New electrodeposited Coating Limits Osteoclastogenesis In Vitro

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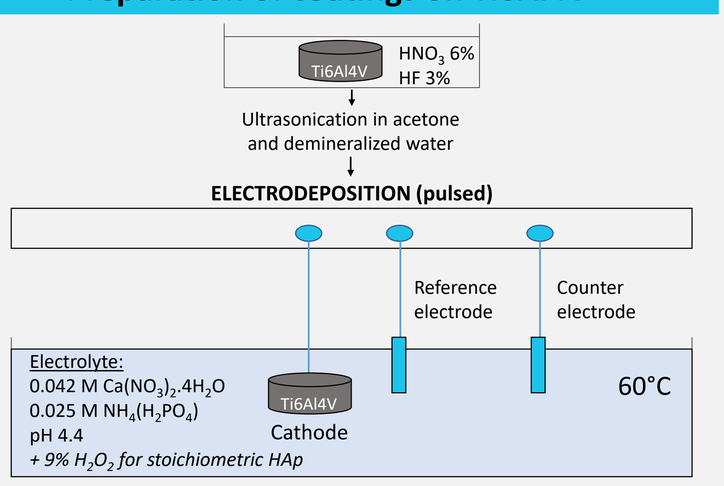
Introduction

Hydroxyapatite (HAp) is widely used for filling bone defects or for prosthesis coating. HAp is known for its capability to mount an inflammatory response which if uncontrolled, could lead to implant loss due to osteoclasts (OCs) differentiation and activation (Velard *et al*, Acta Biomater. 2013). To improve the bioactivity of the implant, electrodeposited calcium-deficient HAp (CaDHAp) was elaborated (Benhayoune H *et al*, Patent WO2010055231; Drevet *et al*, J. Med. Sci. Mat. Med. 2011 and RCS Adv. 2013). Previous short term studies have proved that ceramics coatings enhanced cytokine inflammatory response compared to titanium alloy (Ti6Al4V) and more interestingly, CaDHAp coatings were less inflammatory than HAp (Velard *et al*, ECB 2011). Based on these results we wondered if early cytokine regulation could impact osteoclastogenesis. Here, we have studied human primary monocytes differentiation into OCs in contact with CaDHAp, HAp or Ti6Al4V alone.

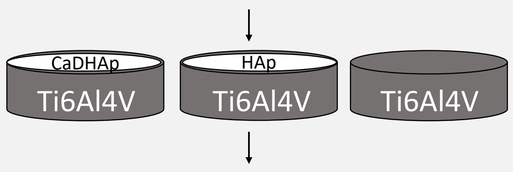
Materials and Method

Preparation of coatings on Ti6Al4V



Cells culture

Counterflow elutriated monocytes
(> 97% by flow cytometry)



2.10^6 cells/mL
± osteoclastic differentiation environment (M-CSF and RANK-L)

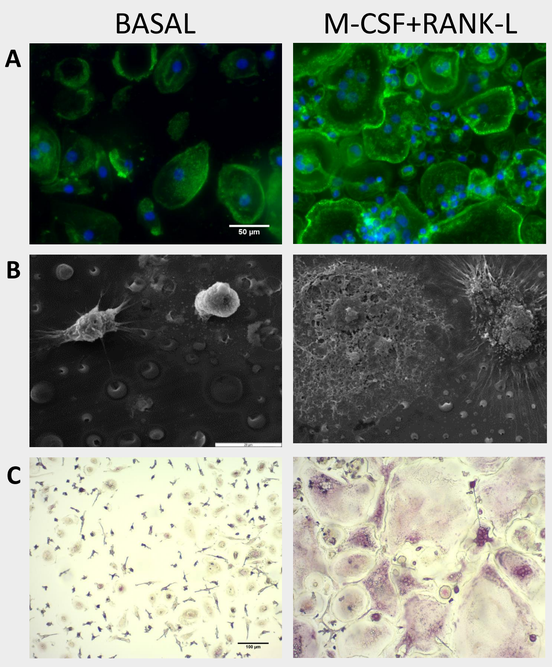
2 days → 21 days

Analyses

- Cells Morphology: SEM /Phalloidin-AlexaFluor®488 /DAPI staining
- OCs Count: Phalloidin-AlexaFluor®488 /DAPI staining /TRAP
- Gelatinolytic activity: Zymography
- mRNA expression of OCs markers: qPCR (CTSK, CALCR, RANK, MMP-9)

