

## of RANKL-induced osteoclastic differentiation in human monocyte cells

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### Background and Aim

• Patients affected by chronic kidney disease (CKD) feature a substantial increase of high phosphate-induced vascular calcification (VC) and iron deficiency-induced anemia (1).

• Increased Pi is known to inhibit both osteoclast differentiation and bone resorption process. Instead, iron citrate treatment enhances osteoclast (OCs) differentiation and bone resorption both in vitro and in vivo (2).

• Little is known about the effects of Pi + Fe<sup>3+</sup> synergy on osteoclastogenesis.

**We aimed to investigate the effect of iron citrate on Pi-induced inhibition of monocyte differentiation in human osteoclast-like cells.**

### Materials & Methods

We consecutively enrolled 5 healthy donors with the following inclusion criteria:

i) Males ii) non smokers iii) age between 25-50 years iv) HCV/HBV negative

#### Osteoclast (OCs) differentiation

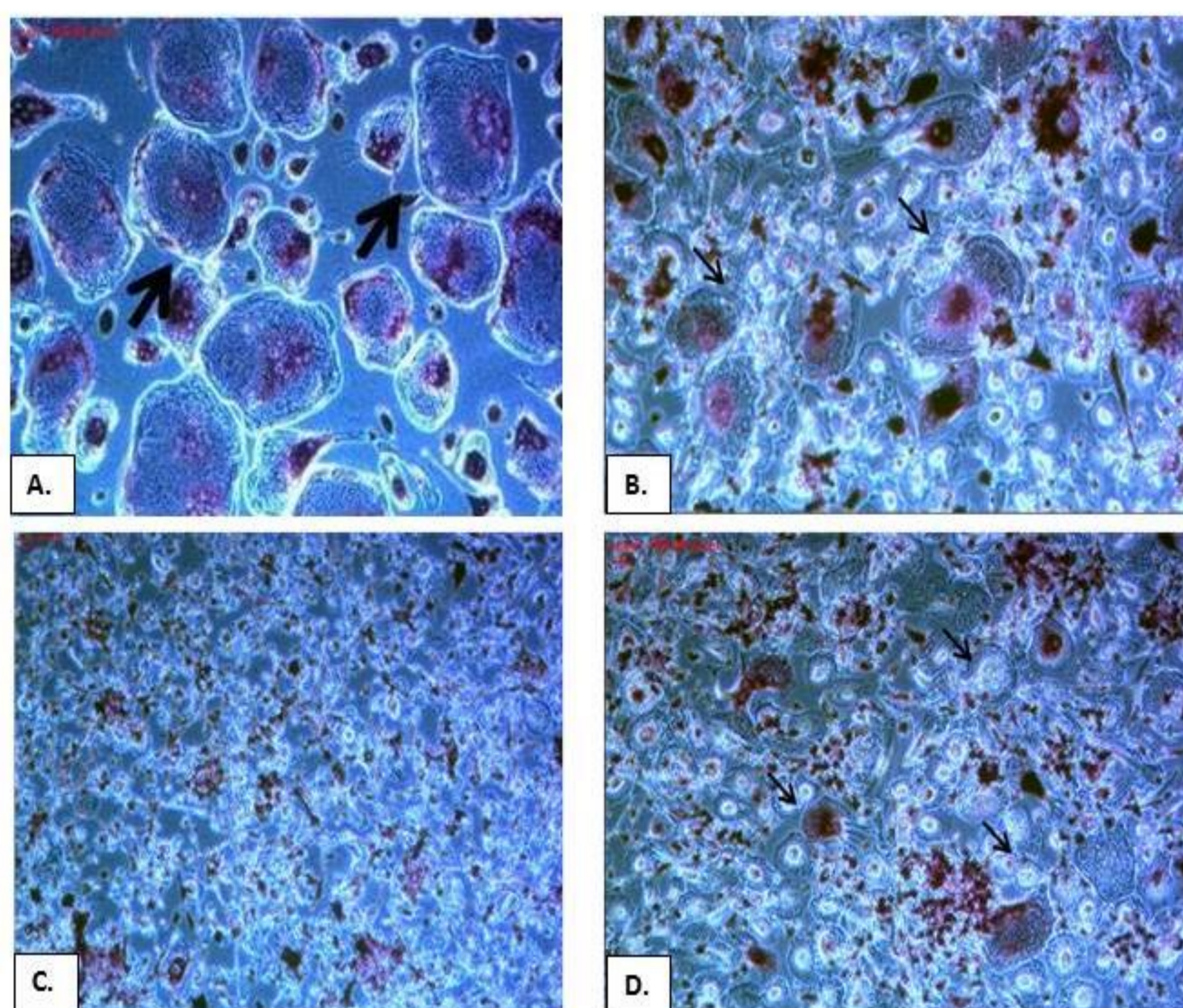
Negative selection on PBMCs was used to isolate monocyte (CD14<sup>+</sup> cells). CD14<sup>+</sup> cells were 7 days cultured in  $\alpha$ -MEM + 25ng/ml M-CSF + 30ng/ml RANKL

