# Copper Doped Tricalcium Phosphate; Phase Composition and *In Vitro* Interaction with Osteoblast Cells

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## Abstract

β-tricalcium phosphate (β-TCP) is an ideal choice as a bone substitute due to its bioactivity and osteoconductivity. However, the lack of osteoinduction is a major concern. Different approaches such as structural modification and incorporation of osteogenic factors are promising tools to enhance the osteoinductivity of TCP. Trace elements can be added in low concentrations to TCP to stimulate osteoinduction. Several dopants such as strontium, magnesium, zinc and iron have been added to TCP and their effects on physical and biological properties of bioceramic have been investigated [1,2]. Among different dopants, copper has received significant attention due to its ability to induce angiogenesis [3]. The purpose of current study was to investigate the effects of copper (Cu) on physical and biological properties of TCP. Our results showed that Cu addition stabilizes the β-TCP phase at high temperature. It has minimal effect on total and open porosity. Although low concentration of Cu did not decrease the osteoblast cell proliferation, high concentration of 1 wt. % resulted in significant toxicity. Cu addition reduced the inflammation at day 5 and upregulated VEGF and COLA-1 expression at early timepoints.

## Background

### Calcium Phosphates (CaPs)

**β-tricalcium phosphate (β-TCP)**

❖Widely used in bone tissue engineering applications due to excellent bioactivity, osteoconductivity, bioresorbability, and compositional similarities to bone mineral.

**Role of trace elements**

❖Alter mechanical properties and resorption kinetics of β-TCP [2].
❖Induce osteoinductivity in CaP bioceramics [3].
❖ Cu plays a role in the metabolism of iron and formation of blood vessels
❖ Cu promotes angiogenesis and the proliferation of osteoblasts [4,5]

### Role of Genes

❖**Osteoprotegerin (OPG):** Decoy receptor for RANKL.

❖**Collagen type 1 (COLA-1):** The dominant fibrous protein in hard tissue

❖**Vascular Endothelial Growth Factor (VEGF):** Associated with blood vessel formation and angiogenesis.

❖**Interleukin-6 (IL-6):** Inflammatory gene and promotes osteoarthritis

### Objective

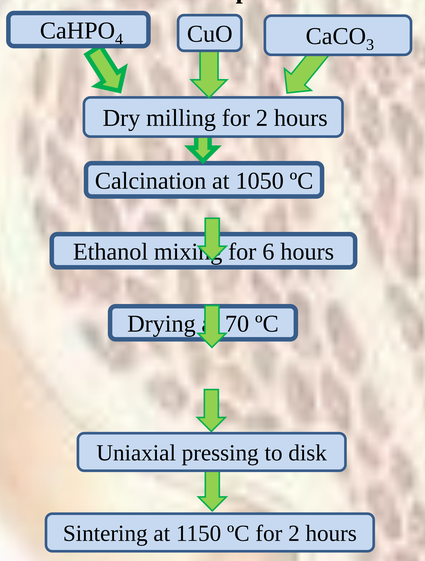
To evaluate the effects CuO addition on β-TCP phase composititon, density, and *in vitro* interaction with osteoblast.

### Hypothesis

Addition of CuO alters the TCP phase composition, porosity, and gene expression by hFOB which in turn can affect the bone remodeling process.

## Experimental Procedure

### Scaffold Preparation




❖ CuO addition in concentrations of 0.25, 0.5, and 1.0 wt%
❖0, 0.25, 0.5, and 1.0 wt% Cu-doped TCP denoted as Pure TCP, 0.25 Cu-TCP, 0.5 Cu-TCP, and 1.0 Cu-TCP, respectively.

### Scaffold Characterization

❖Phase analysis using X-ray diffraction method (XRD).
❖Density measurement using Archimedes' method.

### Biological Characterization

❖Human preosteoblast cell line hFOB 1.19, seeded at density of $50 \times 10^3$ and $2 \times 10^6$ cells/sample for XTT and RT-PCR assays, respectively.

❖Optical cell density was determined by XTT assay,

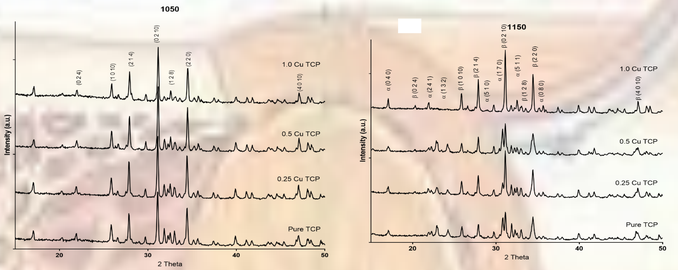
❖Cells were lysed using 1 ml TRIzol reagent

❖Reverse transcription reactions were performed using Moloney murine leukemia virus (M-MLV) reverse transcriptase.

