen species (ROS) generated by **PDT** can
  - ✓ Induce initial oxidative damage to bacterial cytomembranes
  - ✓ Increasing the permeability
  - ✓ Sensitivity of the damaged membrane to heat

Introduction

Common photothermal agents and photosensitizers have shown potential for their application in **PTT** & **PDT**

- Gold nanoparticles
- Black phosphorus
- Upconverting nanoparticles
- Porphyrin
- Cyanine derivatives

- **Tedious preparation** procedures for black phosphorus, porphyrin, and cyanine derivatives
- **Poor ambient stability**, **high cost**, & **low photostability** for gold nanoparticles and upconverting nanoparticles

Introduction

Because lots of functional groups, GQDs surfaces can be easily modified

Low toxicity
Stable photoluminescence
Excellent biocompatibility
Chemical inertness

Why the Graphene quantum dots (GQDs) is remarkable type

With antioxidant activity can produce ROS under visible light irradiation

Introduction

Silver nanoparticle-conjugated GQDs

Exhibited high photothermal conversion efficiency and ROS generation efficiency under **450 nm** light irradiation

The GQDs nanocomposite still had most of its negatively charged carboxyl groups on its surfaces

Introduction

Compared to the **chitosan**

Chitosan oligosaccharide (COS) has many positively charged amino groups on its surface

Can disrupt or permeabilize bacterial cell membranes

COS + GQD

Increase the positive charges of the GQDs' surfaces

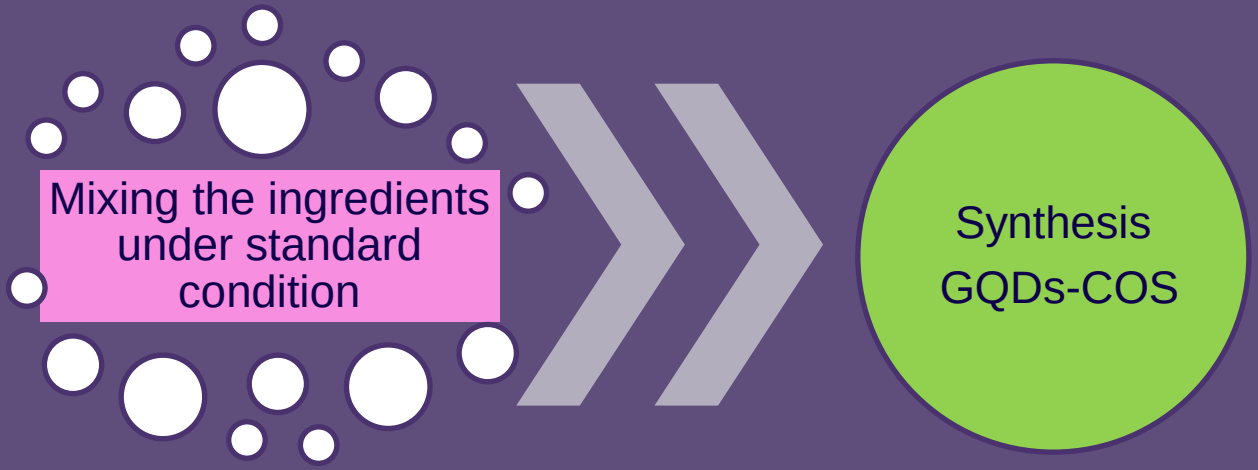
Promote interaction with the negatively charged phospholipid membranes of the bacterial surface

The aim of the study

- Preparation of **GQDs-COS** as a multiple synergetic antibacterial material for using against bacterial infections
- Evaluation three types of antimicrobial activity including

✓ Photodynamic
✓ Photothermal
✓ Chemical

Experimental



Using the UV-vis spectra

Observation morphology by TEM

Experimental

Measurement of Photodynamic

- A **Xe lamp** with a 450 nm band-pass filter was used as the light source
- The generation of $^1O_2$ was measured by using **ADPA** as the $^1O_2$ -trapping agent

Disodium 9,10-anthracendipropionic acid

Measurement of Photothermal Performance

- GQDs-COS with different concentrations **were irradiated** (450 nm) for a given time
- The temperature change was recorded by an **infrared thermal imager**

Experimental

Bacterial Growth Inhibitory

1

Bacterial suspensions were diluted with Luria-Bertani (LB) broth & grown to an optical density of **OD600 = 0.1**

