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# Oxygen nanobubbles for the reversal of hypoxia and drug delivery

**NANO  
MEDICINE  
LAB**

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# THE NANOMEDICINE LAB

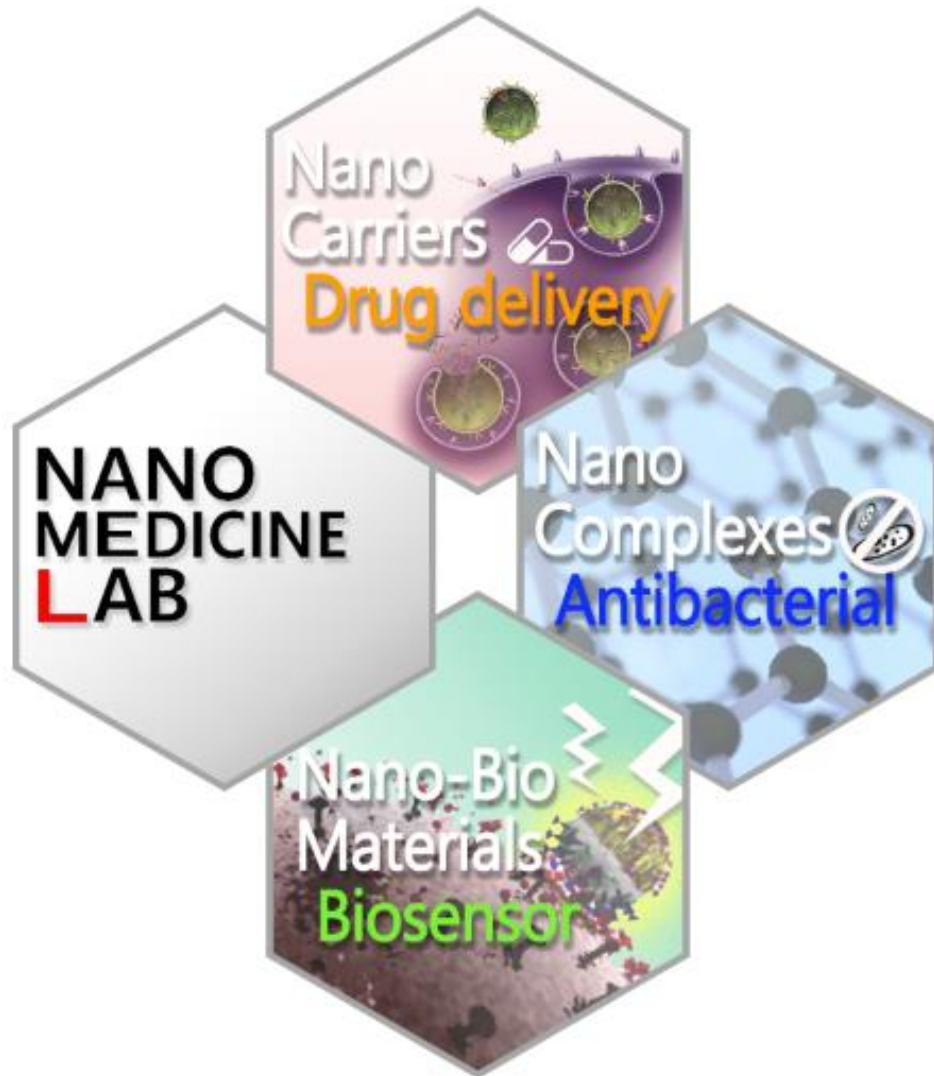


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**M.S. students (5):** Yejin Kwon, Jaehee Jang, Kyungwoo Lee, Chanhwi Park, Dasom Kim