#### OCs characterization

Tartrate-resistant acid phosphatase (TRAP) staining ii) Acid Phosphatase Colorimetric Assay Kit iii) Dentin resorption assay plate

Osteoclast differentiation by TRAP was evaluated as the presence of more than 50 TRAP<sup>+</sup> cells/well (score 1) or the presence of less than 50, but more than 30 TRAP<sup>+</sup> cells/well (score 2); 0 was referred to less than 5 TRAP<sup>+</sup> cells/well.

### Co-administration of Pi and Fe<sup>3+</sup> prevents Pi-induced inhibition of OCs differentiation



TRAP staining (pink) of human osteoclast differentiated from PBMC and cultured for 7 days in presence of MCSF and RANKL.

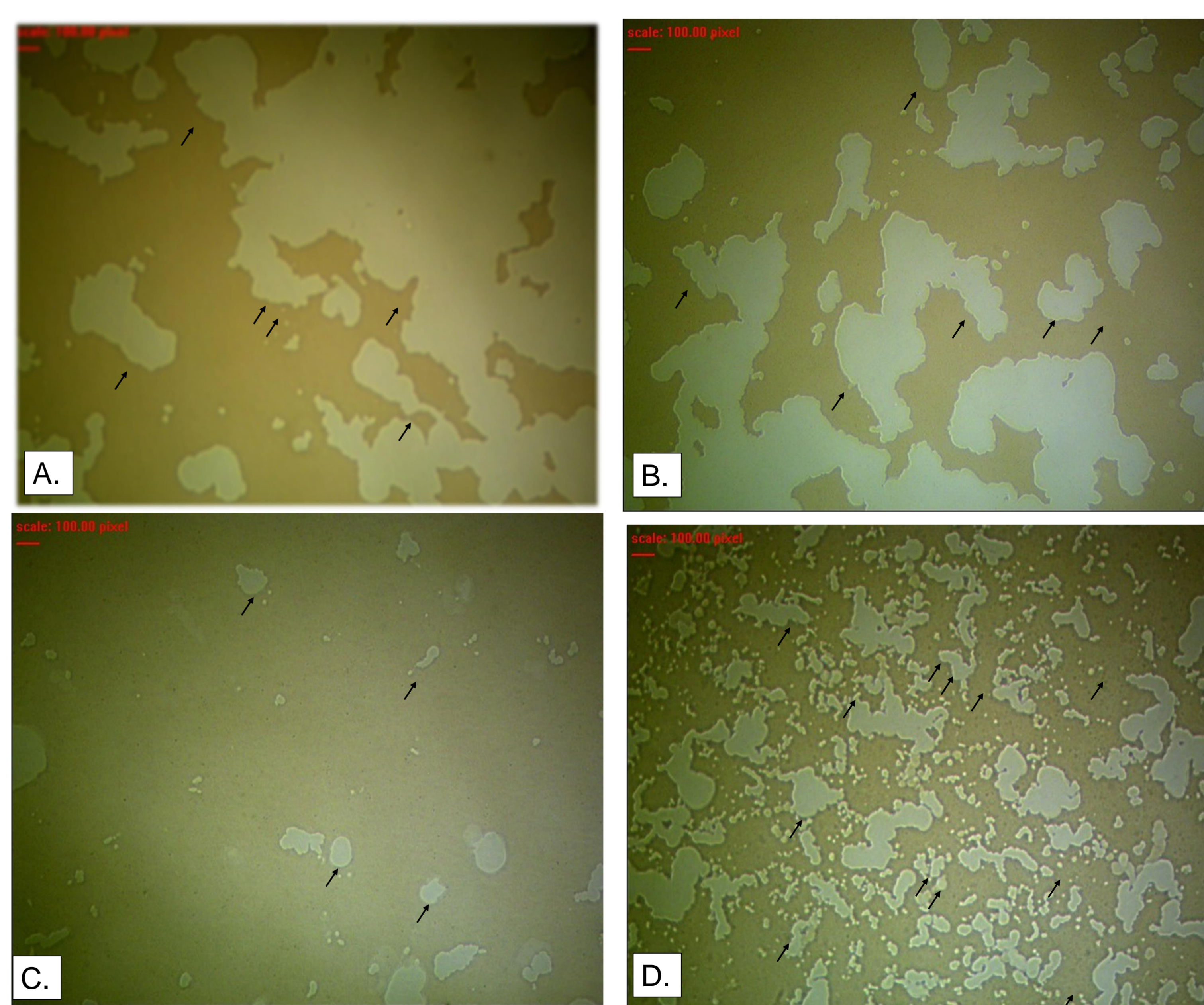
A) healthy control (HC) OCs : **SCORE 1**