2

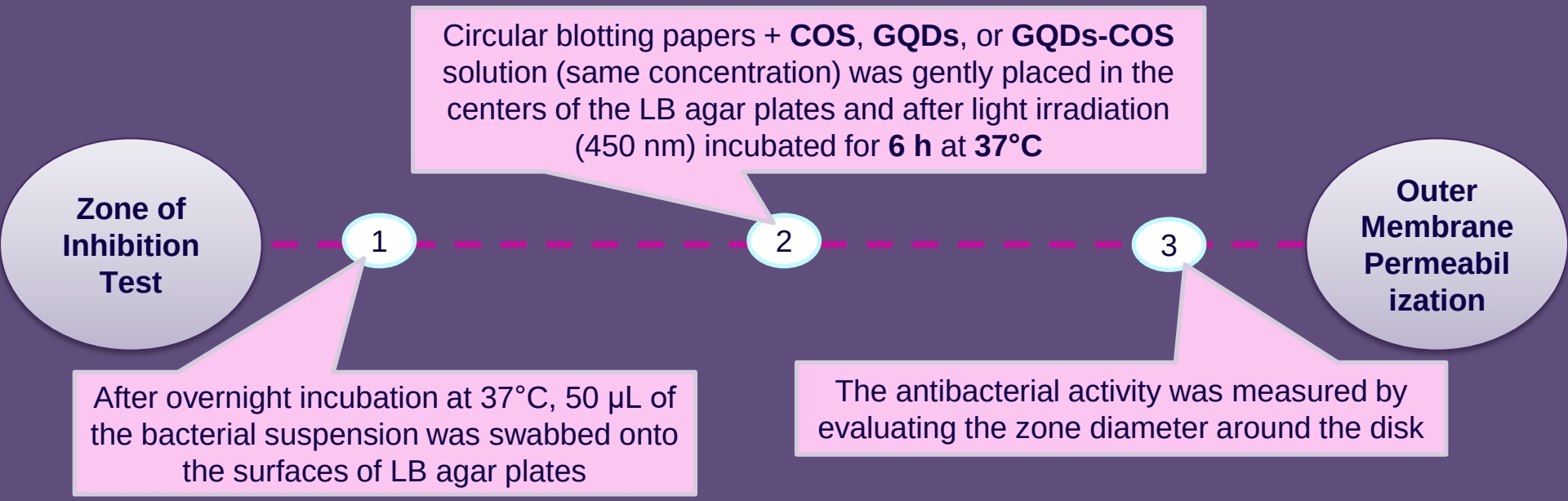
The bacterial suspensions were mixed with **equal** proportions of the GQDs-COS of **different** concentrations

3

Performed irradiation (450 nm) for **10'** & incubation for **8 h**, the OD600 value of the mixed solution was determined

Zone of Inhibition Test

Experimental



Experimental

Outer Membrane Permeabilization

1

E. coli cultures were harvested by centrifugation at **6000 rpm** for **5'** and rinsed with phosphate buffer solution (PBS, 0.01 mol/L, pH 7.4) three times

2

The final bacteria were **resuspended** in **1.5 mL** PBS

3

50 μL of **1 mg/mL NPN** & **GQDs-COS** solution with different final concentrations were added into the bacterial suspension

N-phenyl-1-naphthylamine

4

The fluorescence intensity was determined by a fluorescence spectrophotometer with **excitation** wavelength was **350 nm**, and the **emission** wavelength was **370−550 nm**

Experimental

Inner Membrane Permeabilization

- Using **ONPG** & **GQDs-COS** (150 μL of 10 mg/mL) of various final concentrations
- Adding into the *E. coli* suspension (in PBS, OD600 = 0.4)
- Under light irradiation (450 nm), changes in the **OD420** value were monitored

