Cobalt modified calcium phosphate cement for promotion of angiogenesis during bone repair

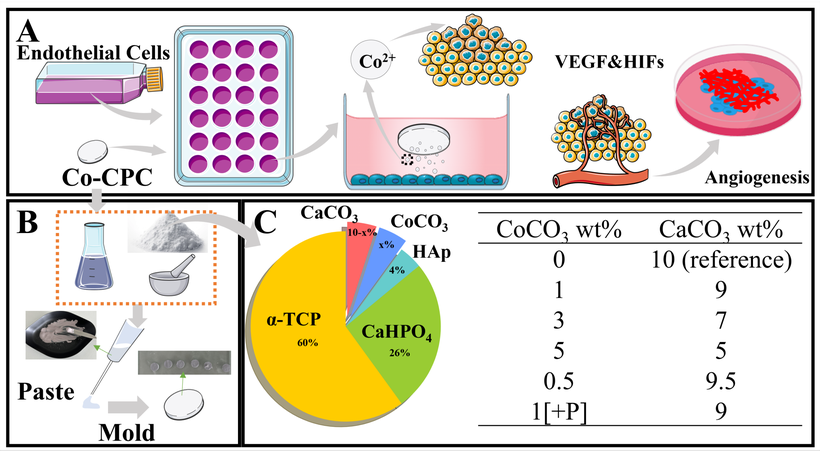
Chuandong Ye[1], Richard Frank Richter[1], Anne Bernhardt[1], Michael Gelinsky[1]

[1]Centre for Translational Bone, Joint and Soft Tissue Research, University Hospital *Carl Gustav Carus* and Faculty of Medicine of Technische Universität Dresden (TU Dresden), Dresden, Germany

Introduction

Calcium phosphate cements (CPC) are a class of bone replacement materials that is widely investigated and already used in clinical applications as it fulfills the general requirements of biocompatibility, osteoconductivity, and injectability. Typically, self-setting CPC consist of a liquid phase and at least one calcium phosphate (CaP) precursor with hydraulic reactivity like α-tricalcium phosphate (α-TCP), making the formulation highly modifiable[1]. The injectability allows the CPC paste to be printed into macroporous scaffolds for implantation. However, at the early stage of implantation, due to the absence of proper vasculature and limited diffusional transport of nutrients and oxygen (the diffusion limit of solutes and gases inside the tissue is 150-200 µm), bone cells present in the interior of the scaffold often die via necrosis[2]. The formation of blood vessels is crucial in the bone repair process as it is essential for delivering oxygen and nutrients. Cobalt ions are known to stimulate angiogenesis, promoting vascularization and bone regeneration[3]. Therefore, this study aims to modify CPC with cobalt(II) (Co-CPC) to endow it with angiogenic properties and enhance its application as bone replacement material.

Results



$CoCO_3$ wt%	$CaCO_3$ wt%
0	10 (reference)
1	9
3	7
5	5
0.5	9.5
1[+P]	9

Fig. 1: Schematic overview of the study. (A) Angiogenesis mechanisms, (B) Preparation process, (C) Formulations tested[4]. ([+P] means that additional Na_2HPO_4 is mixed into the powder).

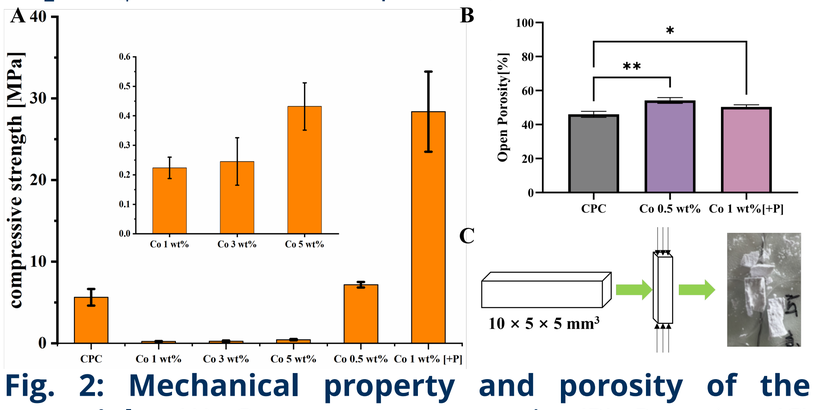


Fig. 2: Mechanical property and porosity of the materials. (A) Compressive strength, (B) Porosity, (C) Mechanical test process.

