Alkaline Phosphatase Physically Immobilized on a DMPA/Ca^{2+} Langmuir-Blodgett Film Induces Mineral Formation in a Homogeneous Media

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INTRODUCTION: The phosphate release in bone mineralization is enzyme-regulated, assigned to tissue nonspecific alkaline phosphatase (TNAP) action over phosphorylated substrates. Different cell membrane models are available to mimic enzyme-guided process of biomineralization. Among them, Langmuir-Blodgett (LB) films are candidates that allow lipids lateral packing control and calcium co-adsorption to further mineralization assays. **OBJECTIVES:** Evaluate phosphohydrolytic activity and calcium phosphate mineralization induction by TNAP immobilized on LB films. **MATERIAL AND METHODS:** Solubilized human-TNAP was processed with hydrophobic bead to detergent remotion. Dimyristoyl phosphatidic acid (DMPA) monolayers were obtained in pure water and 0.1 mM $CaCl_2$ subphases, from which Y-type LB films were constructed at constant surface pressure (30 $mN.m^{-1}$). DMPA/Ca^{2+} films were exposed to TNAP homogeneous solution, monitoring the enzyme adsorption by quartz crystal microbalance. Phosphohydrolytic activity was assayed incubating the TNAP-containing film in a media containing AMPOL buffer (pH 10), *p*-nitrophenylphosphate and $MgCl_2$, and thus continuously monitoring the *p*-nitrophenolate formation spectrophotometrically. Control assay was performed with TNAP in homogeneous media. Mineralization assay was performed incubating those films in a synthetic cartilage lymph, containing Ca^{2+} ions and ATP as a phosphate source, measuring mineral formation through turbidity at 340 nm, using control films in absence of TNAP. **RESULTS AND DISCUSSION:** The deposition of DMPA was optimized with $CaCl_2$ in the subphase as evidenced by higher compressional modulus and linear mass deposited per layer. TNAP film-immobilization followed a Langmuir adsorption isotherm. Even though the phosphohydrolytic activity of immobilized-TNAP was 6% of that estimated in homogeneous media, the enzyme-containing films induced 50 % higher mineral formation compared to control samples. **CONCLUSION:** Physical immobilization of TNAP in DPMA/Ca^{2+}-LB film decreased its phosphohydrolytic activity, possibly due to conformational changes of the amino acid moiety. However, increased mineralization was found compared to control samples. This result is an important achievement in order to obtain osteoinductive biomaterials.

Keywords: Alkaline Phosphatase, Langmuir Monolayers, Langmuir-Blodgett, Cell Membrane Models.

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