B) HC OCs + Fe<sup>3+</sup> (50uM): **SCORE 1**

C) HC OCs+ Pi (3mM) : **SCORE 0**

D) HC OCs+ Pi (3mM)+ Fe<sup>3+</sup> (50 uM): **SCORE 2**

### Co-administration of Pi and Fe<sup>3+</sup> prevents Pi-induced inhibition of OCs bone resorption



Bone resorption assay of human osteoclast differentiated from PBMC and cultured for 7 days on dentin in presence of MCSF and RANKL. Black arrows indicate resorption areas.

A) healthy control (HC) OCs

B) HC OCs + Fe<sup>3+</sup> (50uM)

C) HC OCs+ Pi (3mM)

D) HC OCs+ Pi (3mM)+ Fe<sup>3+</sup> (50 uM)

## Conclusions

- High Pi concentration inhibits osteoclast differentiation induced by RANKL and M-CSF.
- Pi inhibition of osteoclast differentiation was prevented by low dose of Fe<sup>3+</sup>, suggesting a crucial role of Fe<sup>3+</sup> in neutralizing Pi-based effect upon osteoclastogenesis.
- High Pi concentration inhibits osteoclast capacity of dentin resorption
- Low dose of Fe<sup>3+</sup> is able to revert Pi effects on osteoclast resorption, suggesting a role of Fe in osteoclast maturation.
- Further research will clarify the pathways underlying iron citrate role and activity on osteoclastogenesis, understanding the possible effect of iron in CKD patients

## References

- 1) Vervloet, Marc, and Mario Cozzolino. "Vascular calcification in chronic kidney disease: different bricks in the wall?." *Kidney International* (2016).
- 2) Ciceri P, et al. Combined effects of ascorbic acid and phosphate on rat VSMC osteoblastic differentiation. *Nephrol Dial Transplant*. 2012 Jan;27(1):122-7
- 3) M'Baya-Moutoula, Eléonore, et al. "High inorganic phosphate concentration inhibits osteoclastogenesis by modulating miR-223." *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease* 1852.10 (2015): 2202-2212.

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