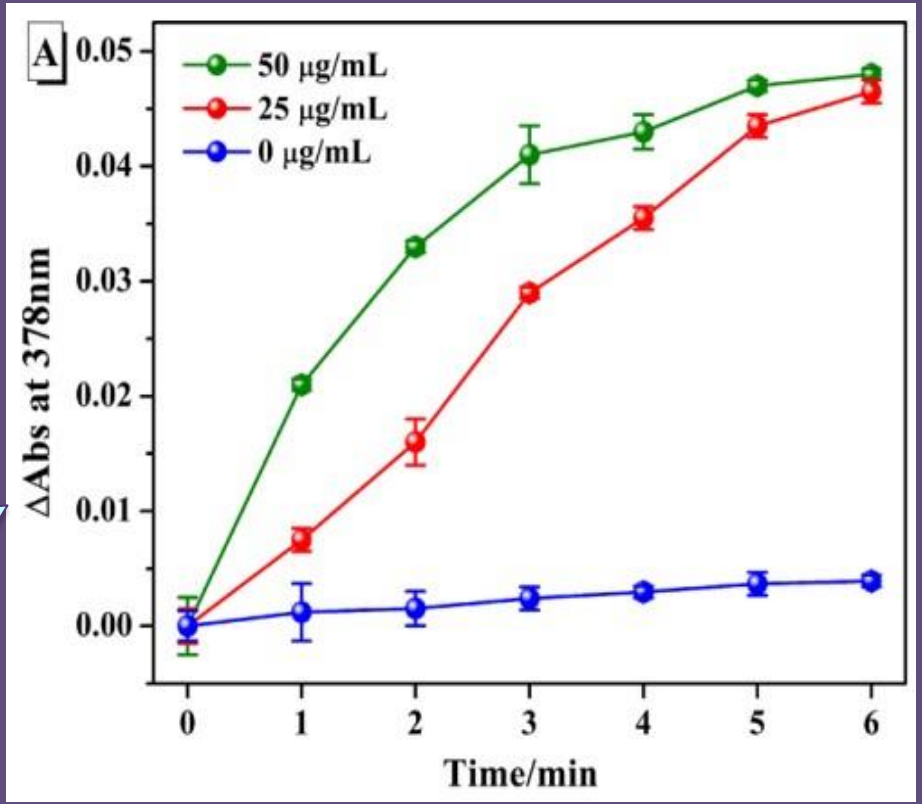
Others

- **Preparation of Bacterial Samples for SEM**
- **Cell Viability Assay**
- **In Vivo Experiments**

Results & Discussion

To investigate $^1O_2$ generation by the GQDs-COS, ADPA was used as the $^1O_2$ trapping agent

A) Absorbance change of ADPA at **378 nm** over time in the presence of GQDs-COS with various concentrations under light irradiation



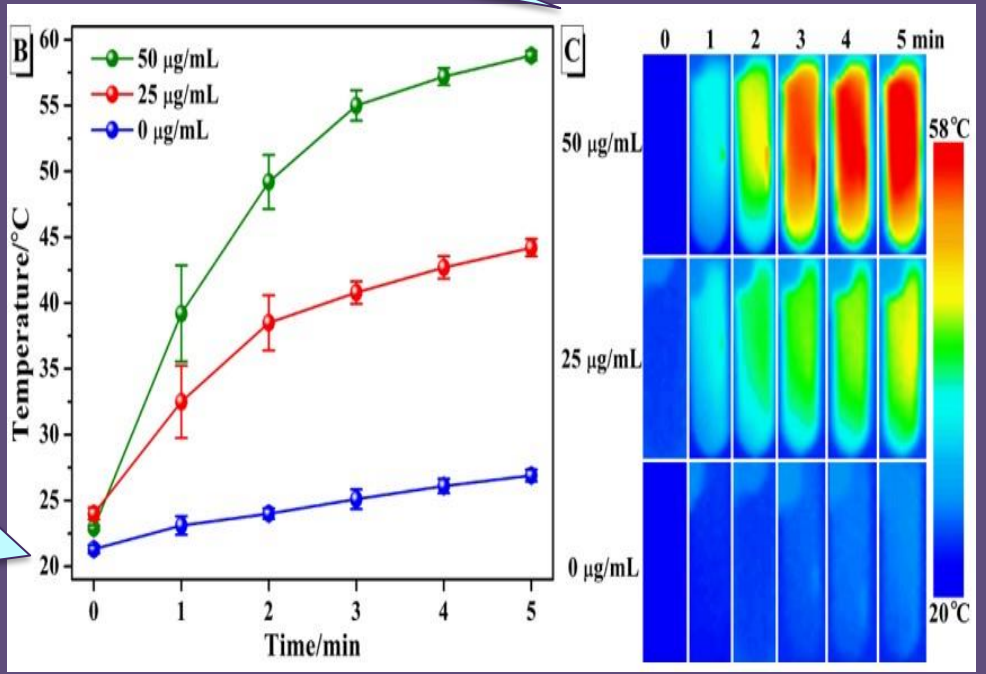
Results & Discussion

After light irradiation for 5', the heating curves of GQDs-COS with different concentrations increased steadily