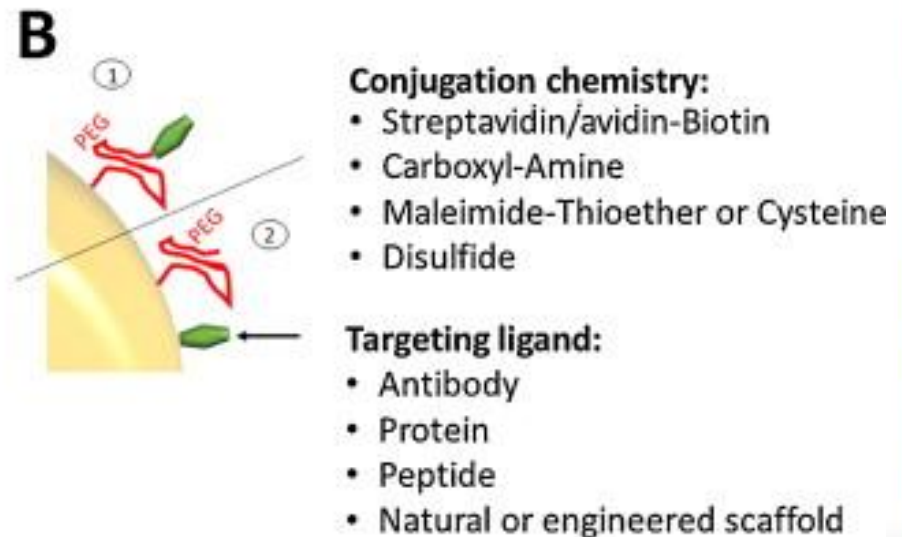
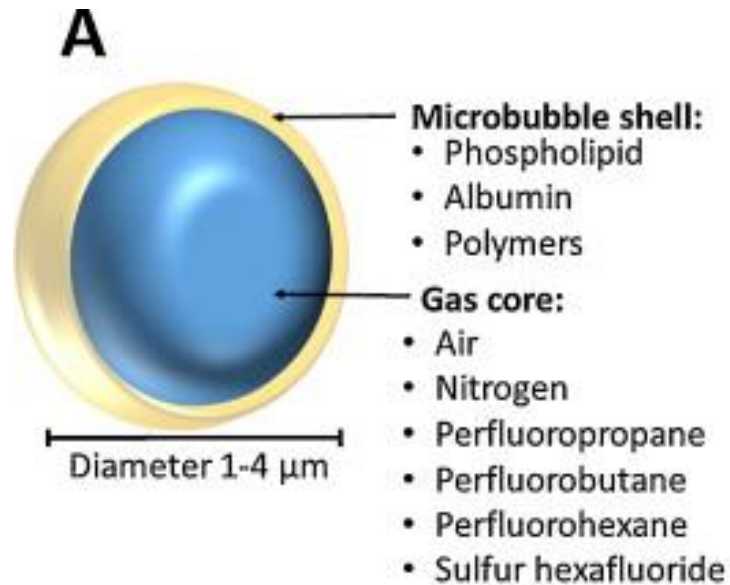
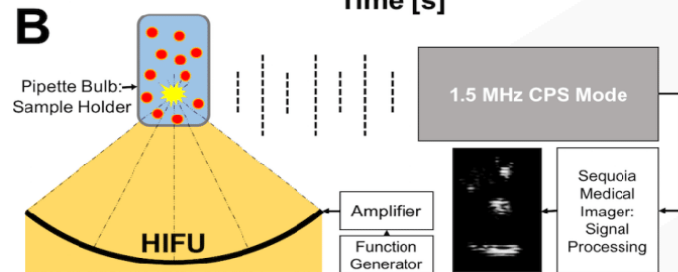
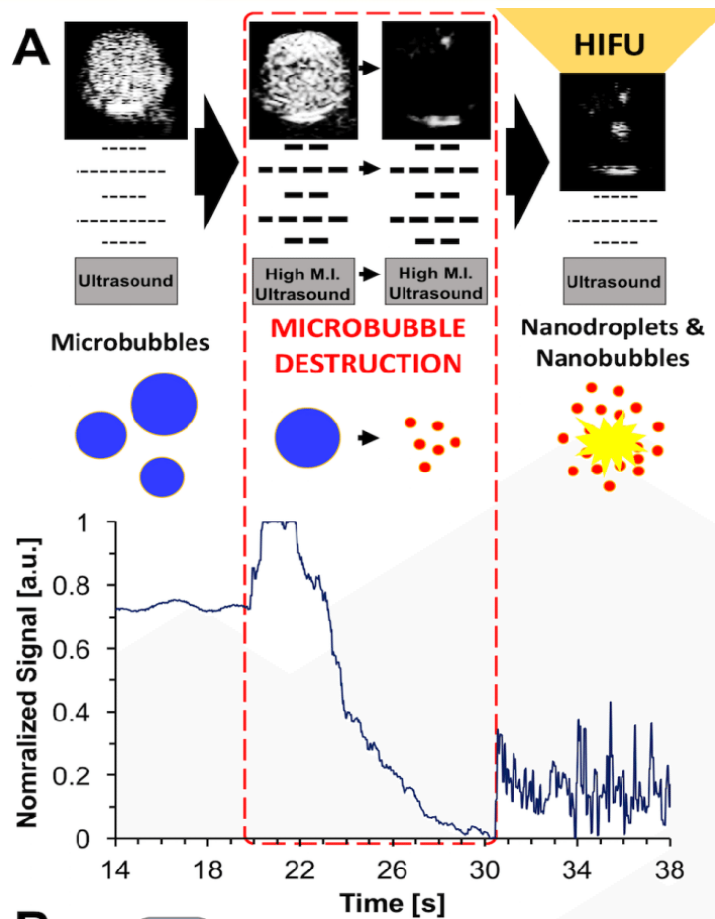
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# THE NANOMEDICINE LAB

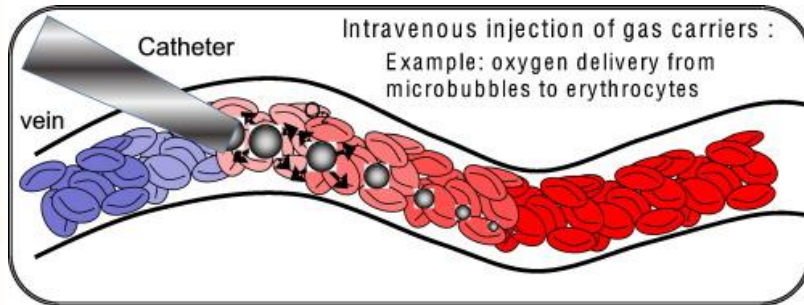


- Nanomaterial synthesis
- Bio-conjugation of nanomaterials
- Biosensor based on nanomaterials
- Functional nanomaterials
- Drug delivery nanocarriers
- Immunologically active nanomaterials
- Theragnostic nanomaterials