❖ Quantitative real-time PCR (qPCR) was conducted using the ViiA7 real-time PCR instrument.

## Results and Discussion

### Scaffold Phase Analysis

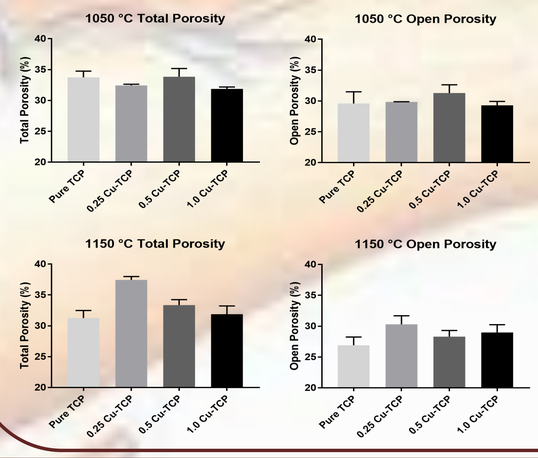



❖β-TCP phase was the only phase present in structure of all samples after calcination and sintering at 1050 °C

❖Increase in sintering temperature resulted in transformation of β to α-TCP.

❖α-TCP percentage was 17.5, 4.9, and 2.8, and 0 for pure TCP, 0.25 Cu-TCP, 0.5 Cu-TCP and 1.0 Cu-TCP, respectively. This shows the stabilization of β-TCP crystal structure during Cu incorporation.

### Open and Closed Porosity

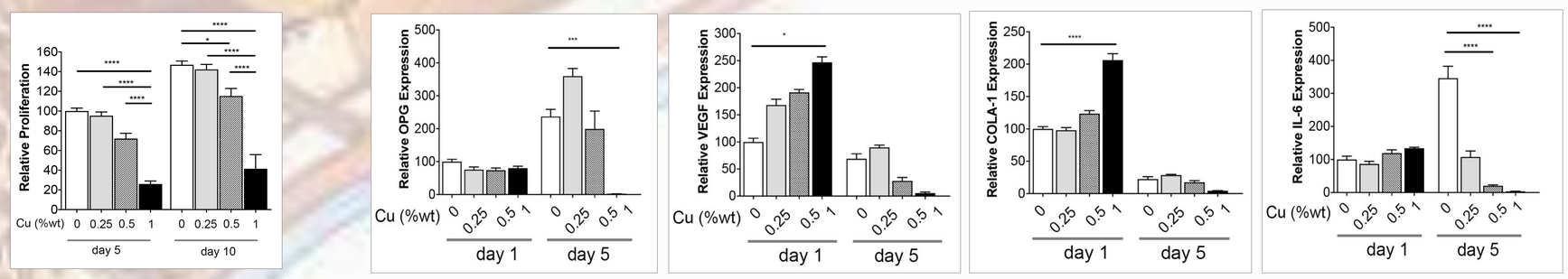



❖In general, the porosity decreased with increase in sintering temperature for all concentrations, except for 0.25 Cu-TCP

❖ At 1050 ºC, among doped samples, 0.5 Cu-TCP had the greatest total and open porosity with no difference in porosities of 0.25 Cu-TCP and 1.0 Cu-TCP. The change was not significant.

❖ 0.25 Cu-TCP had the greatest porosity at 1150 ºC, with total porosity decreasing linearly with dopant amount.

### Osteoblast Cell Proliferation and Differentiation




## Summary

- Decreased transition to α-TCP phase with increasing Cu content due to smaller radius of Cu ion compared to Ca ion for which it substitutes
- Decrease in impact of increase in sintering temperature on open and closed porosity with addition of Cu due to stabilization of crystal structure
- No toxicity at low Cu concentrations, but decreasing cellular proliferation with increasing Cu content indicating Cu toxicity
- Decrease in inflammatory markers and increase in osteoinductive markers with increasing Cu content, indicating therapeutic effect of Cu

## References

1. 1.Vahabzadeh S and Bose S. *Annal Biomed Eng*. 2017;45: 819-828.
2.Tarafder S, Davies NM, Bandyopadhyay A, Bose S. Biomater Sci. 2013;1 :1250-1259.
3.Gbureck U, Hölzel T, Doillon CJ, Müller FA, Barralet JE. Adv Mater; 2007:19: 795-800.
4. S. Bose, G. Fielding, S. Tarafder, and A. Bandyopadhyay, Trends Biotechnol. **31**, 594 (2013).
4. J. Barralet, U. Gbureck, P. Habibovic, E. Vorndran, C. Gerard, and C. J. Doillon, Tissue Eng. Part A **15**, 1601 (2009).
5. M. Schamel, A. Bernhardt, M. Quade, C. Würkner, U. Gbureck, C. Moseke, M. Gelinsky, and A. Lode, Mater. Sci. Eng. C **73**, 99 (2017).

## Acknowledgements