The 50 μg/mL GQDs-COS increased in temperature from **22.9** to **58.8°C**

(C) IR thermal images of GQDs-COS solutions with various concentrations under light irradiation recorded at different time intervals

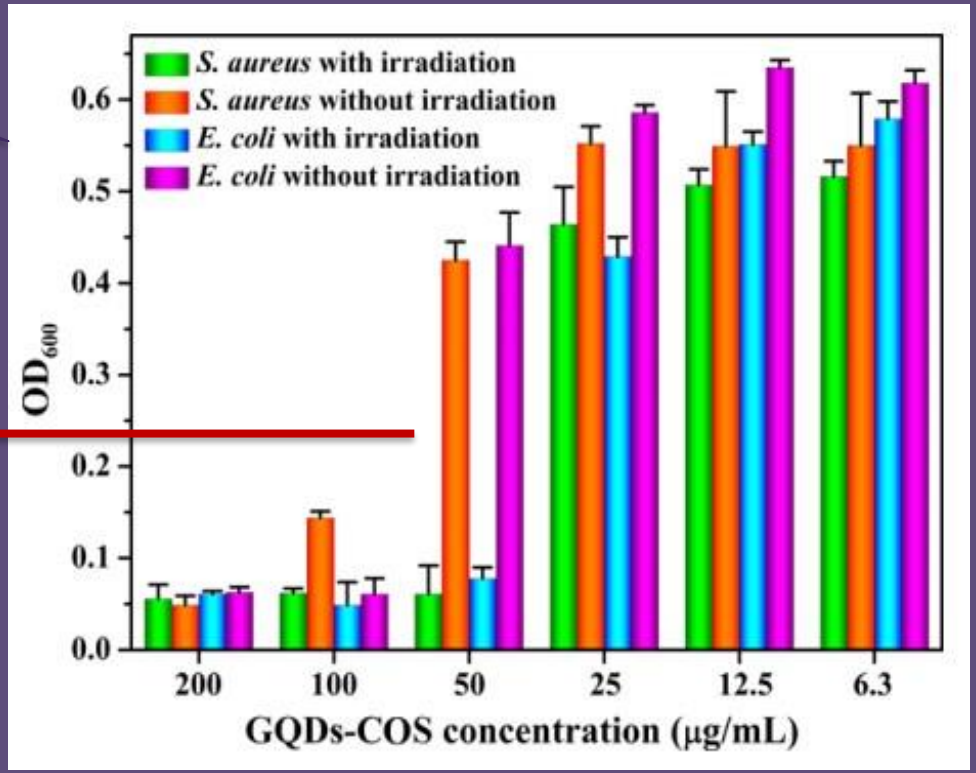
(B) Heating curves



Results & Discussion

Bacterial growth inhibition assays

- *E. coli* & *S. aureus* was inhibited at GQDs-COS concentrations >100 µg/mL without irradiation
- The antibacterial activity was increased 2-fold after irradiation
- An increase in antibacterial activity (decrease in the OD600 value)