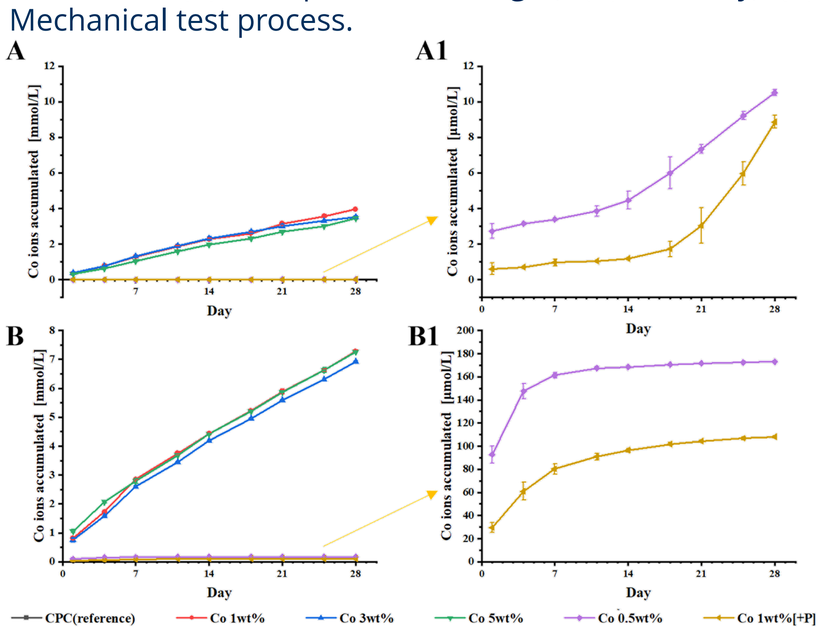


Fig. 3: Accumulated Co^{2+} ion release. *In vitro* Co^{2+} ion release behavior (A&A1 in HEPES, B&B1 in Human Plasma-Like Medium (HPLM)) over 28 days.

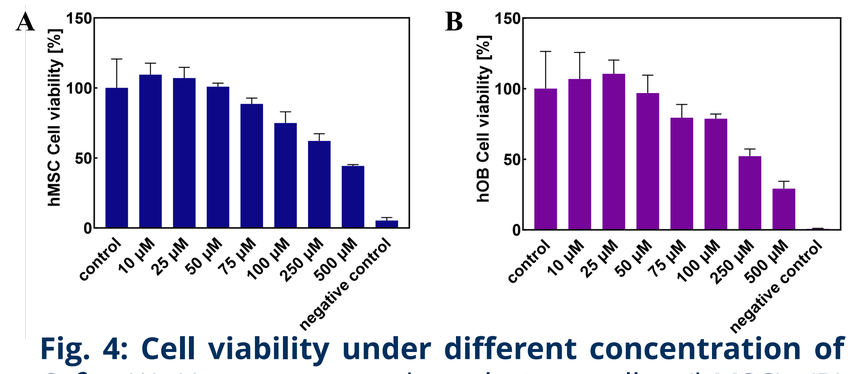


Fig. 4: Cell viability under different concentration of Co^{2+}. (A) Human mesenchymal stem cells (hMSC), (B) human osteoblasts (hOB).

