

ALKALINE PHOSPHATASE-INDUCED MINERAL DEPOSITION TO ANCHOR COLLAGEN FIBRILS TO A SOLID SURFACE

[A.D. Berendsen](#)¹ [T.H. Smit](#)² [X.F. Walboomers](#)³ [A.L.J.J. Bronckers](#)¹ and [V. Everts](#)¹

¹[Academic Center for Dentistry Amsterdam \(ACTA\)-UvA&VU, Amsterdam, The Netherlands,](#)

²[VU Medical Center, Amsterdam, The Netherlands,](#) ³[Radboud University Nijmegen Medical Center, Nijmegen, The Netherlands](#)

INTRODUCTION: Reconstruction of load-bearing tissues requires proper attachment of the tissue-engineered construct to surrounding tissues. Anchoring of collagen-rich soft connective tissues, including tendons and ligaments, to mineralizing tissues is accomplished *in vivo* by embedding collagen bundles into a mineralizing layer. This layer is partially the result of high local activity of alkaline phosphatase (ALP), an enzyme that facilitates mineral deposition both *in vivo* and *in vitro* [1, 2]. A problem of tissue engineering of a collagenous network seeded with fibroblasts *in vitro* is that a collagen gel is contracted by the fibroblasts resulting in a reduced gel volume and detachment from the surrounding solid surface. We tested whether mineral deposition induced by ALP at the interface of collagen gels and culture well surfaces can prevent gel detachment by human periodontal ligament (PDL) fibroblasts *in vitro*. In addition, the effect of these mineral-inducing conditions on collagen I, Runx2, BSP, OPN and DMP1 gene expression by the fibroblasts was studied.

METHODS: Wells were coated with intestinal ALP prior to addition of collagen gels harboring human PDL fibroblasts. Cultures were performed in the presence of the enzyme substrate β -glycerophosphate (β -GP). Gene expression by the fibroblasts was analyzed after culture for 1 (initial mineral deposition) and 3 days (increased mineral deposition) by Quantitative Polymerase Chain Reaction (QPCR).

RESULTS: Coating of culture wells with ALP prevented detachment of gels from the culture well surfaces. Mineral deposition was observed predominantly at the interface between gels and wells. Contraction of free-floating gels was not influenced by the combined presence of ALP and β -GP. Collagen I gene expression was significantly decreased under mineral-inducing conditions after culture for 1 day, whereas DMP1 gene expression was detected after culture for 3 days. Runx2, BSP and OPN gene

expression were not affected under these conditions. Blocking of intracellular phosphate entry into the fibroblasts by foscarnet decreased DMP1 gene expression after culture for 3 days. Addition of phosphate induced DMP1 gene expression in the absence of exogenous ALP but the expression was prevented by blocking the phosphate transporter. DMP1 protein was associated with some fibroblasts in mineralizing gels only.

DISCUSSION & CONCLUSIONS: Coating a solid surface with ALP and providing its substrate to allow mineral deposition can prevent detachment of collagen gels by PDL fibroblasts [3]. This anchoring of collagen fibrils to solid surfaces may be used for reconstruction of load-bearing tissues and temporary attachment of collagenous ligaments to implants. As a consequence of the induction of mineral the fibroblasts are stimulated to differentiate into the osteogenic lineage. This differentiation appears to be mediated by intracellular inorganic phosphate.

REFERENCES: ¹ W. Beertsen and T. Van den Bos (1992) *J Clin Invest* **89**:1974-80. ² T. Van den Bos, J. Steinfort and W. Beertsen (1993) *Bone Miner* **23**:81-93. ³ A.D. Berendsen, T.H. Smit, K.A. Hoeben, et al (2007) *Biomaterials* **28**:3530-6.

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