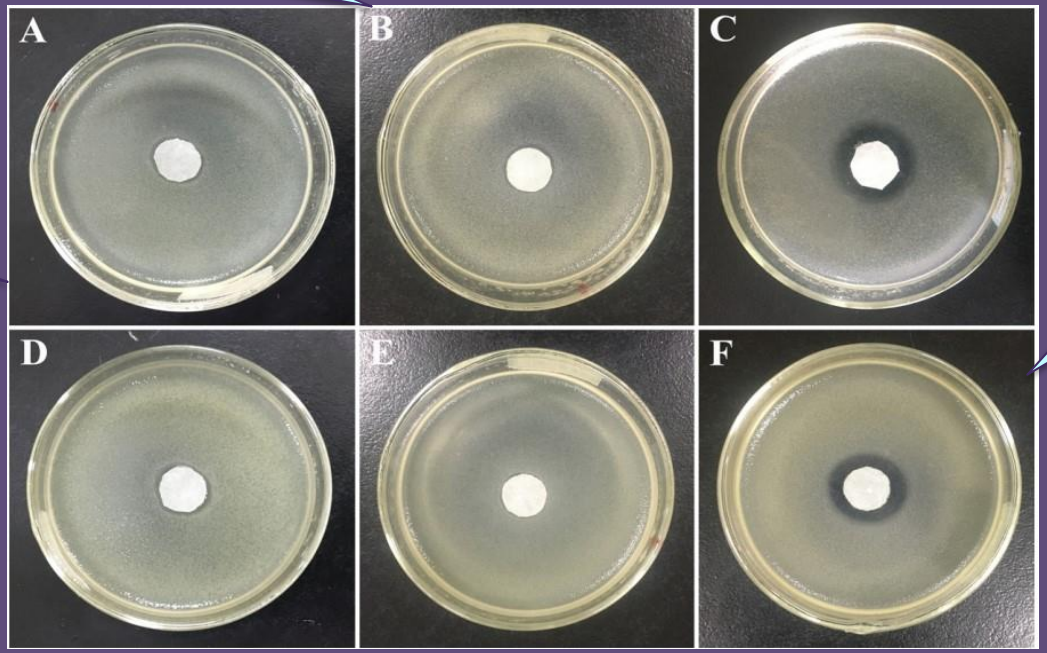


Results & Discussion

Inhibition zones of **GQDs (B, E)**

The inhibition zones with GQDs-COS were **larger** than those with GQDs & COS

Inhibition zones of **COS (A, D)**



Inhibition zones of **GQDs-COS (C, F)**

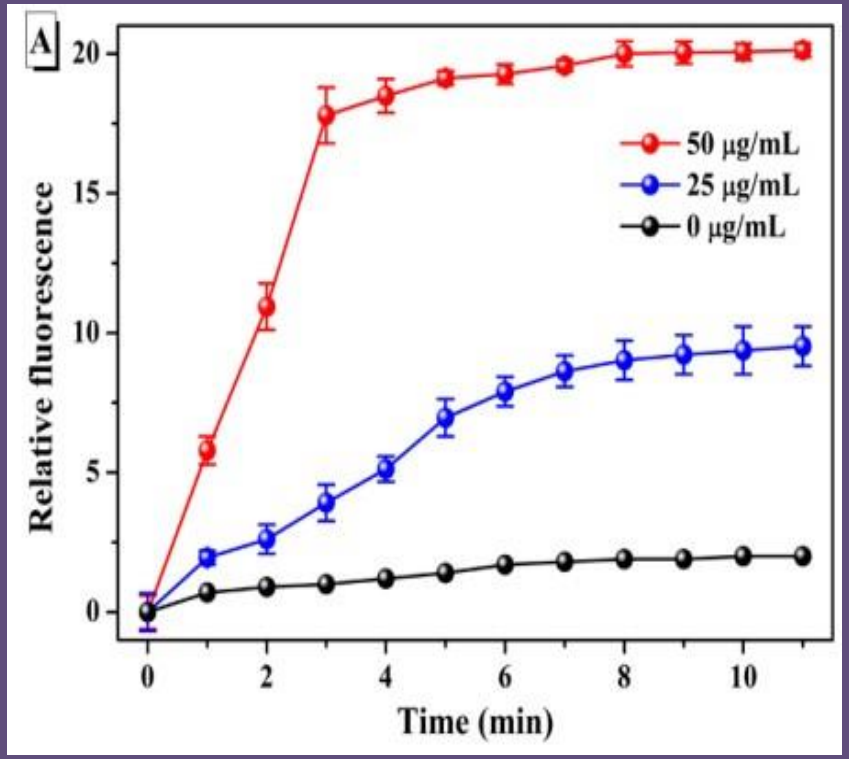
Results & Discussion

Study of the Antibacterial Mechanism

NPN is hydrophobic and without fluorescence in water or LB broth

Once the outer membrane of bacteria is destroyed

NPN reacts with the phospholipids in the cell membrane & shows fluorescence



Results & Discussion

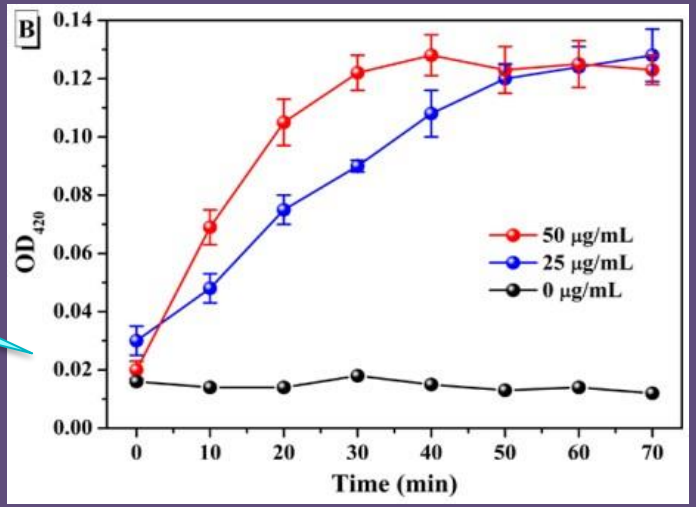
