TGF-β enhances phosphate-driven mineralization of human OA articular chondrocytes

Roderick H.M.J Stassen[1], Guus G.H. van den Akker[1], Don A.M Surtel[1], Andy Cremers[1], Marjolein M.C. Caron[1], Lodewijk W. van Rhijn[2], Tim J.M. Welting[1,2]

[1] Laboratory for Experimental Orthopedics, Maastricht University,[2] Laboratory for experimental Orthopedics, Department of Orthopedic surgery, Maastricht University Medical Center +, Maastricht, The Netherlands

r.stassen@maastrichtuniversity.nl

INTRODUCTION: Mineralization is an intrinsic property of normal physiology, but also occurs in pathological conditions such as osteoarthritis (OA). The presence of calcium-containing crystals has been shown in OA articular cartilage at the time of total knee arthroplasty (1). However, how these crystals form in the joint space remains to be elucidated. One of the mechanisms that has been suggested is a disbalance in inorganic phosphate (2). In this study, an inorganic phosphate disbalance was employed to develop a cell-dependent *in vitro* mineralization model for human OA articular chondrocytes (HACs). This allowed us to study the influence of growth factors on mineralization of HACs. Inspired by the ambivalent role of transforming growth factor β (TGF-β) in cartilage and its role in OA pathology, we investigated the effect of TGF-β on inorganic phosphate-driven mineralization of OA HACs.

METHODS: OA HACs were isolated from surgical waste material originating from total knee arthroplasty (approved by a medical ethics committee). A pool of HACs (N=6) was cultured in DMEM/F12 supplemented with 10% FCS, 1% P/S and 1% NEAA. Inorganic phosphate disbalance was induced by addition of ATP (1 mM) and β-glycerophosphate (BGP, 10 mM) up to 7 days in the absence or presence of 10 ng/ml rhTGF-β with or without ALK5 inhibitor (SB505124). Cell cultures were analysed by von Kossa staining and SEM-EDX. Quantification of total calcium and phosphate was performed after crystal hydrolysis with the Randox calcium (Randox) and phosphate colorimetric assays (Sigma). Gene expression analysis was performed by RT-qPCR and normalized to *PPIA* as a reference gene. Normal distribution of the data was assumed with equal variance. Statistical analysis was performed with use of an unpaired Students T-test. Results were considered significant with P.value < 0.05.

RESULTS: After 7 days under mineralizing conditions, nodules were observed our HAC cultures. These nodules were positive for von Kossa staining. Additionally, these cultures showed a significant increase in both calcium and phosphate ion content. SEM-EDX revealed the presence of calcium, phosphorus and oxygen atoms in the deposited mineral fraction (Figure 1A). The culture medium was supplemented with TGF-β3 in the presence or absence of SB505124 as an ALK5 inhibitor. TGF-β3 induced the total mineral content in mineralizing HAC cultures, while this effect was dose-dependently abolished by the ALK5 inhibitor (Figure 1B). SEM-EDX revealed that in the TGF-β3 supplemented cultures mineral nodules were larger compared to the non-supplemented mineralization cultures. These findings were further corroborated by von Kossa stainings that revealed a significant increase in total positive mineral area. Besides the gross increase in mineralization induced by TGF-β3, SEM revealed a different crystal morphology. Crystals deposited in the non-supplemented condition had a Ca:P ratio of 1:1.41, while in the TGF-β3 supplemented condition this ratio was 1:1.05. To find a possible underlying mechanism of this TGF-β3-driven HAC mineralization, we investigated the effect of TGF-β supplementation on gene expression levels of cartilage extracellular matrix components and matrix degrading enzymes. Mineralization in the presence of TGF-β significantly increased expression of *COL10A1, COL1A1* and *COL3A1*. This was accompanied by decreased expression of *MMP-1*, *MMP-3,* while *MMP-13* was slightly upregulated. These results indicate an altered ECM composition *in vitro*.

DISCUSSION: Due to the high prevalence of calcium-containing crystals and their emerging role in OA pathobiology, there is a need for better understanding the underlying mechanisms involved in cartilage and chondrocyte mineralization. In this study, an inorganic phosphate-driven mineralization model for OA HACs was developed. This model allowed us to investigate the role of TGF-β in HAC mineralization under imbalanced phosphate conditions. We found a pro-mineralizing role for TGF-β, resulting in the formation of larger calcium phosphate mineral nodules, which was accompanied by changes in the crystal morphology. A likely effector of this TGF-β3-driven mineralization is an altered ECM composition, with an increased expression of collagens potentially facilitating additional crystal nucleation centers and increasing mineral nodule formation.

SIGNIFICANCE/CLINICAL RELEVANCE: The high prevalence of calcium-containing crystals in OA articular cartilage and their role in OA pathobiology urge the need for a better understanding of how these crystals emerge and can be treated. Our results shed a new light on the formation of calcium-containing crystals *in vitro* and underpin important crosstalk between intra-articular growth factors and pathological crystal formation in the OA joint micro-environment. This is expected to uncover novel opportunities to identify of drugable targets for OA therapy based on inhibition of pathological crystal formation.

REFERENCS: 1. Stassen *et al* .OAC 2022 2. Yan *et al* Biol. Rev. Camb. Philos. Soc. 2020.

IMAGES AND TABLES:

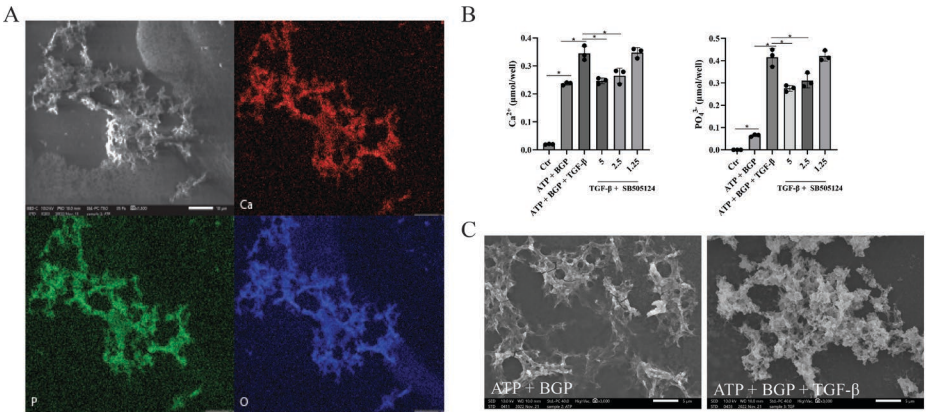

Figure 1 TGF-β enhances phosphate-driven mineralization. A) SEM-EDX analysis of OA HACs after 7 days in mineralization medium. B) Calcium and phosphate quantification with colorimetric assays Data are presented as Mean ± SD. * P.value <0.05. C) SEM imaging of OA HACs in mineralization medium with or without TGF-β.