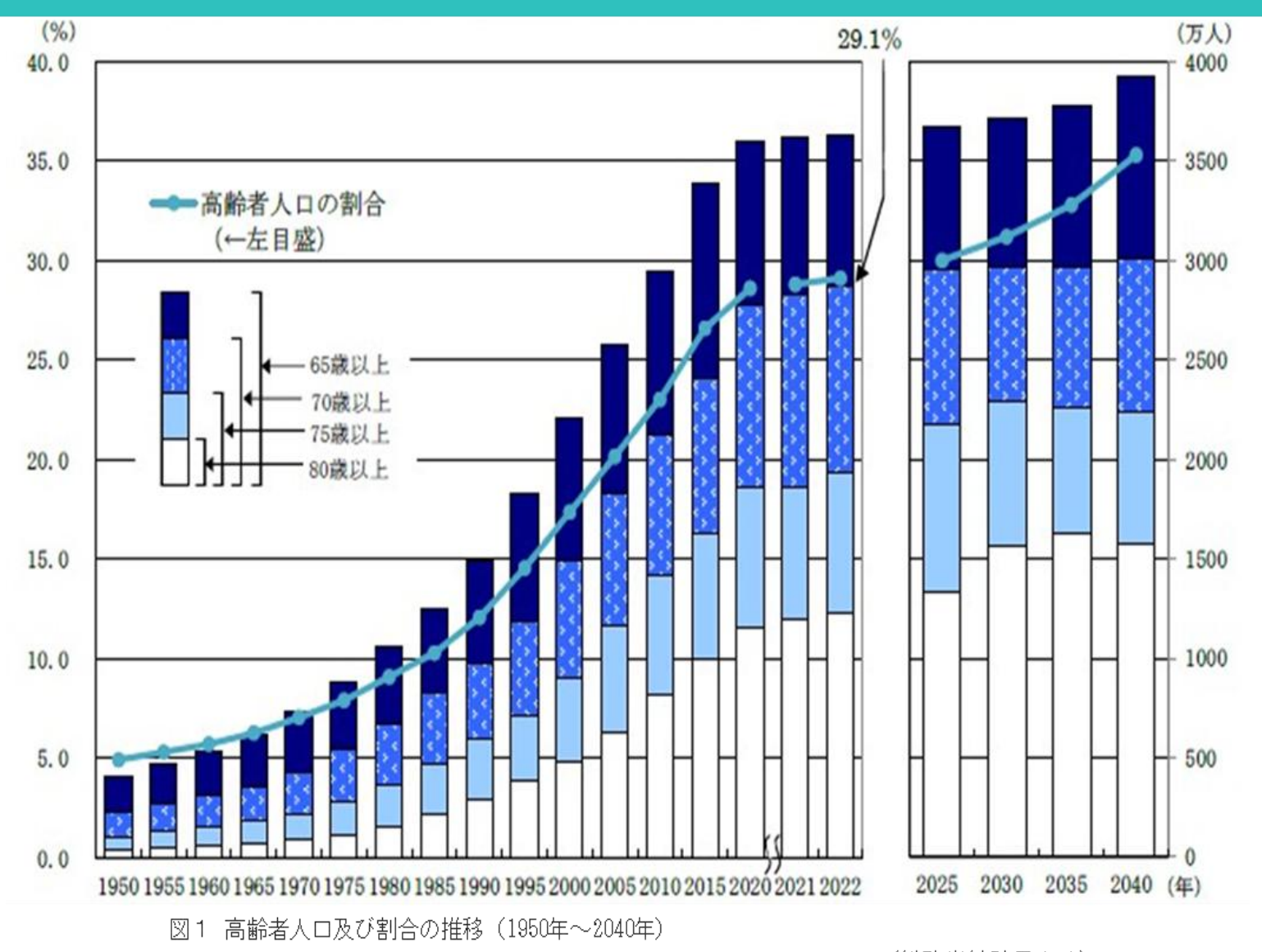


# Additive Manufacturing of Bio-Functionalized Ti-CuO Alloys: Mechanical and Biological Insights

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## INTRODUCTION & AIM

The elderly population aged 65 and over accounts for approximately 29.1% of Japan's total population (right figure), with masticatory dysfunction due to tooth loss significantly impacting quality of life. In an aging society, dental implants have become increasingly vital for restoring lost teeth. Titanium (Ti) and its alloys are widely utilized as dental implant materials.