Investigate whether GQDs-COS disrupt the permeabilization

Adding ONPG to the bacterial suspension

The Cytoplasmic β-galactosidase leak from damaged bacterial membranes and reacts with ONPG to produce **ONP**

Can be monitored by absorbance at 420 nm

Shows that the OD420 values increased with increasing GQDs-COS concentrations



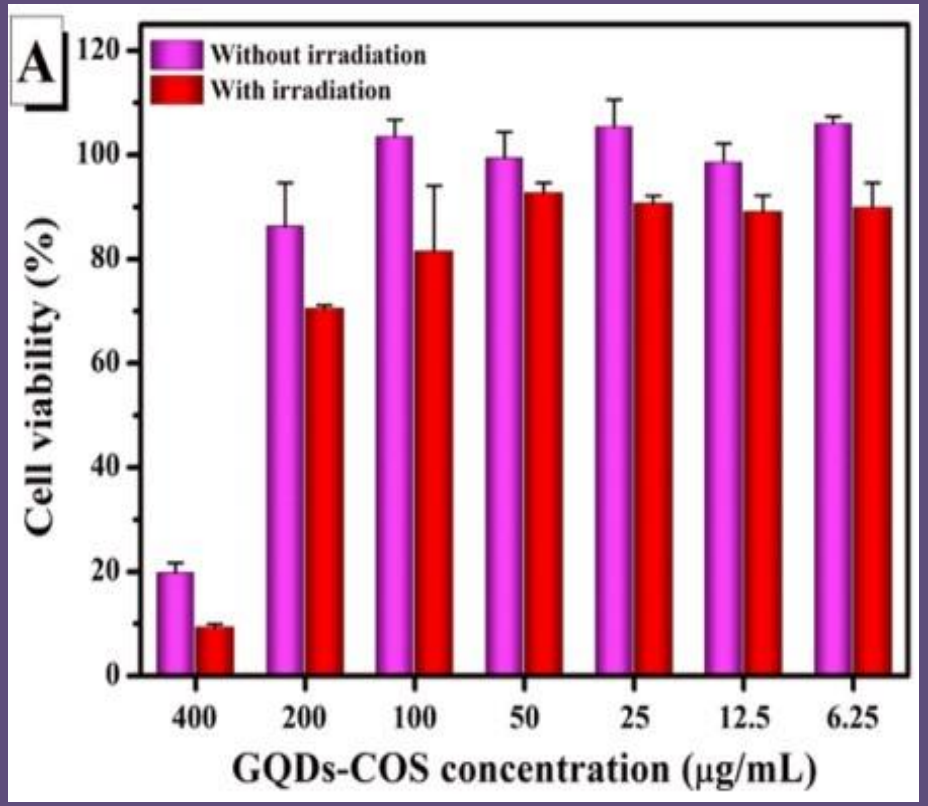
Results & Discussion

The cytotoxicity of GQDs-COS was determined by an MTT assay

Cell viability decreased with increasing GQDs-COS concentrations and light irradiation

The cell viability **with irradiation** was over 80% at concentrations of **≤100 μg/mL**