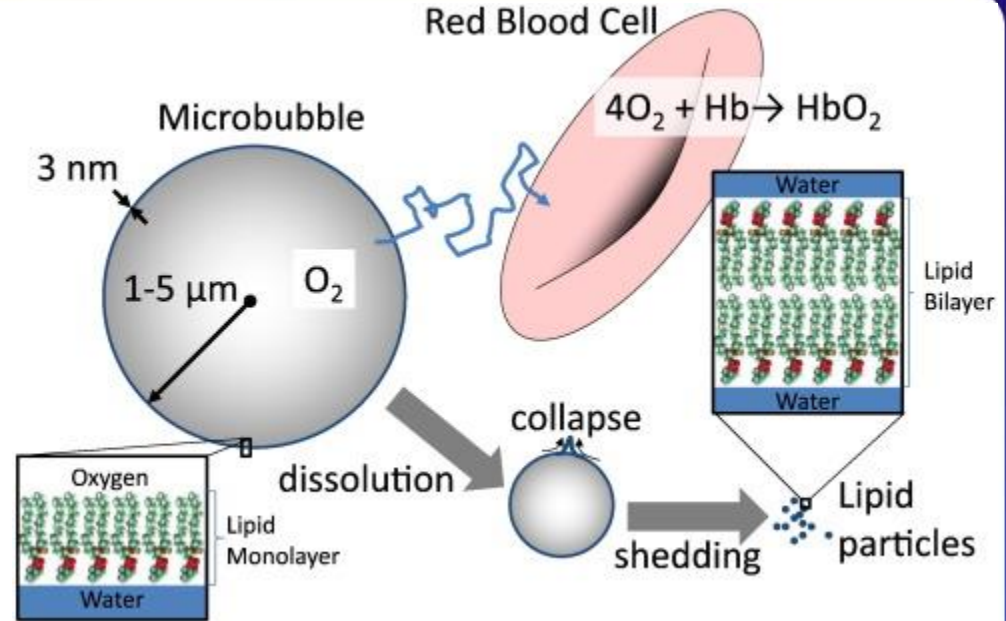
# Microbubble research in biomolecular imaging



# Oxygen microbubble research



Journal of Controlled Release, 2015, 209:139-149.



## PURE BUBBLES

### FOUNTAIN OF YOUTH

Immerse yourself in an effervescent bath with millions of oxygen-rich micro bubbles that will make you feel better and look younger by moisturizing and hydrating at the same time. See immediate results after only 20 minutes; your skin will feel softer and rejuvenated.

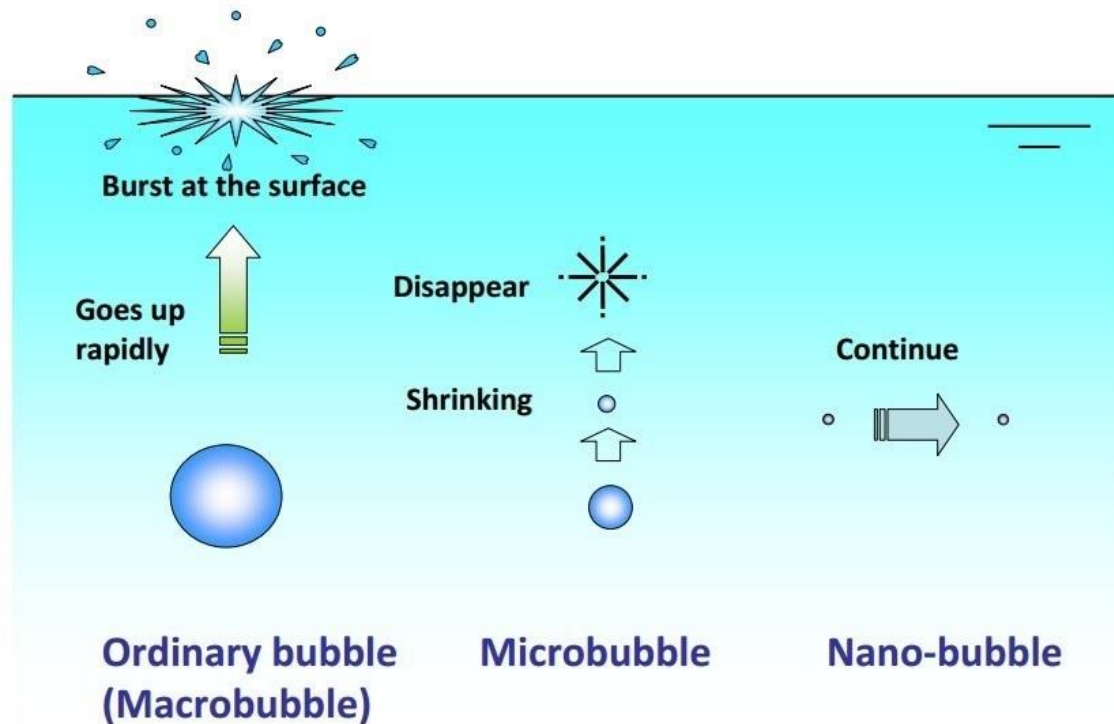
ENJOY THE FEELING OF A SUPER OXYGENATED AND EFFERVESCENT WATER SENSATION.

### THE SCIENCE

Micro bubbles are 10 microns in diameter. Since they are smaller than the skin pores which are around 50 microns in diameter, they will **exfoliate and clean all skin pore impurities while removing** the fatty acid and bacteria that cause body odor.

