The micro(µm)- and nano (nm) structure of modern and fossil braquiopod achives

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1. Introducction

Biological hard tissues are the result of a biologically controlled mineralization wich is a regulated process that produces materials that have specific biological functions and strutures (Mann, 1983). This type of materials have two main properties, they are hybrid composites of organic componets and minerals and have an hierarchical architecture.

Biological hard tissues are widely recognized in nanotechnology and materials science as an important source for designe conceps for cutting-edge materials (e.g. Currey, 1999; Currey, 2006; Gao, 2006; Fratzl & Weinkammer, 2007). They form one of the most important source of information on the evolution of life and enviromental conditions. The key features for the improvement of mechanical performance of the mineral or polymer components are the microstructure and texture. In this context the investigation of the designe principles of shells of those phyla which were succesful and abundant throughout the geological record is truely important.

The phylum Braquiopoda presents a huge fossil record in space and time as they exist in a variety of habitats since the early Cambrian - late Precambrian (Williams *et al.*, 2000). Brachiopods are sessile marine invertebrates and one of the pioneering phyla to develop two main chemical groups of exo- and endoskeleton biomaterials (Lowestam, 1981): Calciumcarbonate and organocalciumphosphate.

2. Braquiopods shell

Brachiopod shells consists of two different valves connected near the umbo at a hinge. They can be opened by a muscular pedicle with attaches the animal to the substrate. The valves have a mirror plane (median plane).

The general structure of calcitic brachiopods consist of calcite interlaced with an organic matrix. It is still a matter of research how calcite crystals are deposited and transported to the final location in the shell. Some hypothesis were proposed to explain the formation of this structure, some support the crystallization from a gel produced by the brachiopod others support that the structure can be formed by a nanoparticle assembly (Meldrum & Cölfen, 2008; Weiner & Addadi, 2011). These proposals nevertheless need to be proved by modern studies.

Calcite shell brachiopods produce crystals principally in three different fabrics:

1) Nano-scale dendrite-like crystalline units.
2) Fibrous calcite mesocrystals with cross-sections of 10 micrometer range and more than 200 micrometers lengths.
3) Columnar calcite composed of crystals with diameters in the 100 range and lengths getting almost 1 mm.

These fabrics defined the three major microstructures of modern brachiopods (Figure 1):

- **Nanostructure external primary layer:** this layer is divided into a nanostruture material in the outside and a micrometer size columnar or platelet-shaped crystals toward the inward part of the valve (e.g. Goetz et al. 2009 and Griesshaber et al. 2010).
- **Fibrous layer** consist in mesocrystal fibers wich are stacked in parallel. In this layer can be distinguished sub-layers with different orientations: longitudinal and transverse.
- **Columnar layer** is composed of large columns in comparison with the primary layer and the axial crystallographic preferred orientation becomes increasingly sharp; it even reaches as three dimensional single crystal-like texture (Smahl *et al.,* 2012).

