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Biomineralization Studies on Cellulose Membrane Exposed to Biological Fluids of *Anodonta cygnea*

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Abstract The present work proposes to analyse the results obtained under in vitro conditions where cellulose artificial membranes were incubated with biological fluids from the freshwater bivalve *Anodonta cygnea*. The membranes were mounted between two half 'Ussing chambers' with different composition solutions in order to simulate epithelial surfaces separating organic fluid compartments. The membrane surfaces were submitted to two synthetic calcium and phosphate solutions on opposite sides, at pH 6.0, 7.0 or 9.0 during a period of 6 hours. Additional assays were accomplished mixing these solutions with haemolymph or extrapallial fluid from *A. cygnea,* only on the calcium side. A selective ion movement, mainly dependent on the membrane pore size and/or cationic affinity, occurred with higher permeability for calcium ions to the opposite phosphate chamber supported by calcium diffusion forces across the cellulose membrane. In general, this promoted a more intense mineral precipitation on the phosphate membrane surface. A strong deposition of calcium phosphate mineral was observed at pH 9.0 as a primary layer with a homogeneous microstructure, being totally absent at pH 6.0. The membrane showed an additional crystal phase at pH 7.0 exhibiting a very particular hexagonal or *cuttlebone* shape, mainly on the phosphate surface. When organic fluids of *A. cygnea* were included, these crystal forms presented a high tendency to aggregate under *rosaceous* shapes, also predominantly in the phosphate side. The cellulose membrane was permeable to small organic molecules that diffused from the calcium towards the phosphate side. In the calcium side, very few similar crystals were observed. The presence of organic matrix from *A. cygnea* fluids induced a preliminary apatite–brushite crystal polymorphism. So, the present results suggest that cellulose membranes can be used as surrogates of biological epithelia with preferential ionic diffusion from the calcium to the phosphate side where the main mineral precipitation events occurred. Additionally, the organic fluids from freshwater bivalves should be also thoroughly researched in the applied biomedical field, as mineral nucleators and crystal modulators on biosynthetic systems.

Keywords Cellulose membranes · Biomineralization · *Anodonta cygnea* · Biological fluids

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Introduction

Biomineralization is the process by which organisms produce minerals from biological solutions for their own functional requirements (Carter 1990; Lopez et al. 1992;