Acta Biomaterialia xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Acta Biomaterialia

journal homepage: www.elsevier.com/locate/actabiomat

Mapping nanomechanical properties of freshly grown, native, interlamellar organic sheets on flat pearl nacre ☆

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ARTICLE INFO

Article history:
Available online xxxx

Keywords:
AFM
Force mapping
Hertz–Sneddon model
Young's modulus
Haliotis tuberculata

ABSTRACT

We imaged surfaces of freshly grown flat pearl nacre (*Haliotis tuberculata*) in different stages of growth in seawater using an atomic force microscope (AFM). Characteristic mineral phases of nacre, such as aragonitic stacks of coins, as well as the associated organic sheets, could be detected. Apart from imaging, the acquisition of force volumes on freshly grown organic surface areas on flat pearl nacre was conducted with the AFM. The evaluation of the force volumes with the Hertz–Sneddon model resulted in Young's moduli in the MPa range. The presented values are considerably smaller than values previously determined from macroscopic tensile tests. This might reflect the anisotropy of the organic nacre layers.

1. Introduction

Nacre is a natural composite with a high content of a mineral phase (~98 wt.%) and a small portion of organic material (~2 wt.%). This small portion of organic material is responsible for the distinct structure of nacre. As can be seen from Fig. 1, mineral platelets (aragonite) grow between organic sheets (interlamellar matrix). This distinct structure – mineral platelets of a defined geometry and organic sheets between them – is responsible for the interesting mechanical properties of nacre. This biocomposite is a tough material although its mineral components do not have this property. Often, common minerals show stiff and brittle behaviour. Less is known about the properties of the thin organic sheets (see e.g. Ref. [1] for a recent study on the interfaces within nacre). The mechanical testing of the whole composite material and the mineral parts has been the subject of many studies (for recent reviews of nacre and its properties, see e.g. Refs. [2–4]).

Recently, two approaches have been used to access the mechanical properties of the organic sheets of demineralized nacre pieces of *Haliotis* sp. in wet conditions. Bezares et al. [5] probed macroscopic specimens of demineralized nacre pieces from *Haliotis rufescens* in different conditions in a macroscopic tensile test system. They described the organic sheets in terms of a standard linear solid model. Dastjerdi et al. [1] probed demineralized organic sheets from red abalone, pearl oyster and top shell with a similar approach. They reported a modulus of 136 MPa for the organic matrix from red abalone.

Our group [6] probed the demineralized organic sheets of nacre of *Haliotis laevigata* in aqueous solution with the atomic force microscopy (AFM) force mapping technique and derived values for the Young's modulus below 1 MPa in the fully hydrated state.

Flat pearls [7] (cf. Fig. 1E and F) can be used to prepare freshly grown, native, hydrated interlamellar organic sheets from growth front nacre (cf. Fig. 1A) as well as other organic phases close to their natural state. The animal deposits shell material on glass slides in a defined sequence [8], when inserted between mantle epithelium and shell as a support (cf. Fig. 1D–F). In a polymer cushion crystals are grown.

In contrast to the flat pearls of *H. rufescens* (e.g. [8–10]) the different layers of flat pearls from *H. tuberculata* seem not to be characterized yet. However, Fleury et al. [11] investigated repair processes in the shell of *H. tuberculata*. They imaged the sequence of different layers during the shell formation process. Fritz et al. [7], Zaremba et al. [8] and Fleury et al. [11] could show in *H. rufescens* and *H. tuberculata*, respectively, that the deposition of shell material on glass and the deposition during the repair

☆ Part of the Biomineralization Special Issue, organized by Professor Hermann Ehrlich.

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http://dx.doi.org/10.1016/j.actbio.2014.02.050
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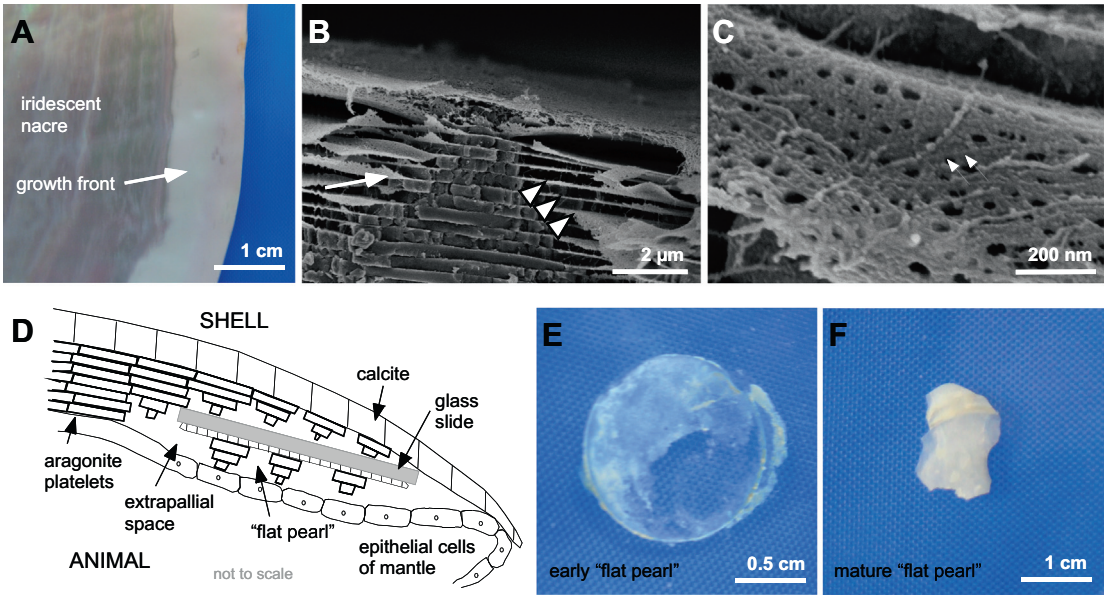