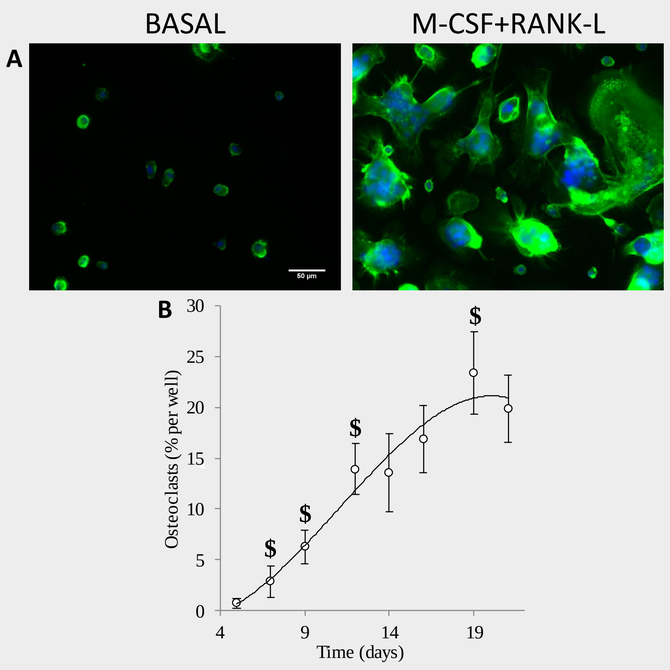
Results and Discussion

Osteoclasts formation requires M-CSF and RANK-L



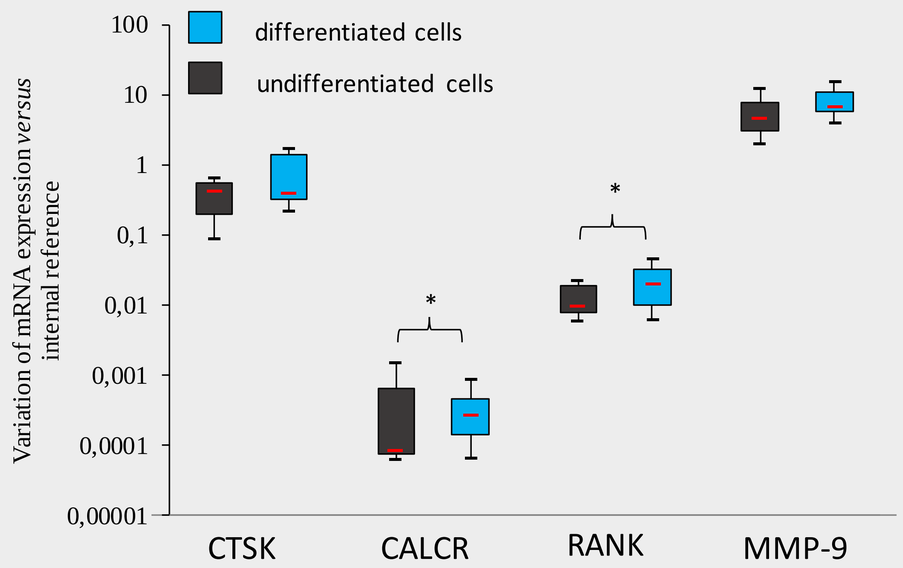
(A) Representative photographs of Phalloidin-AlexaFluor®488/DAPI staining, (B) Scanning Electron Microscopy and (C) TRAP staining of undifferentiated or differentiated monocytes after 12 days, n=8. Magnification: (A) x 20, (B) x 1500 and (C) x 10

