

# IN VITRO WOUND HEALING CHARACTERISTICS OF AMELOGENINS

## Akademisk avhandling

Som för avläggande av medicine doktorsexamen vid Göteborgs Universitet kommer att offentligas försvaras i föreläsningssalen på våning 5, Avdelningen för Biomaterialvetenskap, Arvid Wallgrens backe 20, torsdagen den 26 maj 2011, kl 13:00 av

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Avhandlingen baseras på följande delarbeten:

- I. Almqvist S., Werthén M., Lyngstadaas S.P., Ågren M.S., Thomsen P. *Amelogenins Promote an Alternatively Activated Macrophage Phenotype in Vitro*. International Journal of Nano and Biomaterials, In press
- II. Almqvist S., Werthén M., Lyngstadaas S.P., Ågren M.S., Gretzer C., Thomsen P. *Amelogenins Modulate the Inflammatory Response in LPS-Stimulated Monocyte-Derived Macrophages*. Submitted manuscript
- III. Almqvist S., Werthén M., Johansson A., Ågren M.S., Thomsen P., Lyngstadaas S.P. *Amelogenin is Phagocytized and Induces Changes in Integrin Configuration, Gene Expression and Proliferation of Cultured Normal Human Dermal Fibroblasts*. Journal of Materials Science: Materials in Medicine, 2010; 21(3):947-54
- IV. Almqvist S., Werthén M., Johansson A., Törnqvist J., Ågren M.S., Thomsen P. *Evaluation of a Near-Senescent Human Dermal Fibroblast Cell Line and Effect of Amelogenin*. British Journal of Dermatology, 2009;160(6):1163-71
- V. Almqvist S., Kleinman H.K., Werthén M., Thomsen P., Ågren M.S. *Effects of Amelogenins on Angiogenesis-Associated Processes of Endothelial Cells*. Journal of Wound Care, 2011; 20(2): 68,70-75

## ABSTRACT

Wound healing involves the co-ordinated actions of several cell types, soluble cell mediators and extracellular matrix (ECM). This research project aimed at investigating the role of certain ECM proteins in different processes during tissue repair by studying the interaction between dermal cells and ECM. The focus has been on amelogenins, ECM proteins that under physiological conditions aggregate into spherical structures. As a resorbable biomaterial, amelogenins enhances periodontal tissue regeneration and have been introduced in the treatment of hard-to-heal ulcers. However, the mechanisms of action need to be delineated. The aim of this project was to increase the knowledge on the effects of amelogenins on cell behaviour, to further understand the role of this specific ECM protein in tissue repair and regeneration.

To study the *in vitro* effects of amelogenins on wound healing, three human cell types; macrophages, fibroblasts, and endothelial cells, all essential for successful tissue repair, were utilised. The study designs included cell cultures, in monolayer, 3D-culture and an *ex vivo* model (chick aortic arch assay) for the angiogenesis studies. The evaluation methods included cell quantification, mitogenesis and apoptosis studies by BrdU incorporation and TUNEL measurements respectively, cytokine analysis by ELISA and multiplex bead array, cell surface integrin adhesion assay, gene microarray analysis, phase contrast and fluorescence microscopy for morphology and viability, and ultrastructural studies by electron microscopy.

The results demonstrate that amelogenins influence the *in vitro* cell behaviour of all three cell types investigated. The interaction and uptake of amelogenin aggregates was demonstrated for both macrophages and fibroblasts. In addition, the possible involvement of integrin-dependent adhesion was demonstrated for fibroblasts and endothelial cells, with increased cell binding by multiple integrins subunits and  $\alpha v\beta 3$ ,  $\alpha v\beta 5$  and  $\alpha 5\beta 1$ . Amelogenin treatment of cultured macrophages displayed anti-inflammatory properties, directing the release of several pro- and anti-inflammatory cytokines. In particular, induced secretion of the specific marker of alternative macrophage activation AMAC-1, along with vascular endothelial growth factor was seen, most probably resulting from a switch of macrophage phenotype to an alternatively activated cell, with tissue repair characteristics. Also, amelogenins increased cell proliferation and induced the expression of genes involved in cellular growth, migration and differentiation in normal dermal fibroblasts. Moreover, amelogenins had the capacity to restore an acute-like phenotype in senescent fibroblasts. Finally, amelogenins displayed pro-angiogenic properties *in vitro* and *ex vivo*.

In conclusion, the effects of amelogenins on wound healing are plausibly, at least partly, conducted by providing macrophages, fibroblasts, and endothelial cells with tissue repair characteristics. These effects are most probably conducted through cell adhesion via integrin interaction.

**Keywords:** Amelogenins, extracellular matrix, cell culture, macrophage, fibroblast, endothelial cell, integrin, cytokine, inflammation, wound healing/tissue repair

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