GLOBAL SKIN CARE  
OXYGEN PURITY ANTI AGE

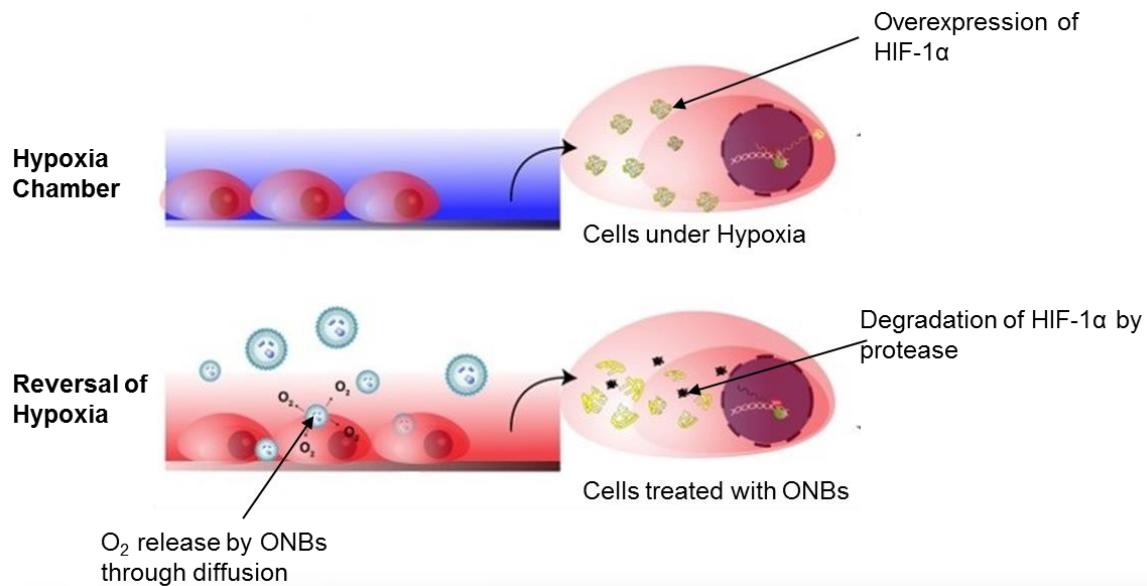
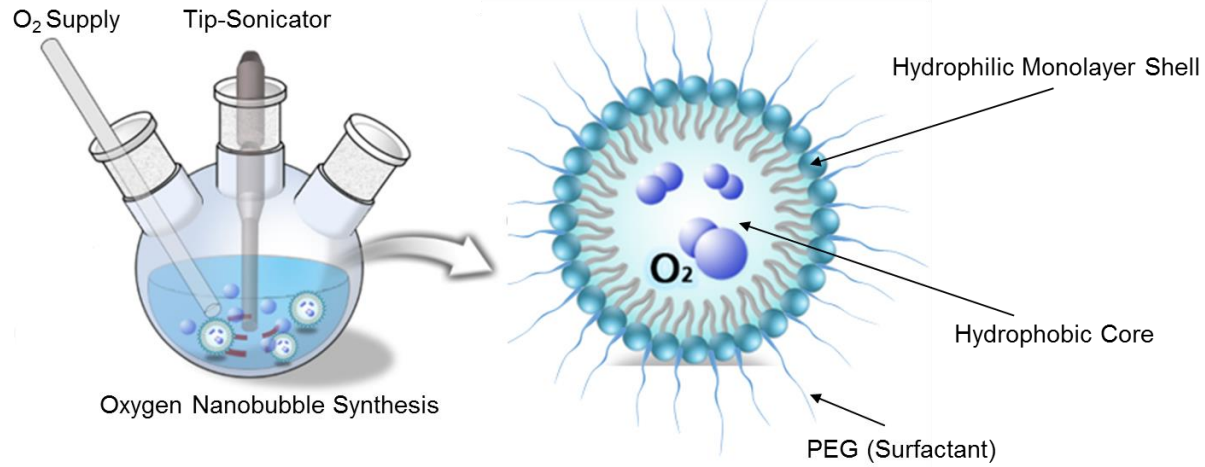
# Why oxygen nanobubbles?



- High stability
- Controllable surface composition
- Carrier for biomolecules & drug molecules
- Cell & tissue penetration