5 days of culture were sufficient to observe osteoclasts formation



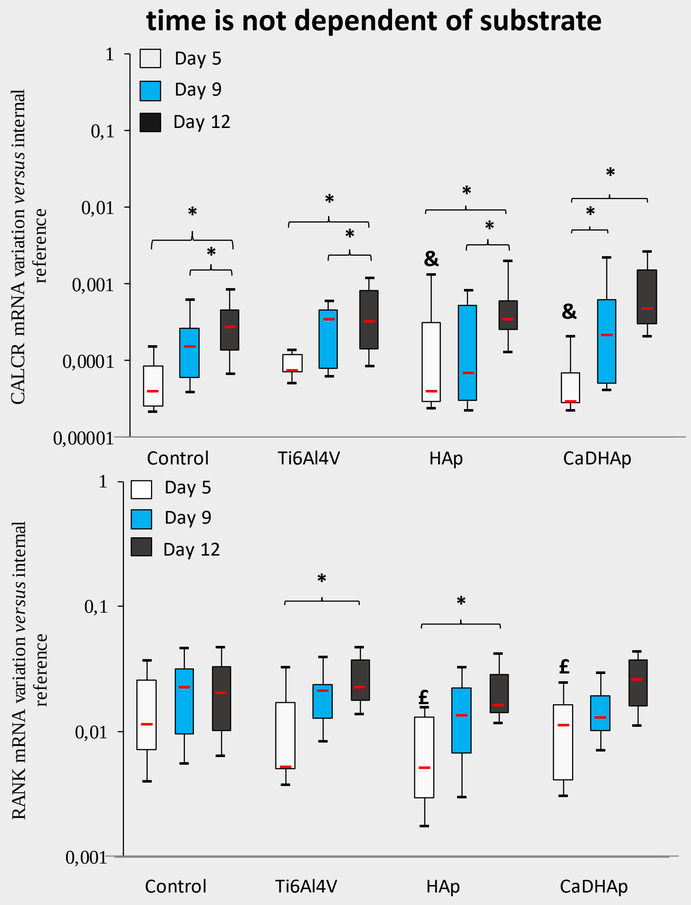
(A) Representative photographs of Phalloidin-AlexaFluor®488/DAPI staining after 21 days of culture without or with M-CSF and RANK-L (Magnification x20). (B) Kinetic of OCs differentiation based on cell count per field from Phalloidin-AlexaFluor®488/DAPI staining. n=13. $ means $p < 0.05$ when compared with previous time point. Non parametric exact stratified Wilcoxon Mann Whitney test.

Markers of Osteoclastic differentiation expression



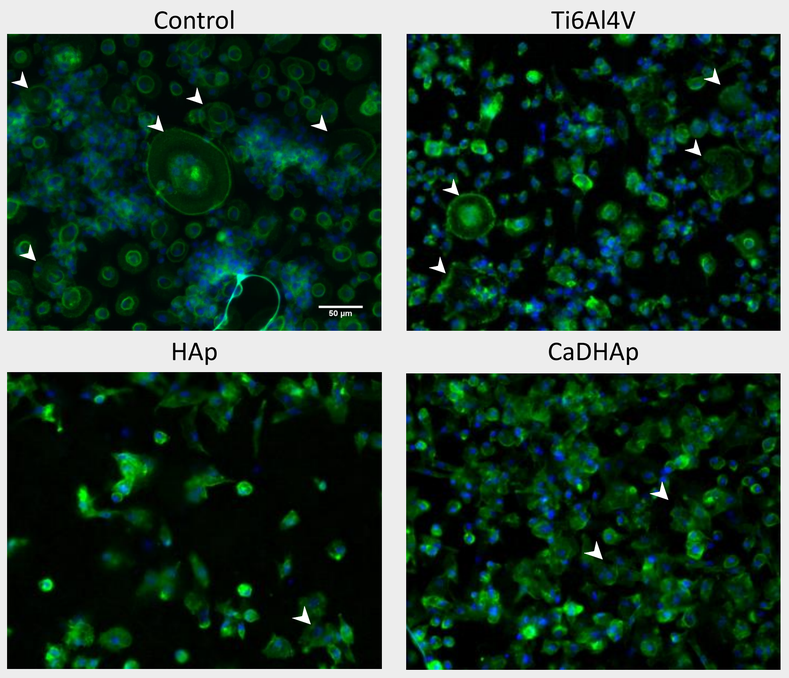
mRNA production was evaluated by RT-qPCR analysis (n=7). Data were shown as variation of CTSK, CALCR, RANK and MMP-9 mRNA using the $2^{-\Delta\Delta Ct}$ method (*versus* internal control RPS18). Red bar represents median value. * means $p < 0.05$ when compared with non-stimulated cells. Non parametric exact stratified Wilcoxon Mann Whitney test.

