

# Crystallization on the eggshell membrane: calcium phosphate nucleation on a calcium carbonate nucleating membrane

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The eggshell membrane (ESM) is a biopolymeric network that naturally nucleates calcium carbonate (CaCO<sub>3</sub>) during shell formation [1]. It consists mainly of cysteine-rich eggshell membrane protein (abbreviated CREMP), collagen (namely collagen type I, V and X), and globular proteins (ovocalyxin-36, lysozyme, lysyl oxidases, etc.) [2]. ESM not only provides a surface for heterogeneous nucleation but also possesses specialized organic aggregates composed of “Mammilan,” a calcium-binding molecule containing keratan sulfate proteoglycan. These aggregates, known as “mamillary knobs,” randomly arrange over the ESM outer surface and act as nucleation centers of CaCO<sub>3</sub> during eggshell formation [3]. In this study, the membrane was carefully peeled out from the shell of an egg and employed as a heteronucleant surface of calcium phosphate by the sitting drop vapor diffusion technique [1]. Results after 1, 7, and 15 days of experiment show that the outer surface of ESM promotes the nucleation of nanocrystalline apatite, which is associated with the ESM fibers, while the inner opposite side does not. The physicochemical and crystallographic characteristics of the mineral coating, populated with apatite nanocrystals doped with A- and B-type carbonate substitutions and low crystallinity, resembled those of the mineralized bone tissue. In addition, biological tests performed on human mesenchymal stromal cells (hMSCs) indicated the enhanced biocompatibility of the mineralized membrane compared with the non-mineralized one and the ability for osteogenic differentiation of hMSCs after 21 days in culture. Hence, the ESM proved to be an excellent heteronucleant scaffold for calcium salts (e.g., calcium phosphates) different from the ones for which it was designed over millions of years of evolution. Moreover, the precipitated phase depicted biomimetic bonelike features. Because of its singular characteristics, the mineralized ESM is an excellent candidate for use in bone tissue regeneration.

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## References

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