However, failure rates for dental implants are reported to be approximately 5% to 10%, with postoperative infection being the one of the major cause (left pic.). This infection occurs when bacteria adhere to the implant-tissue interface, triggering inflammation. Consequently, surface modifications that antibacterial functionality are highly demanded.



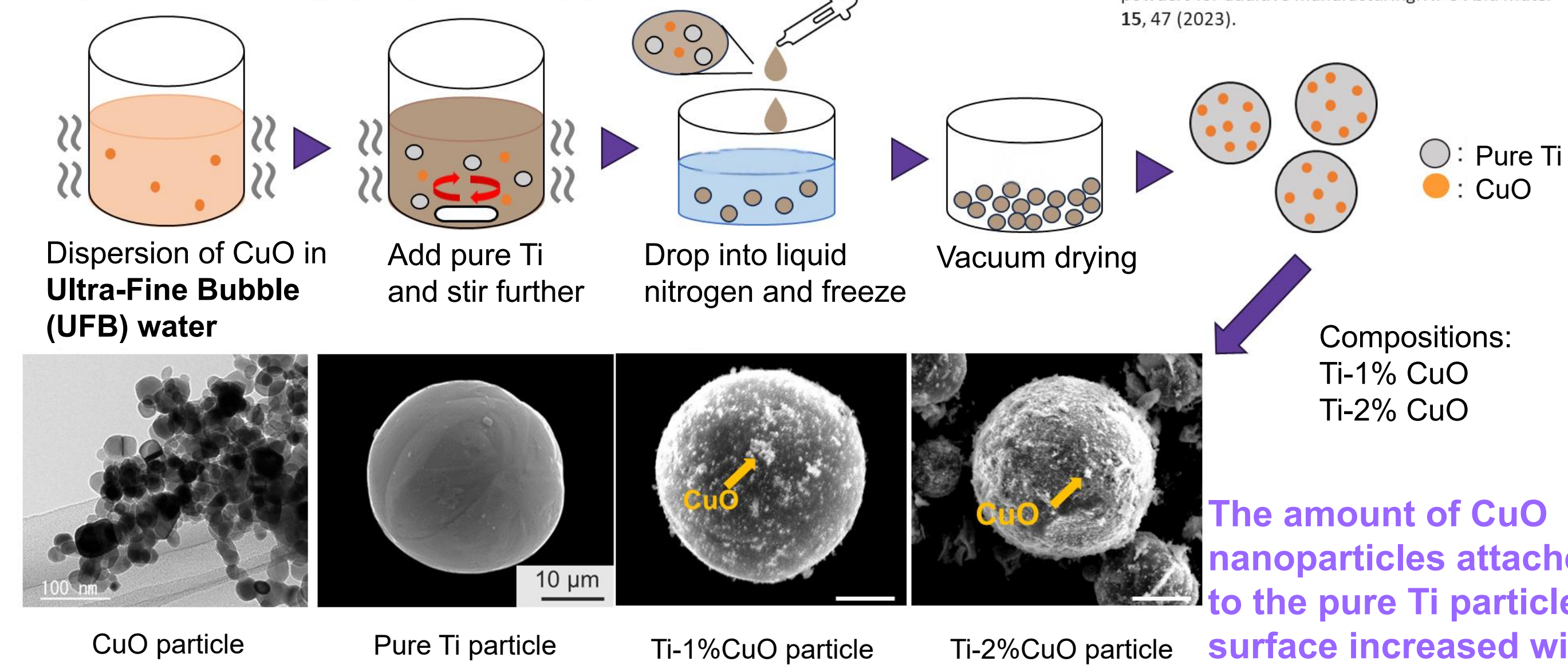
Incorporating antimicrobial metals (Cu, Ag, Zn) effectively enhances antibacterial properties. Concurrently, solid-solution strengthening serves as a promising approach to increase titanium alloy strength, supporting next-generation implant systems.