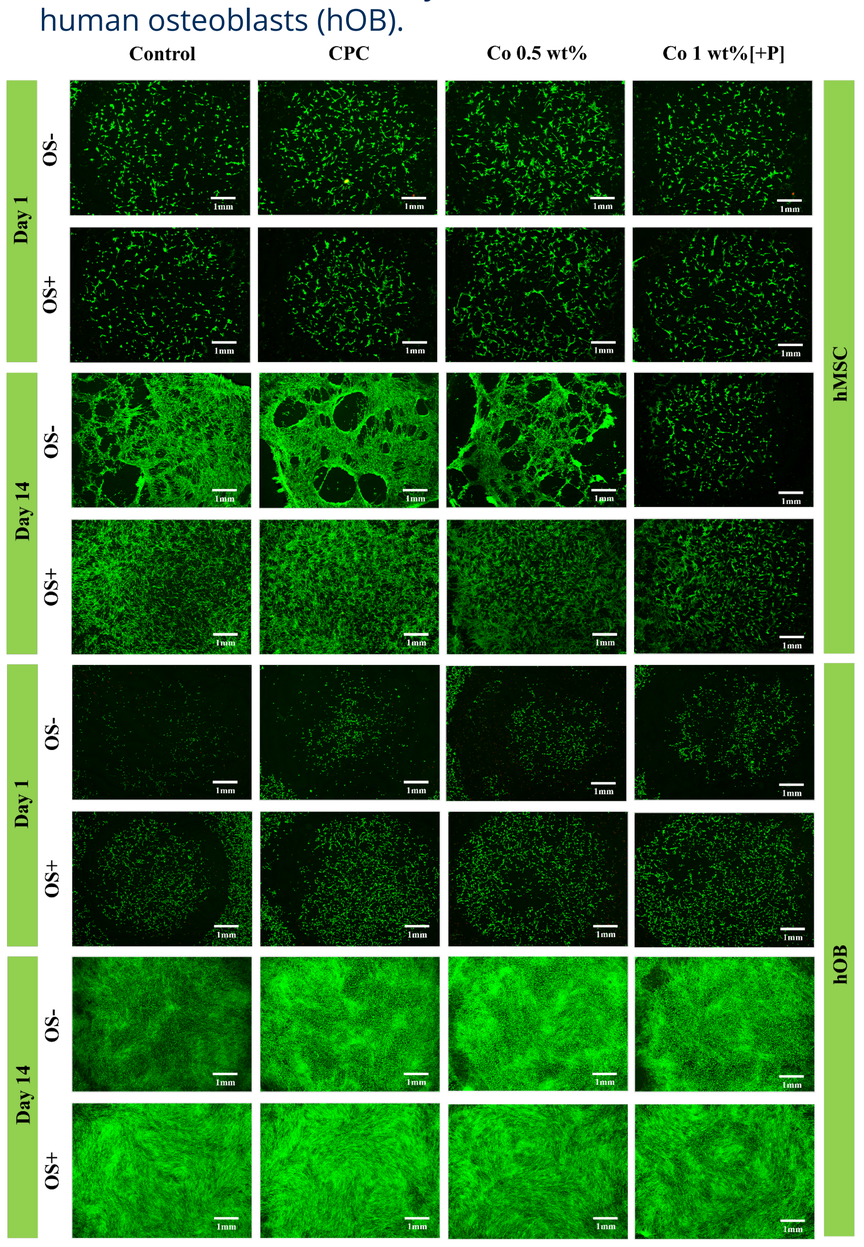


Fig. 5: Live/Dead staining of hMSC and hOB under different extracts of Co-CPC. Fluorescent microscopy images show live (green) and dead (red) cells, OS+: Culture medium with osteogenic supplements.