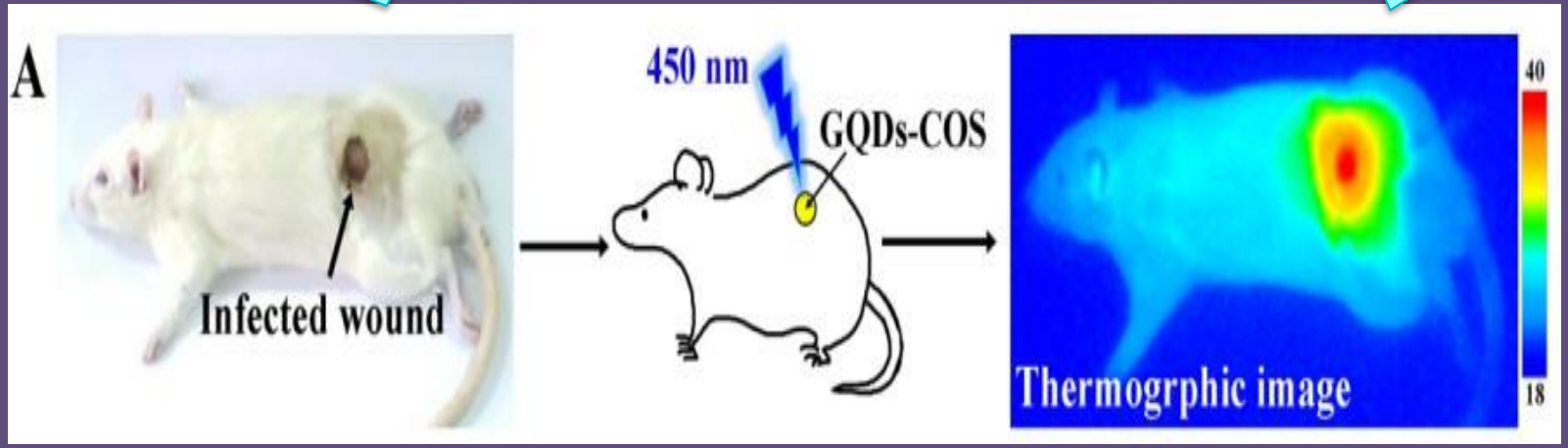
This is **greater** than the antibacterial concentration of GQDs-COS against *E. coli* and *S. aureus*



Results & Discussion

The rat wound was infected by *S. aureus* & coated with 100 µL GQDs-COS under light illumination for 10 min

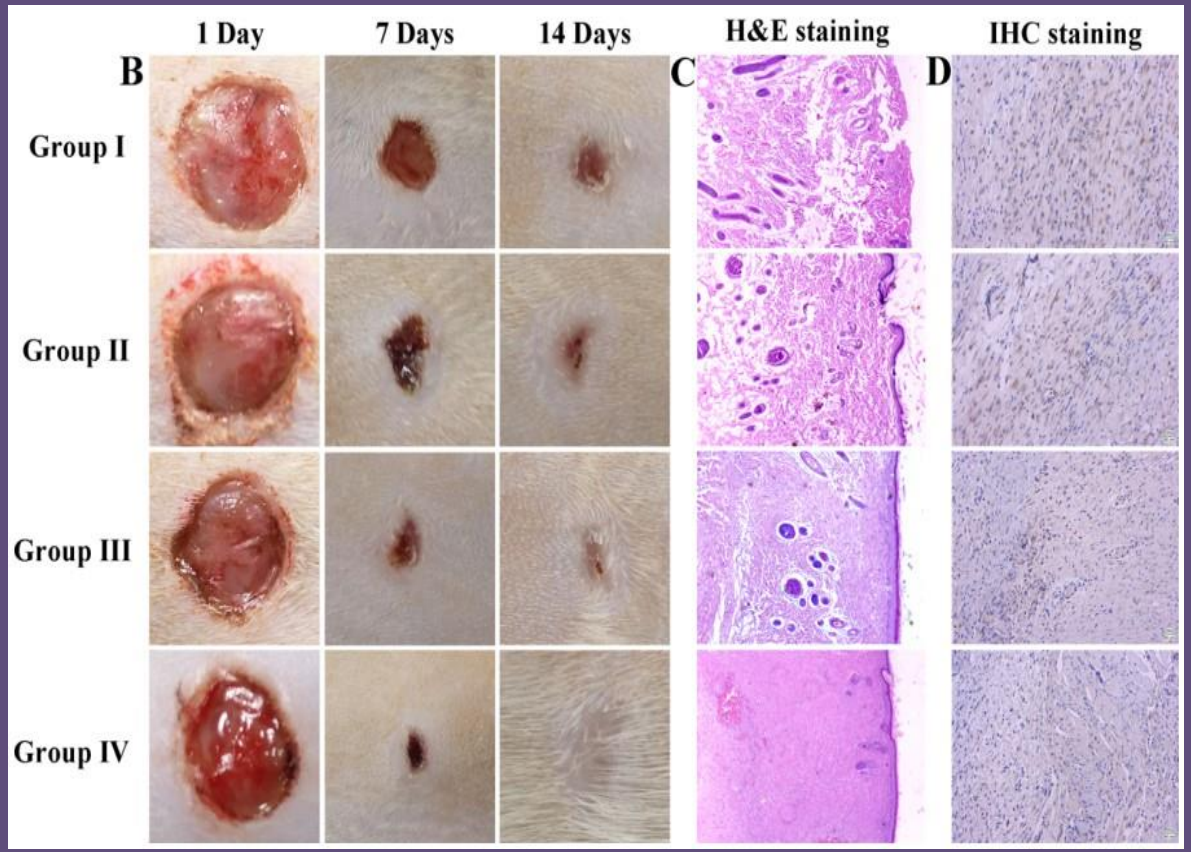
Temperature around the wound increased to **40 °C**