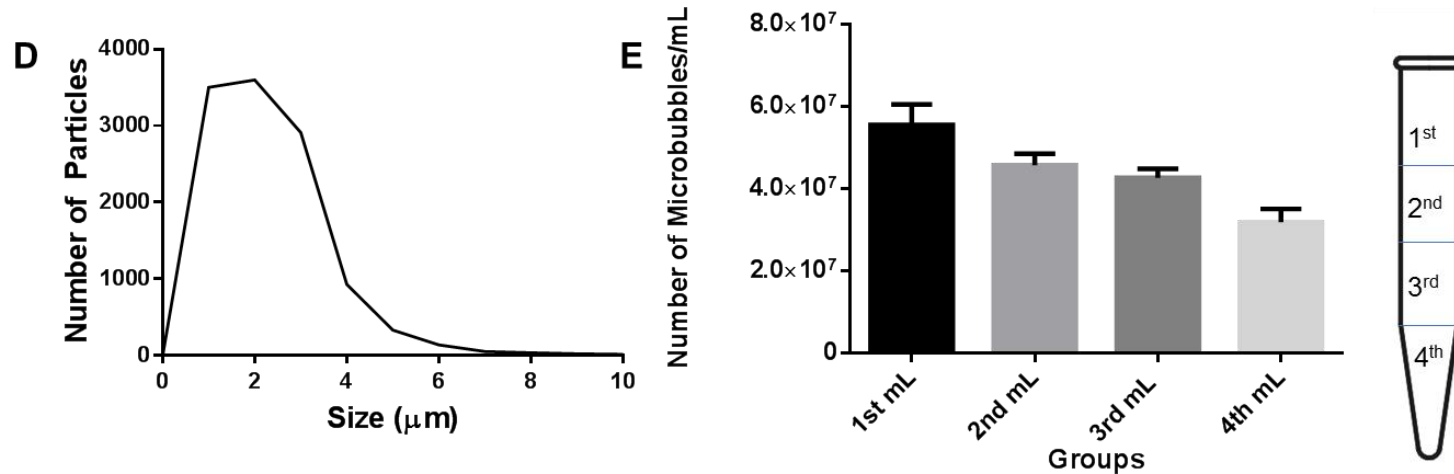
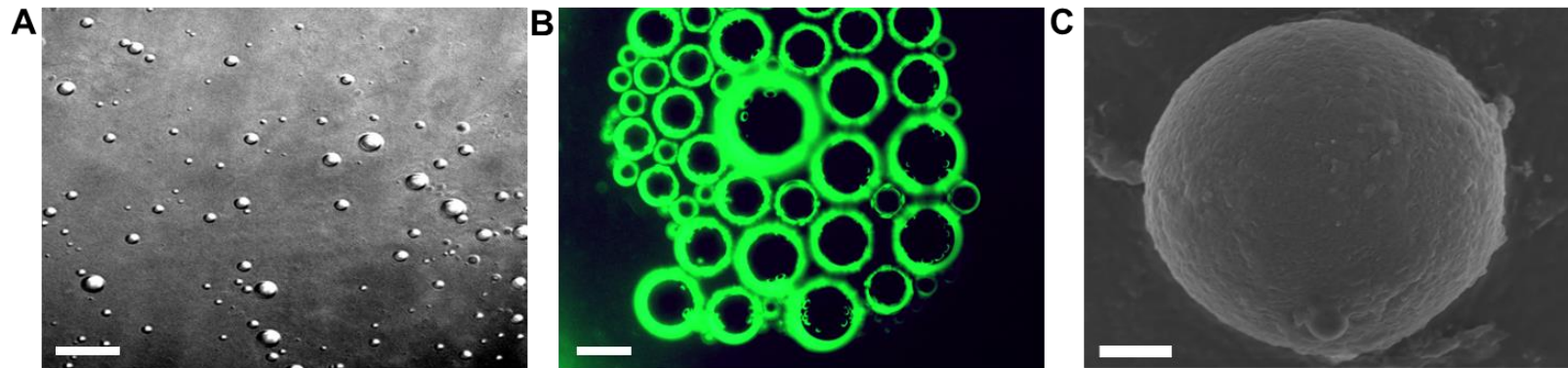
# 1. Hypoxia reverse

# Preparation of oxygen nanobubbles



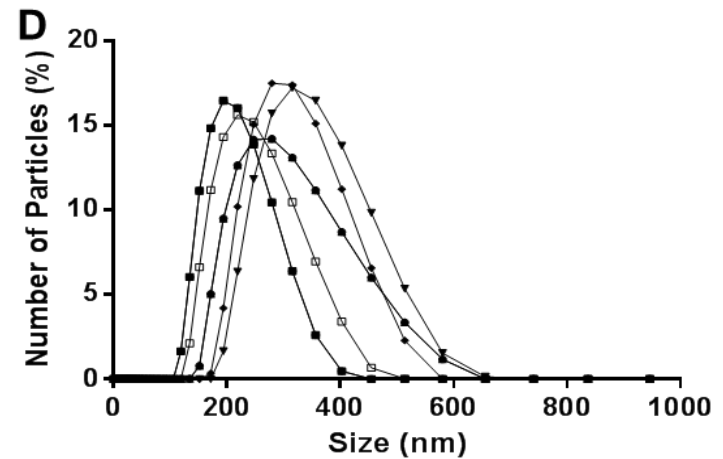
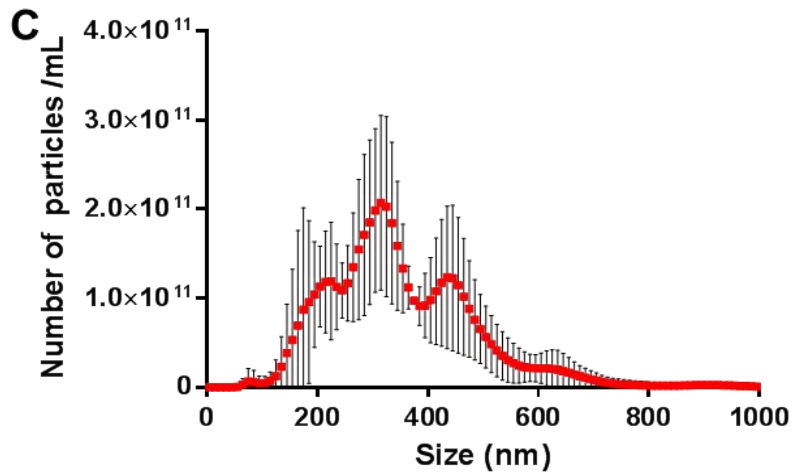
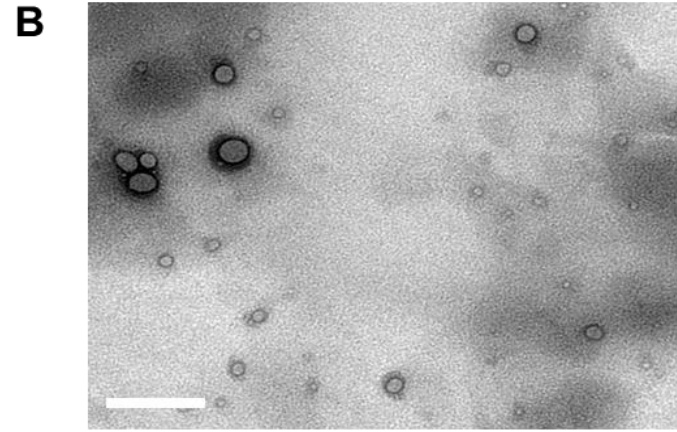
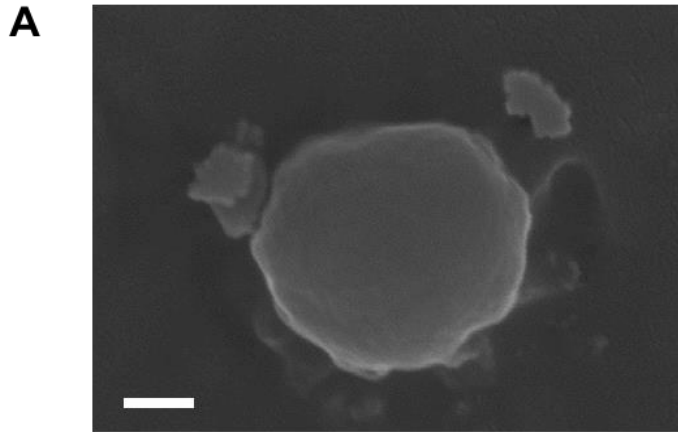


