

Atomic force microscopy of mineralization-competent matrix vesicles: a focus on the role of PHOSPHO1

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INTRODUCTION. Matrix vesicles (MVs) are a special class of extracellular vesicles (EVs) that initiate mineralization in cartilage and other vertebrate tissues. **OBJECTIVES.** We investigate the morphology and development of the nucleation core (NC) within MVs with different mineralization capabilities. **MATERIALS AND METHODS.** MVs were secreted by chondrocytes isolated from WT and *Phospho1*^{-/-} mice and analyzed through atomic force microscope (AFM) and Raman spectroscopy. **RESULTS AND DISCUSSION.** AFM images showed that WT MVs were in greater number than *Phospho1*^{-/-} MVs. Both WT and *Phospho1*^{-/-} MVs were oblate spheroids either individually dispersed or connected to mat-like features, which were interpreted as type-X collagen monomers. Individually dispersed WT MVs had volumes ranging from few to several tens of thousands of cubic nanometers, whereas *Phospho1*^{-/-} MVs showed volumes in a smaller range of values. AFM topographic and phase images showed that WT MVs had surface morphology and phase angle (ϕ) values dependent on the volume. Vesicles with volumes smaller than $\sim 5 \times 10^3 \text{ nm}^3$ showed a surface that was smooth and with a narrow distribution of ϕ values slightly smaller than those of mica substrate. Bigger vesicles showed an irregular surface with several angstrom-tall bumps that increased in size with vesicle volume and corresponded to areas with ϕ values similar to that of mica substrate. We interpreted the bumps as caused by the presence of the NC under the MVs' membrane. The surface of *Phospho1*^{-/-} MVs was smooth and showed small irregularities only for bigger vesicles. *Phospho1*^{-/-} MV ϕ values were constant, slightly bigger than those of mica substrate and increasing with MV volume. These results suggest the absence of a NC in most of *Phospho1*^{-/-} MVs. Finally, Raman spectroscopy validated the differences between the material inside WT and *Phospho1*^{-/-} MVs. **CONCLUSIONS.** Our data document the decreased ability of *Phospho1*^{-/-} MVs to initiate mineralization.

Keywords: matrix vesicles, atomic force microscope, nucleation core.