In Vivo

Results & Discussion

- **Group I** (100 μL of physiological saline)
- **Group II** (100 μL of physiological saline with light illumination)
- **Group III** (100 μL of 100 μg/mL GQDs-COS)
- **Group IV** (100 μL of 100 μg/mL GQDs-COS with light illumination



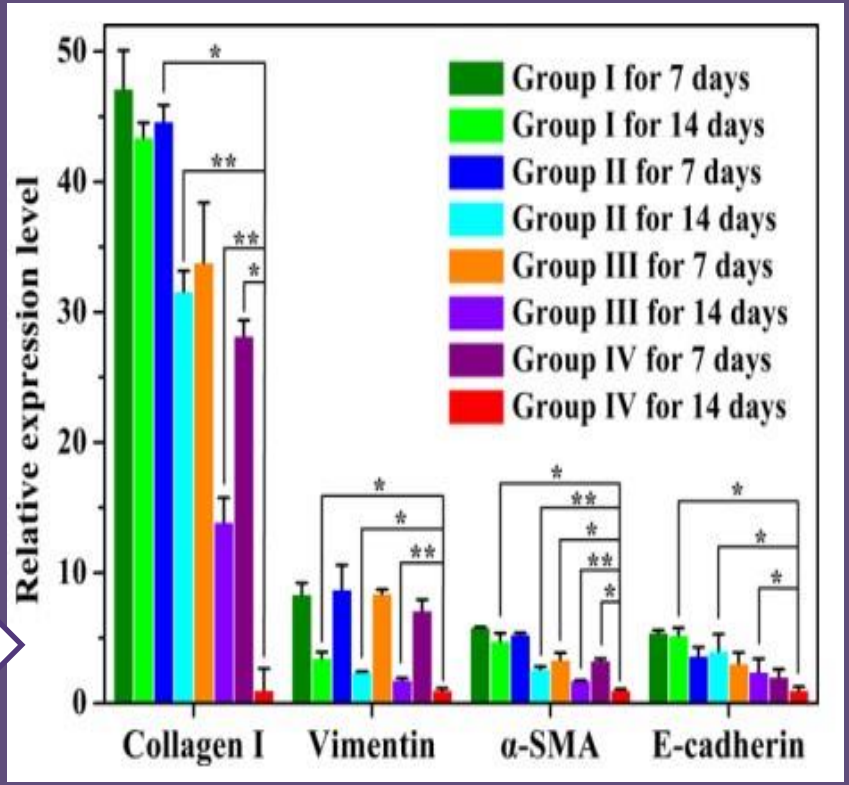
Results & Discussion

The epithelial-to-mesenchymal transition (EMT)

The mainly biological function of EMT is to produce fibroblasts to repair tissue damage

The α-SMA, collagen I & vimentin are EMT makers

Bacterial infection can induce the high expression of EMT makers in the sites of inflammation



Conclusion

- ✓ This paper proposes a new antibacterial system based on PDT, PTT, and chemotherapy
- ✓ The positively charged surfaces of these nanosheets can easily capture bacteria
- ✓ The epithelial & stromal cell expressions of inflammatory markers in wound skin were analyzed and confirm that inflammation was eliminated


Thank
you