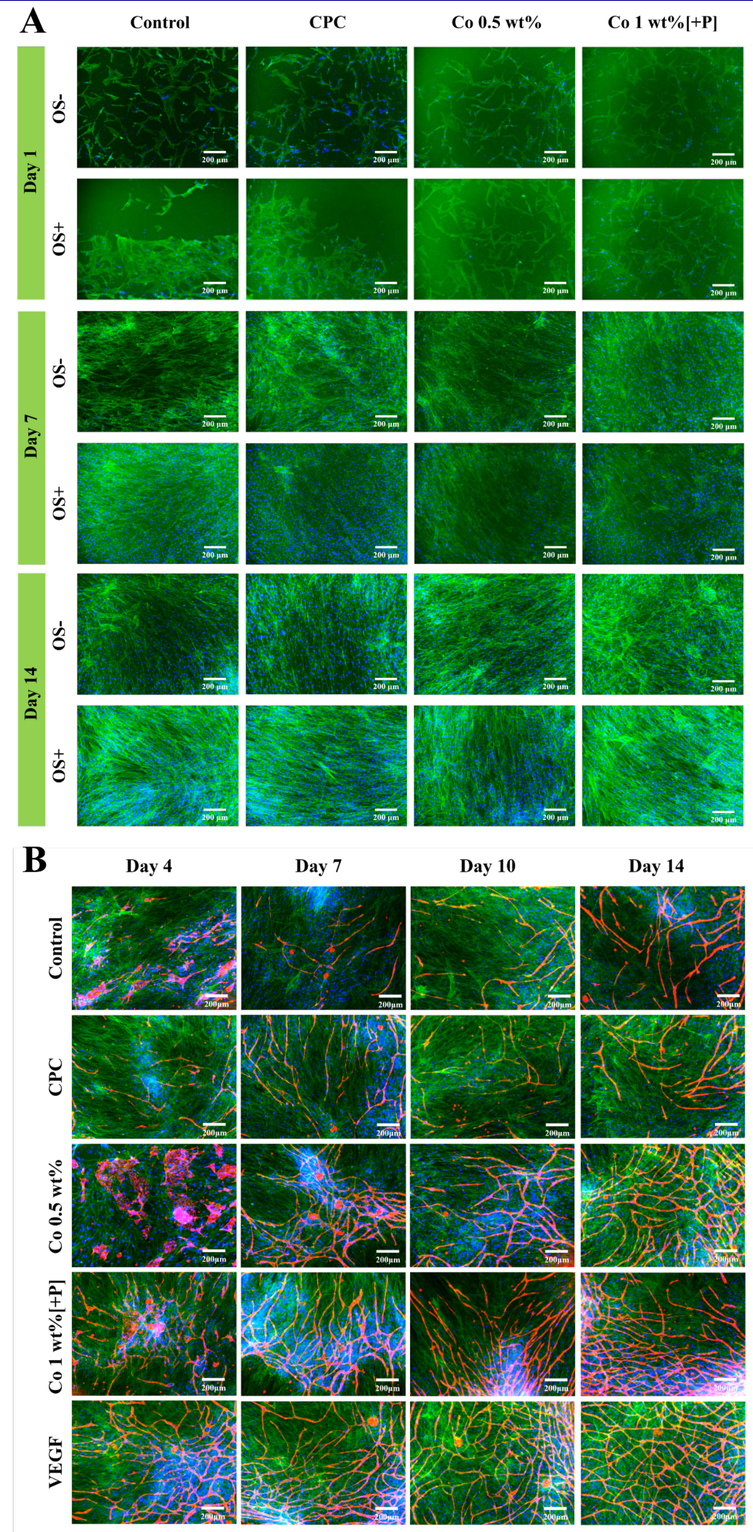


Fig. 6: Fluorescence microscopic images of hOB and endothelial cells (HUVEC). (A) hOB monoculture under different extracts of Co-CPC, (B) coculture of hOB and HUVEC: The cytoskeleton was stained green using Alexa Flour Phalloidin 488, cell nuclei were stained blue with DAPI, and endothelial cells were stained red using CD31 antibody staining.

Conclusion

The partial substitution of $CaCO_3$ by $CoCO_3$ in CPC resulted in a controlled release of Co^{2+}, as illustrated in Fig. 3. According to the mechanism outlined in Fig. 1A, the release of Co^{2+} ions is expected to enhance vascularization (Fig. 6) by promoting the expression of VEGF and HIF-1α and results reveal that Co doping also enhances the mechanical properties, particularly in the Co 0.5 wt% and Co 1 wt% [+P] group. This study confirms that Co substitution in CPC (Co-CPC) not only preserves essential mechanical properties, but does not interfere with the growth of osteoblasts and osteogenically differentiated hMSC, but also significantly enhances angiogenesis *in vitro*, making it a promising material for bone replacement.

Contact:

Chuandong Ye
Centre for Translational Bone, Joint and Soft Tissue Research
TU Dresden, Faculty of Medicine
Fetscherstr. 74, 01307 Dresden, Germany
Tel: +49-15236333451
chuandong.ye@mailbox.tu-dresden.de

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