To develop a novel Cu-incorporated Ti alloy that simultaneously achieves antibacterial/anti-inflammatory properties and enhanced mechanical strength associated with lattice distortion induced by CuO incorporation.

## METHOD

### Heterocoagulation and lyophilization (freeze-drying)

Heterocoagulation is the formation of aggregates by the addition of a polyelectrolyte to a suspension of organic nanoparticles.



The amount of CuO nanoparticles attached to the pure Ti particle surface increased with increasing CuO content.

### Antibacterial Evaluation

#### Experimental Specimens:

- ① Mirror-polished pure titanium: Ti
- ② As-fabricated L-PBF titanium: L-PBF Ti
- ③ As-fabricated L-PBF titanium alloy: L-PBF Ti-1%CuO

#### Sterilization Process:

Ultrasonic cleaning in 70% ethanol for 15 min  
Thorough rinsing with autoclaved Milli-Q water

Evaluation Method: Film-contact method (JIS Z 2801)

#### Bacterial Strains Used:

*Escherichia coli* (*E. coli*)  
*Staphylococcus aureus* (*S. aureus*)

Medium: Nutrient Agar

Inoculation Density: 10<sup>6</sup> CFU/mL

Incubation Time: 24 h

#### Statistical Analysis:

one-way ANOVA followed by Student's t-test  
(\*p < 0.05; n.s.: not significant).

### Anti-inflammatory Evaluation

#### Experimental Specimens:

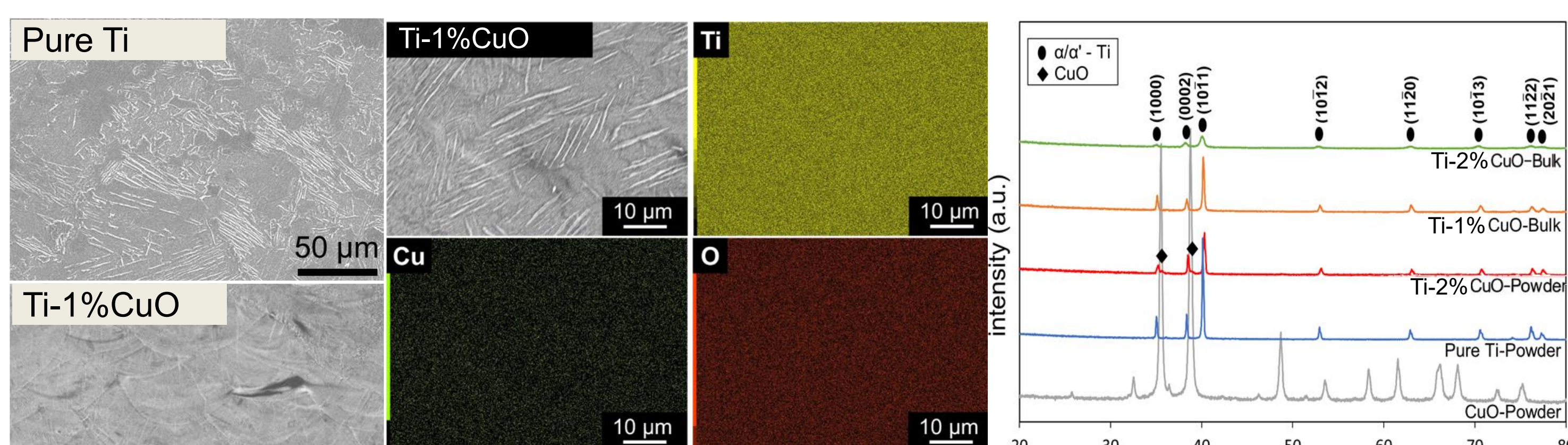
- ① Tissue culture polystyrene: TCPS
- ② As-fabricated L-PBF titanium: L-PBF Ti
- ③ As-fabricated L-PBF titanium alloy: L-PBF Ti-1%CuO