# Characterization of oxygen microbubbles



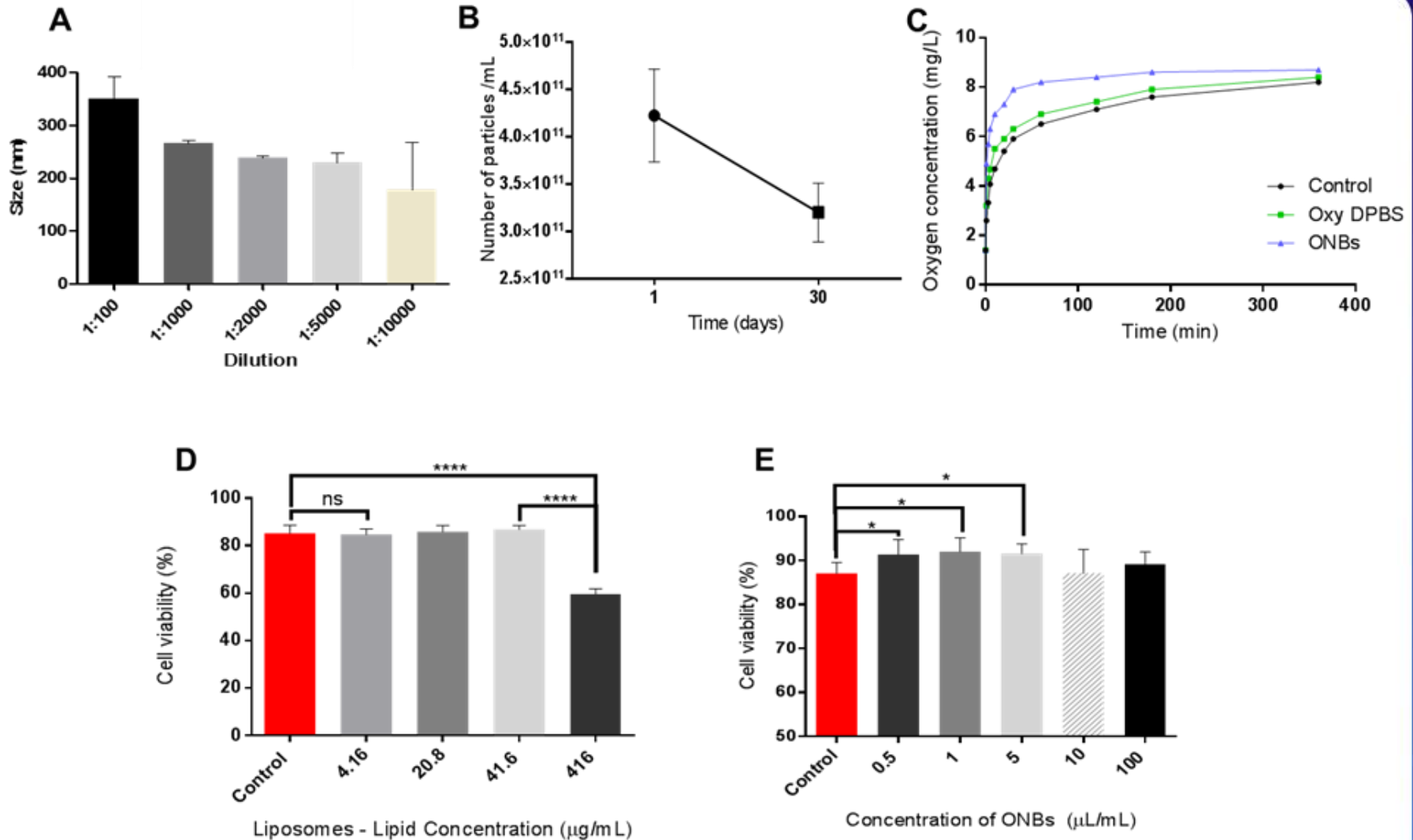
Characterization of microsize bubbles. **A.** Confocal microscopy image of microbubbles, scale bar = 20  $\mu\text{m}$ . **B.** Fluorescence microscopy image showing microbubbles, scale bar = 20  $\mu\text{m}$ . **C.** SEM image showing a microbubble, scale bar = 1  $\mu\text{m}$ . **D.** Size distribution of microbubbles calculated using ImageJ software. **E.** Concentration of microbubbles calculated in various sample groups taken from the top to the bottom of a 15 mL conical tube.