Increased expression of osteoclastic markers over time is not dependent of substrate



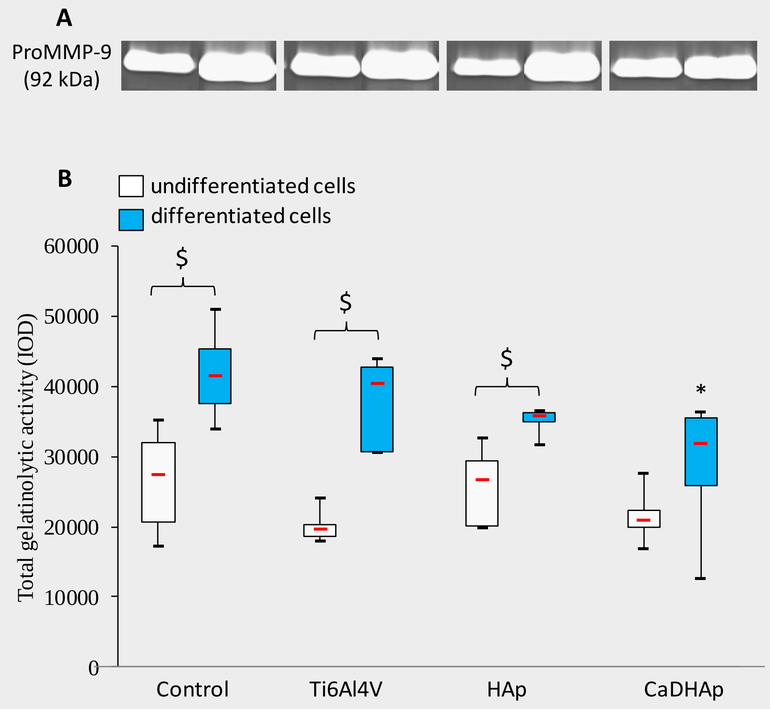
Osteoclastic markers kinetics of mRNA expression on different materials (n=7). CALCR and RANK mRNA variation was evaluated by RT-PCR analysis using the $2^{-\Delta\Delta Ct}$ method (*versus* internal control RPS18). * means $p < 0.05$ between considered conditions, £ *versus* control and & *versus* Ti6Al4V. Non parametric exact stratified Wilcoxon Mann Whitney test.

Ceramics coatings reduce osteoclastic formation *versus* Ti6Al4V substrate



Representative photographs (n=2) of Phalloidin-AlexaFluor®488/DAPI staining after 12 days of culture with M-CSF and RANK-L on Ti6Al4V, HAp and CaDHAp materials. Arrowheads highlight osteoclastic cells. Magnification x20.

CaDHAp decreases MMP-9 activity



Effect of the different materials on proMMP-9-related gelatinolytic activity in cell culture supernatants. (A) Representative photographs of zymograms (gelatinolytic activity is seen as white bands against dark background). (B) Quantification of gelatinolytic activity by image analysis. (n=7). $ means $p < 0.05$ when compared with non-stimulated cells. * means $p < 0.05$ when compared with control. Non parametric exact stratified Wilcoxon Mann Whitney test.

Conclusion and Perspectives

Combined with our previous data collected on early monocytic inflammatory response, these results suggest that CaDHAp might be used as a novel coating for prosthesis to improve implant integration and lifespan as it controls inflammatory environment and limits osteoclastogenesis, both responsible for aseptic loosening.

The authors warmly thank Pr P Nguyen and Ms C Macé (EA 3800 HERVI, URCA) for elutriations and PICT (URCA) platform for imagery.
This work was supported by région Champagne-Ardenne Emergence Nanosurf and ANR program Nanobonefiller.