Cell Line Used: Macrophages (RAW264.7)

Incubation Time: 6h and 24h

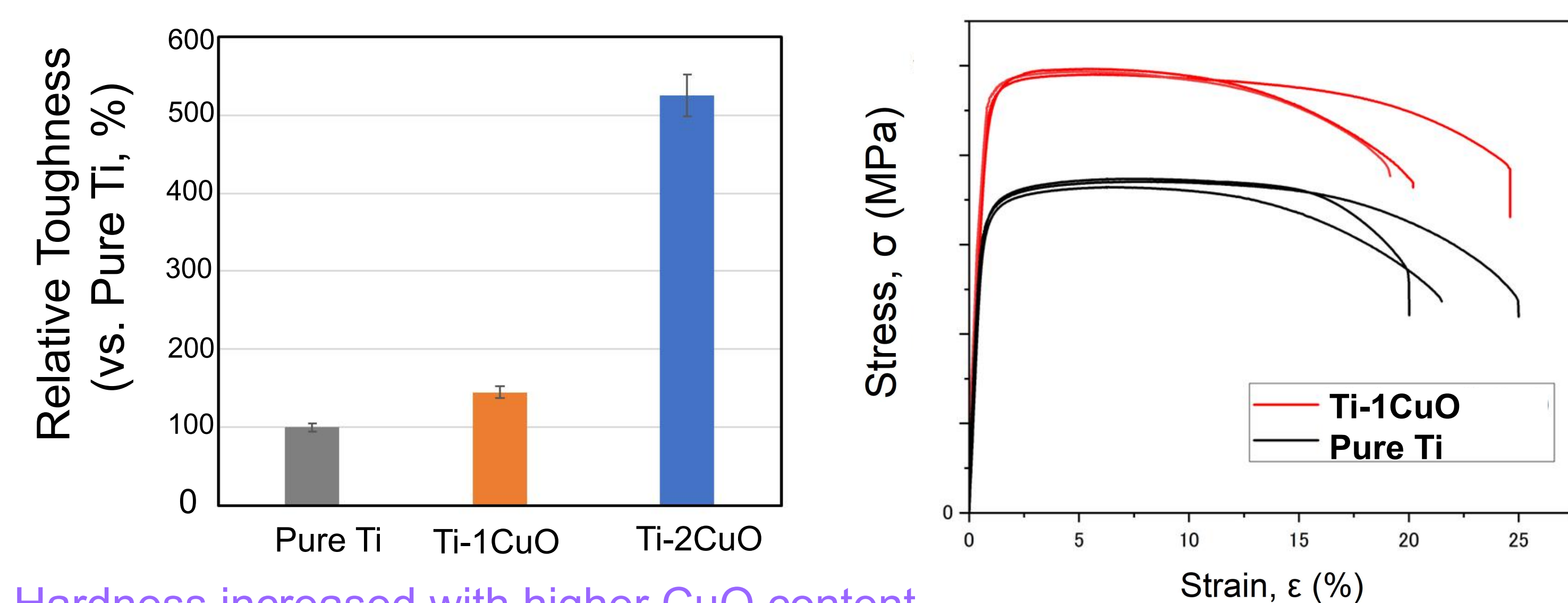
Assay Kit: NO<sub>2</sub>/NO<sub>3</sub> Assay Kit-C -Griess Reagent Kit

## RESULTS & DISCUSSION



The Ti-CuO specimen was composed of  $\alpha/\alpha'$  phases, just like the Pure Ti specimen. The peaks corresponding to the (100) and (002) planes of the Ti-CuO specimen shifted to lower angles, indicating lattice expansion associated with CuO addition.

Alloying can induce phase transformation-related lattice distortion, generating internal defects. Transformation-induced volume expansion creates severe internal stress, causing lattice distortion that leads to microcracks and voids.

