

## Hydroxyapatite growth induced by extra cellular matrix deposition on solid surfaces

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**INTRODUCTION:** Biological systems have a remarkable capability to produce perfect fine structures such as seashells, pearls, bones, teeth, corals, etc. These structures are composites of interacting inorganic (calcium phosphate or carbonate minerals) and organic (molecules) parts. Which part has the primary role is difficult to say unambiguously.

In this work the growth of hydroxyapatite (HA) by two methods is reported: (i) a simple soaking process in a simulated body fluid (SBF) and (ii) a laser-liquid-solid interaction (LLSI) process which allows interaction between a scanning laser beam and the substrate immersed in SBF. Metallic and non-metallic materials are used as substrates. Their surfaces are modified by a deposition of extra cellular matrix (ECM) proteins. The deposited layers are investigated by FTIR Spectroscopy and Scanning Electron Microscopy.

**METHODS:** Samples are prepared by coating of ECM on AISI 316 stainless steel (further named ECM/SS), (100) n-type silicon (ECM/S), and silica glass Herasil (ECM/SG). The osteoblast-like cell line SAOS-2 is allowed to synthesize and assemble its own ECM on the substrates under standard cell culture conditions. Cells are then selectively removed resulting in surfaces coated with a thin film of ECM. To evaluate the ability of the as-modified samples to precipitate HA two methods are applied: (i) a simple soaking process in SBF for 4 and 24 h in which the samples are immersed simultaneously in horizontal and vertical positions and (ii) a laser-liquid-solid interaction process which allows laser beam-substrate interaction in the fluid and subsequent soaking in SBF for 4 and 24 h. Some samples are taken out immediately after the laser interaction to study the instantaneous effect of the laser energy on the HA nucleation and growth (named ECM/SS-LLSI 0h). CuBr vapor pulsed laser ( $\lambda = 578.2$  nm, 330 mW) equipped with a scanning system is applied. The laser beam is focused on the substrate surface and the interaction time is approx. 2 min. A container with 400 ml SBF kept at physiological conditions (temperature 37°C and pH 7.4) is used in the experiments.

**RESULTS:** FTIR shows the formation of HA layers on all materials, modified by the two methods (Fig. 1). An estimation of the layer thickness shows that

the applied LLSI leads to a thicker layer in comparison to the soaked samples, as well as to an effective formation of nuclei within few minutes for facilitation of further layer growth. Such fast formation of nuclei is not observed in the case of the simple soaking. The vertically soaked samples induce a HA layer on their surfaces, which shows that the deposition is not simply a question of gravity.

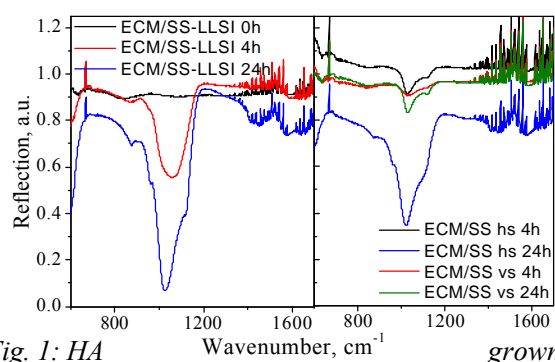


Fig. 1: HA grown on ECM-stainless steel samples modified by a laser-liquid-solid interaction in SBF (left) and by a simple soaking in SBF (right)

HA morphology on the samples modified with surface ECM proteins presents regular sphere-like particles, grouped homogeneously in a network and embedded in the ECM structure as in a matrix. The latter observation is ascribed to the ability of the organic molecules to control and define the crystallization process.

**DISCUSSION & CONCLUSIONS:** It was found that (1) the surface deposited ECM proteins serve as a matrix for a homogeneous HA growth and influence the size of the HA spherules; (2) the HA nucleation and growth on as-modified materials, prepared by two methods is strongly influenced by the application of laser energy; (3) the layer growth in the case of the soaked samples is not simply a question of gravitational forces.

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