# Characterization of oxygen nanobubbles



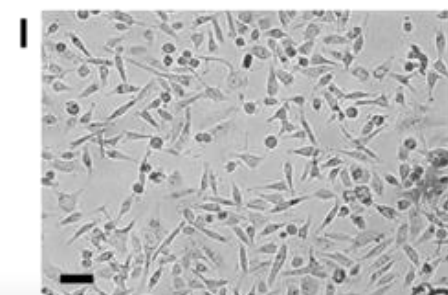
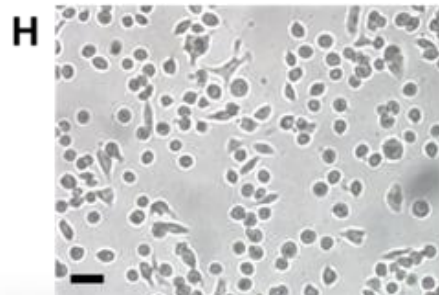
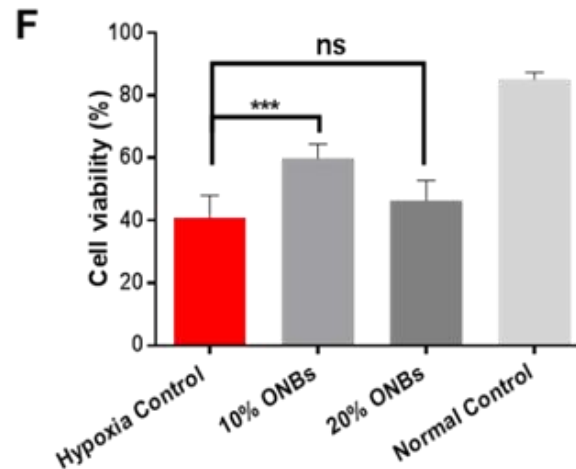
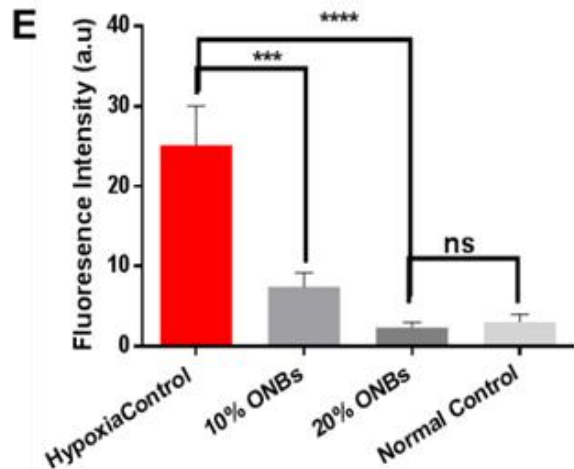
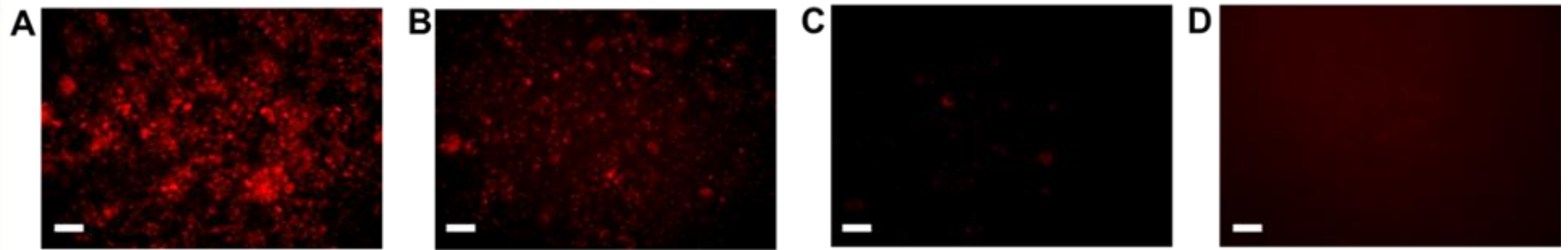
Size distribution of nanobubbles. **A.** SEM image of nanobubbles, scale bar = 100 nm. **B.** TEM image of nanobubbles, scale bar = 100 nm. **C.** NTA results for particle count and size distribution with mean size distribution in red color. **D.** DLS results of seven samples of ONBs plotted together to indicate particle size distribution.