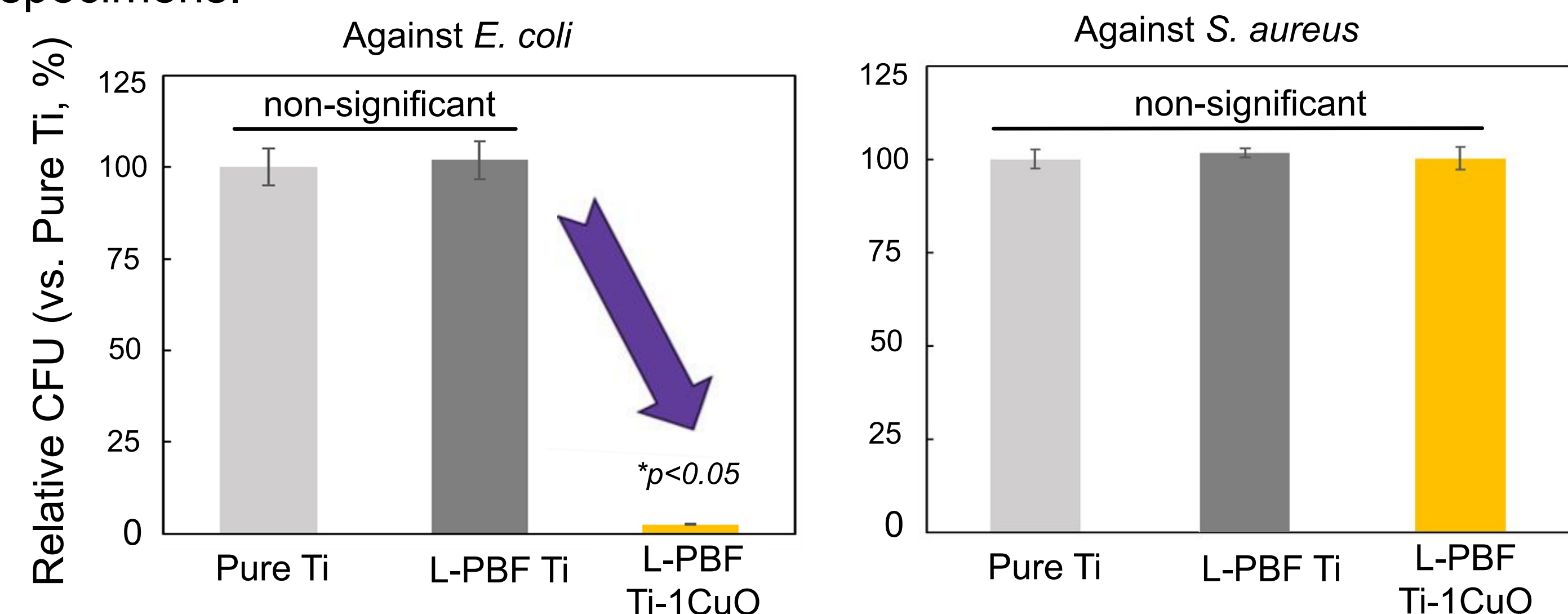


Hardness increased with higher CuO content. However, the addition of 2% CuO induced significant lattice expansion, resulting in embrittlement and leading to a reduction in ductility.

