

## Collagen I, Chondroitin 4-Sulfate and Collagenase Interact with Hydroxyapatite Particles

Carvalho, R.G.<sup>1</sup>; Lima, M.A.<sup>1</sup>; Nader, H.B.<sup>1</sup>; Nascimento, F.D.<sup>2</sup> and Tersariol, I.L.S.<sup>1</sup>

<sup>1</sup>Departamento de Bioquímica, UNIFESP, SP, Brazil. <sup>2</sup>Biotechnology in Health Program, Universidade Anhanguera de São Paulo, SP, Brazil.

**INTRODUCTION.** The mechanical properties of biomineralized tissues are determined by the organization and strength of binding at the hydroxyapatite (HAP)–collagen interface. **OBJECTIVE.** Here, we show the influence of biomolecules collagen I, chondroitin 4-sulfate (C-4S) and collagenase type IA in the formation of HAP particles *in vitro*. **MATERIAL AND METHODS:** The HAP nucleation kinetics experiments were performed in light-scattering system. The transition time of amorphous calcium phosphate particle (ACP) for HAP was monitored by pH titration of the solutions in the presence or in the absence of biomolecules. The enzymatic activity of collagenase was analyzed during HAP nucleation process with the aid of fluorogenic substrate. **RESULTS AND DISCUSSION.** The transition time ( $t_{1/2}$ ) of ACP for HAP in the absence of biomolecules was  $35 \pm 4$  min and in presence of collagen I ( $1 \mu\text{g/mL}$ ) or C-4S ( $10 \mu\text{g/mL}$ ) were  $65 \pm 6$  min,  $135 \pm 12$  min, respectively. In addition, the  $t_{1/2}$  values for the nucleation of HAP particles (200 nm) were  $2.9 \pm 0.3$  min in the absence of biomolecules and in the presence of collagen I ( $1 \mu\text{g/mL}$ ) or C-4S ( $10 \mu\text{g/mL}$ ) were  $3.2 \pm 0.4$  min and  $68 \pm 7$  min, respectively. Collagenase IA adsorbs in the early HAP particles formed, removing this enzyme of the solution either in the absence of biomolecules or in the presence of collagen I and C-4S. The results show that collagen I and C-4S increased the transition time of ACP for HAP. However, the time of crystals nucleation was elongated in the presence of C-4S, but not in the presence of collagen I, suggesting that collagen I did not disturb the rate of ACP formation. **CONCLUSION.** Taken together, the present results show that collagen I, C-4S and collagenase I adsorb in HAP particles and change the HAP nucleation kinetics.

Keywords: Chondroitin 4-Sulfate, Collagen I, Collagenase, Hydroxyapatite.  
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