# Preparation of oxygen nanobubbles

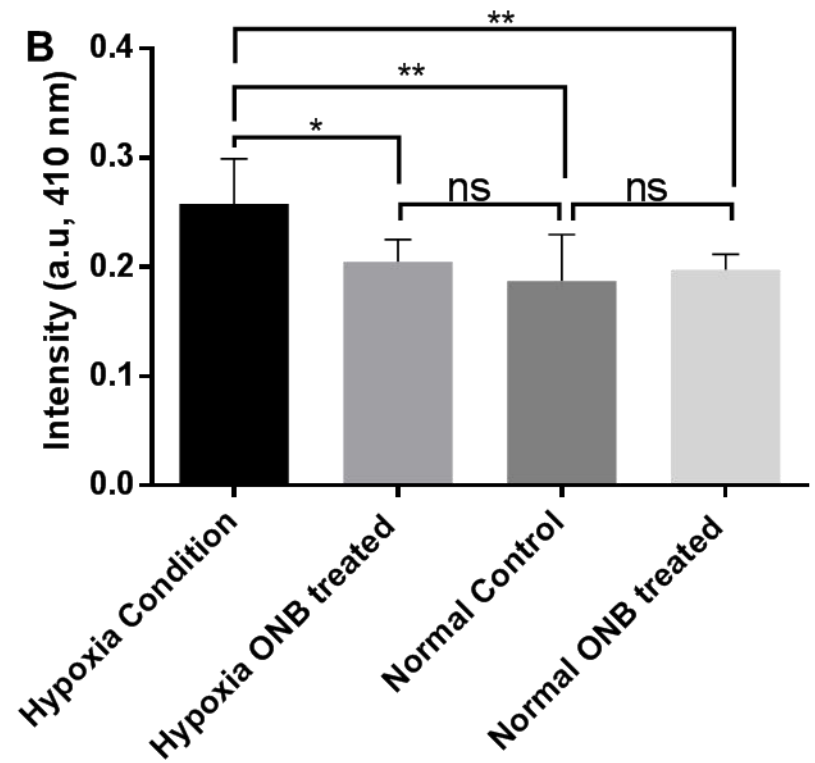
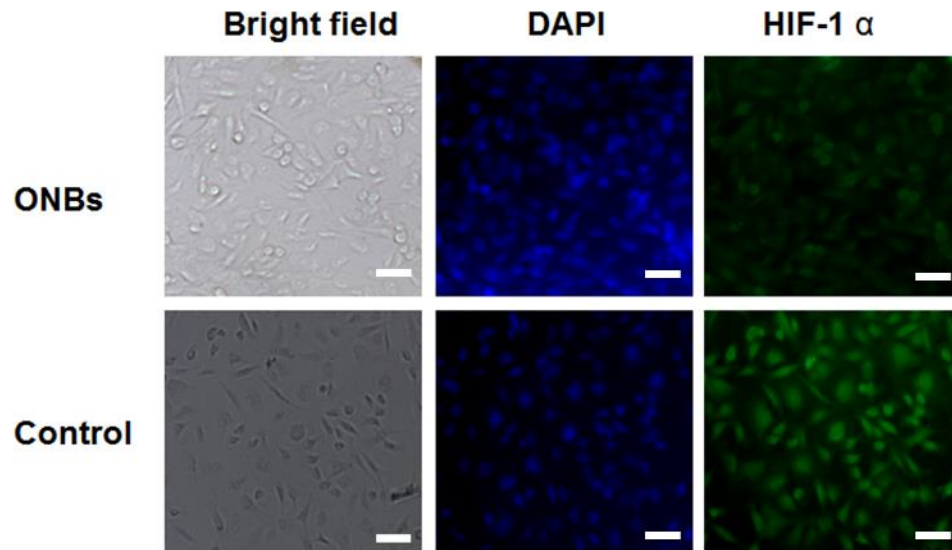


Stability, oxygen delivery, and cytotoxicity tests. **A**. Size distribution using various dilution ratios of ONBs, obtained through an NTA. **B**. Reduction in bubble count after 30 days of storage. **C**. Increase in oxygen concentration of deoxygenated water after injection of ONBs and oxygenated DPBS. **D**. Cytotoxicity of the lipids used in synthesis of nanobubbles; ns means no significance, \*\*\*\* indicates P value < 0.0001. **E**. Cytotoxicity of ONBs for varying concentrations; \* indicates P < 0.05.

# Reversal of hypoxia by oxygen nanobubbles



# HIF-1 $\alpha$ downregulation induced by oxygen nanobubbles



HIF-1 $\alpha$  expression assay. **A.** Comparison of HIF-1 $\alpha$  expression in control and after the reversal of hypoxic conditions. FITC-conjugated anti-HIF-1 $\alpha$  antibodies were used along with DAPI staining of MDA-MB-231 cells. The reduced expression of anti-HIF-1 $\alpha$  is clearly observable in the fluorescence image, indicating successful degradation of HIF-1 $\alpha$  protein due to ONBs. Scale bars = 20  $\mu$ m. **B.** HIF-1 $\alpha$  expression evaluated through the indirect ELISA method. The hypoxia control is the untreated sample in hypoxic conditions. Hypoxia bubble means the samples in the hypoxia chamber treated with ONBs. Normal control is an untreated sample in normal conditions. Normal bubble means the samples treated with ONBs in normal conditions; \* represents  $P < 0.05$ , \*\* represents  $P < 0.01$ , ns means no significance,  $n = 3$ .

## Summary

- Oxygen nanobubbles were prepared and characterized for their size, surface chemistry and oxygen delivery
- Lipid surface of bubbles can protect bubbles and also provide moiety for drug or other biomolecules
- Delivery of oxygen nanobubbles reverse the hypoxic condition of cells
- Delivery of doxorubicin drug molecules with oxygen nanobubbles provides enhanced drug effects

# Acknowledgement



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JC Bio Inc.

Thank you!

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