## CONCLUSIONS

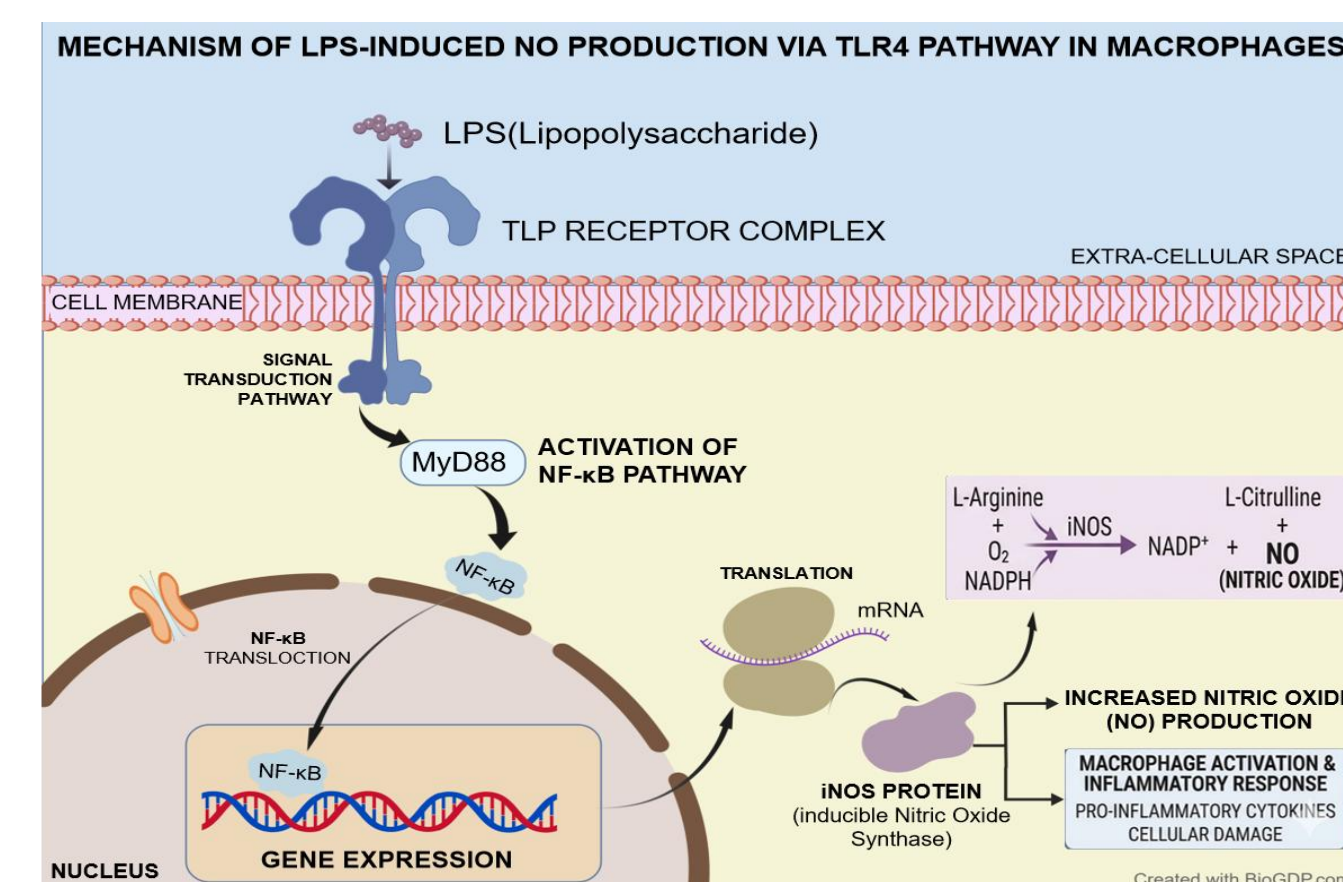
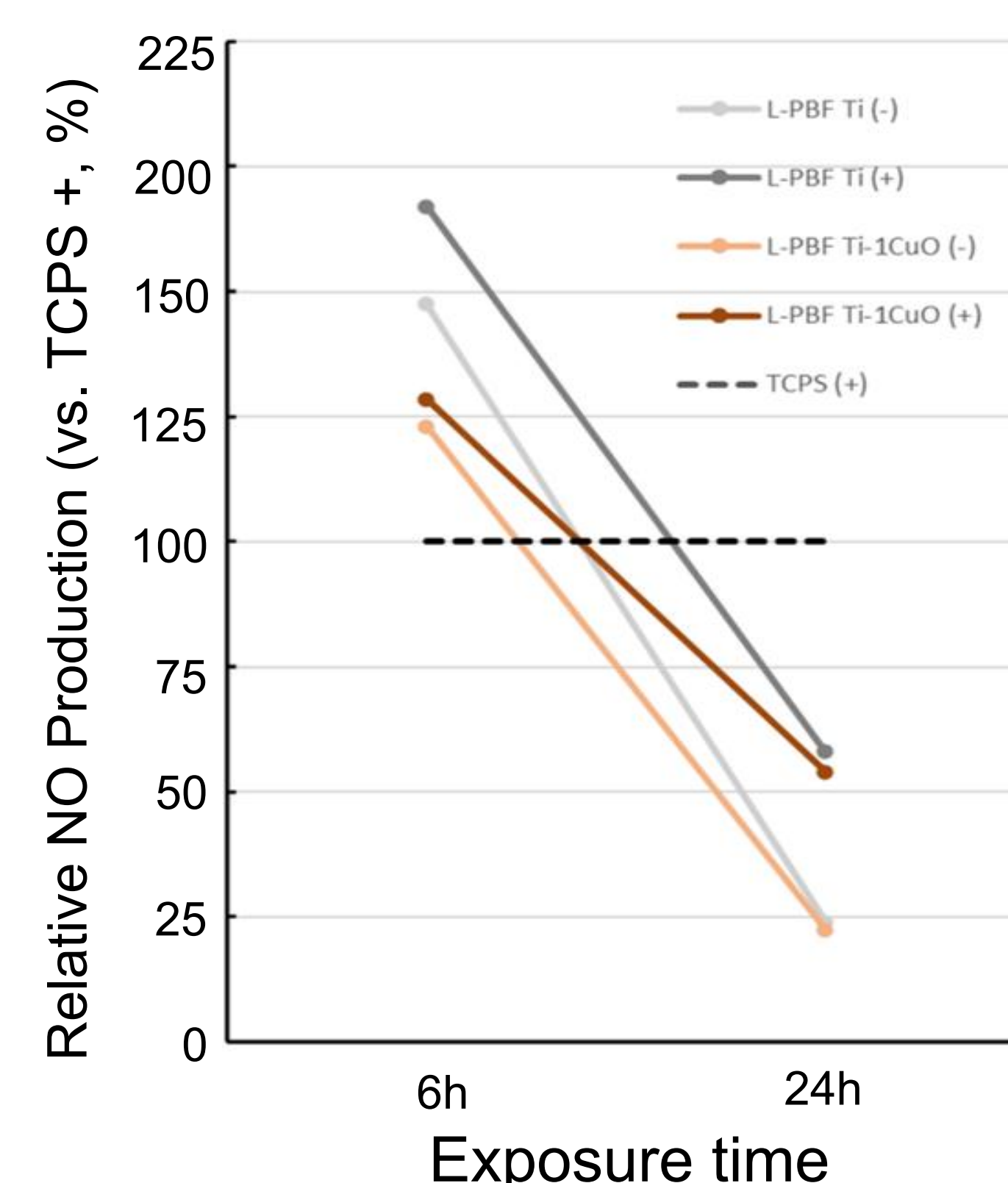
Evaluation of Ti-CuO composite powders and L-PBF specimens confirmed that CuO addition enhances mechanical properties and imparts antibacterial efficacy. Furthermore, these materials exhibit potential anti-inflammatory properties. These findings demonstrate the potential of CuO-added L-PBF Ti materials for antibacterial biomedical applications.

Antibacterial evaluation were conducted via the film-contact method using *E. coli* and *S. aureus*. These evaluations were performed to investigate the effects of CuO addition on the antibacterial properties of the specimens.



L-PBF Ti-1.0CuO exhibited significant antibacterial efficacy against *E. coli*, whereas no significant antibacterial effect was observed against *S. aureus*.

Anti-inflammatory evaluation was evaluated by measuring NO production via the Griess assay, utilizing LPS-stimulated macrophages.



Under inflammatory stimulation, the L-PBF Ti-1CuO specimen exhibited lower NO production (24h) compared to TCPS and L-PBF Ti. This suggests that the Ti-CuO alloy may modulate macrophage inflammatory responses.