Fig. 1. Nacre of natural shell of *Haliotis laevigata* (A) and biogenic flat pearl of the seawater snail *Haliotis tuberculata* (B, C, E, F). (A) Photograph taken from the outermost part of nacre of a shell (*H. laevigata*). The growth front of the shell (white arrow) has a whitish appearance due to light scattering on the stacks of coins (see mineral part in (B)). Iridescent nacre is formed by thin confluent aragonite layers due to interference of visible light. (B) SEM image of stacks of coins (white arrow) of aragonite crystals horizontally intercalated by multilamellar organic sheets (white arrowheads) of protein decorated chitin filaments (white arrows in (C)). (C) SEM image of a network with holes/pores constructed by protein decorated chitin filaments (white arrows). (D) Sketch of a "flat pearl" inserted between mantle epithelium and the shell. The inserted glass slide is overgrown by shell material in a defined sequence. (E) Photograph of an "early flat pearl", which was inserted for less than 3 weeks. (F) Photograph of a "mature flat pearl", which was inserted for more than 5 weeks. The overgrowth with growth front nacre is clearly visible.

process are comparable to the deposition of shell material in the natural shell.

Therefore we assume in the following that the sequence and type of layers deposited during flat pearl growth are similar to the genus *Haliotis*. As a first step of flat pearl mineralization, islands of prismatic calcite (or in some cases spherulitic aragonite) expand until they cover the entire surface. If the crystals are prismatic calcite then this organic surface resembles the periostracum of the natural shell. A mixture of secreted proteins is necessary for an ongoing calcite growth. On a certain signal, which is not understood yet, the epithelial cells seem to switch to the secretion of a protein set, which then enables the growth of another calcium carbonate crystal polymorph: aragonite. Aragonite tablets start to grow in a preformed organic matrix (cf. Fig. 1C), where multilamellar sheets of organic material are deposited first. The tablets grow in so-called stacks of coins, where the first crystal is nucleated in an unknown way on one of these organic sheets in its crystallographic *c*-direction pointing perpendicular to the surface. The crystal grows until it reaches a height of ~0.5 µm as a small stencil and later in the crystallographic *a*- and *b*-directions. Long before it reaches its final lateral dimensions, a new crystal starts to grow on top of it (Fig. 1B and D), passing the crystal orientation to the next layer [12] by a so-called mineral bridge. Horizontally between each crystal layer one multilamellar sheet of a protein embedded chitin network is located (Fig. 1B, white arrowheads, and Fig. 1C) [2,13].

In this study we measured the elastic properties of the native, hydrated organic matrix, which is freely accessible between the freshly grown aragonitic stacks of coins and other calcium carbonate mineral phases.

The advantage of the thus prepared samples is that organic sheets are of a defined age and may be used without any further preparatory means. They may be imaged and probed in aqueous solution (here: seawater) by AFM to investigate their mechanical properties.

We will show in the next paragraphs the topography of the sequentially deposited material from the first mineral layers to the first nucleation of aragonite tablets to a fully covered sample by stacks of coins, which resembles the growth front of a natural shell (Fig. 1A).

Furthermore, we will show the results of force–indentation measurements conducted with the AFM on three fully hydrated flat pearl samples in aqueous solution. The flat pearls can provide organic layers that can be probed with the AFM without further treatments like demineralization. The Hertz–Sneddon model is used to obtain information on the Young's modulus of the probed samples.

2. Materials and methods

2.1. Sample preparation

Cover glass discs with a diameter of 5 or 10 mm were inserted between the shell and the mantle epithelium of the snail *H. tuberculata*. The animals are kept in a seawater aquarium at 15–17 °C at our institute. The seawater snail *H. tuberculata* is abundant in the Mediterranean Sea and the East Atlantic Ocean, e.g. at the coast of France. The animals, which deposited nacre on an inserted glass slide, were raised at abalone farms in France and Germany (SCEA France Haliotis, Plougerneau, France and Sylter Algenfarm GmbH, List/Sylt, Germany) and specimens of different ages were purchased by us. They have lived for several years in a seawater tank with natural seawater collected from the North Sea (close to Helgoland) provided by the Alfred Wegener Institute, Bremerhaven (Germany).

After different periods of time (for different growth stages) the cover glasses were removed from the snails and prepared for imaging. From the removal until imaging the so-formed flat pearls were stored in filtered (Millex-GV, PVDF membrane, pore size 0.22 µm, Millipore Ireland Ltd) seawater (North Sea water, collected and kindly provided by the Alfred-Wegener-Institut für Polar- und Meeresforschung, Bremerhaven, Germany). The absence of plankton and bacteria, which could otherwise influence our measurements, was tested by light microscopy after filtering the water.

Please cite this article in press as: Launspach M et al. Mapping nanomechanical properties of freshly grown, native, interlamellar organic sheets on flat pearl nacre. Acta Biomater (2014), http://dx.doi.org/10.1016/j.actbio.2014.02.050