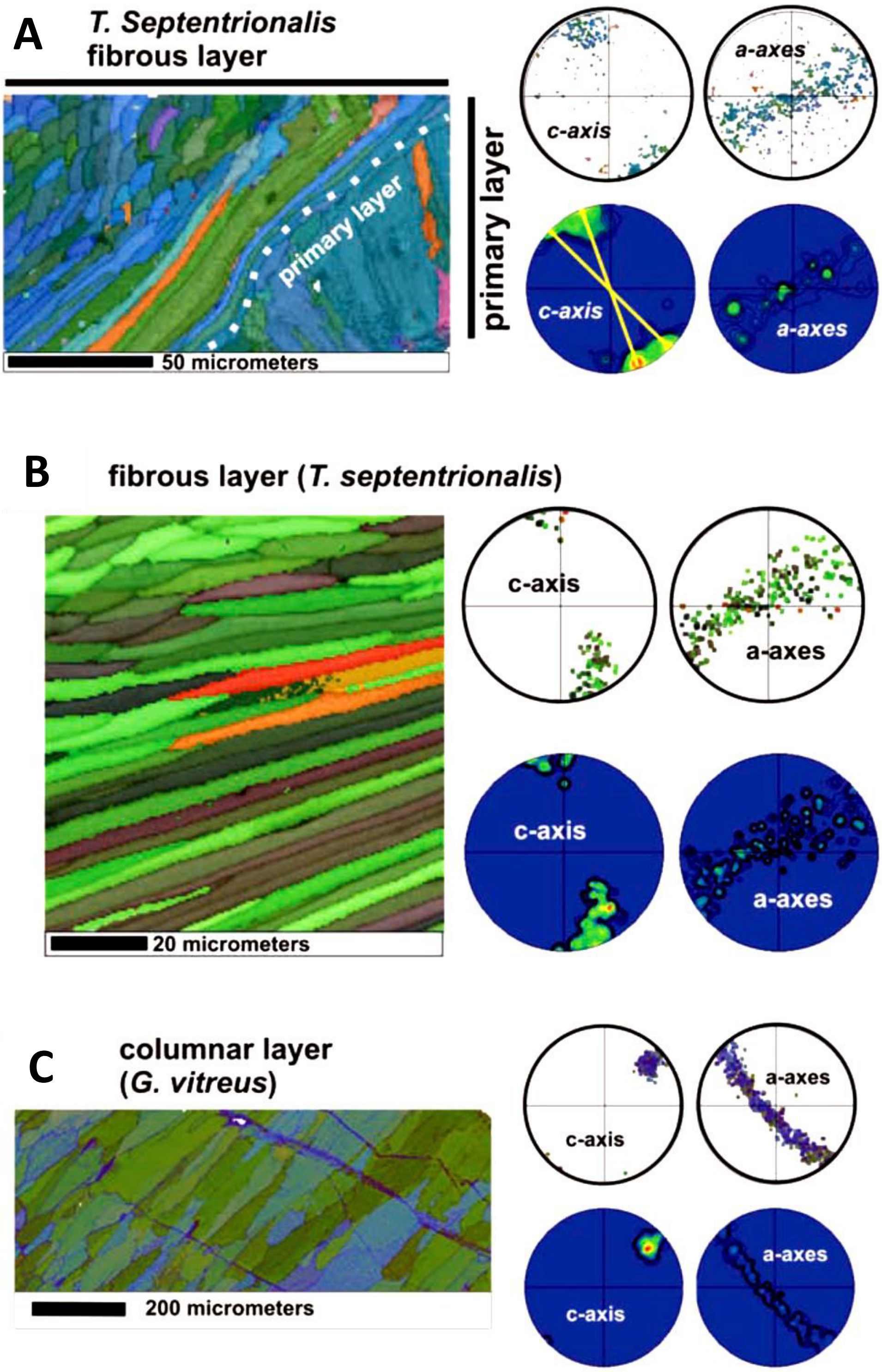

Figure 1. EBSD images of the main layers of the modern brachiopods shell Terebratulina septentrionalis (A and B) and Gryphus vitreus (C). The different colours represents different crystallographic orientations. The crystas axes were plot in stereographic projections with the colours used in the EBSD images. From Schmahl *et al.*, 2010.

Biomineralization does not occur in an equilibrium with the sea water, it is referred to as the vital effect (Weiner & Dove, 2003). The mineralization of the shell is completely controlled by complex physiological processes in the organism (Schmahl *et al.,* 2012). Therefore, the knowlege of the original structure and the processes of morphogenesis and biosythesis are essencial for interpretations of hard materials.

Looking into the texture or micro- and nanostruture of the shells we can infer that during the shell formation different metabolic enviroments are present at distinct parts of the shell and create the high functional specilaization of the different shell parts and thus induced and marked scatter of stable-isotope results whithin the same shell (e.g. Carpenter & Lohmann, 1992; Samtleben *et al.*, 2001; Parkinson *et al.*, 2005).

3. Hierarchical structure. Micro and nano structure of the shell.

Mineral substances and organic molecules form a hybrid composite material with the components inter-weaved on many length scales. Brachiopods are hybrid composite materials and have a hierarchical architecture as this they grow and mineralize calcite continuously. The mineralization used to occurs at the commissural margin of the shell, where the epithelial cells of the mantle tissue secrete the $CaCO_3$ (Williams, 1968, Hiller, 1988, Chuang, 1996) since the mineralization is needed to increase the thickness on the posterior parts of the shell.

The brachiopod calcite shows a systematic pattern of crystallographic preferred orientation (Schmahl et al., 2004, Griesshaber et al., 2007, Griesshaber et al., 2010), which connects the molecular scale structure with the architecture on the macroscopic scale: the [001] axes of the trigonal calcite crystals show a maximum of the orientational probability density in the perpendicular or sub-perpendicular orientation to the shell vault (Schmahl et al., 2004, Griesshaber et al., 2007).

The study of the hierarchical structure involves diffraction and microscopy techniques at different length scales. The main methods to to visualize the structure are, at nano scale, the Transmition Electrons Microscopy (TEM), at micro scale, Scanning Electrons Mircoscopy (SEM) and at all scales Tomography and Atomic Force Mircoscope (AFM). The Electron Backscatter diffraction (EBSD) is use to see the texture and crystallographic orientation of the crystals. Other characterization methods as nanoindentation or micro Raman, Electronic Probe Micro-Analyzer, (EPMA) and X-Ray Diffraction are use to analyze the material porperties and for phase characterization.

4. Main objectives of the PhD

- Decipher the interaction of organic components and hard tissues by chemical experiments and imaging the disposition of the carbonate crystals.
- Decipher processes and pathways of chemical alteration, as diagenesis, and the eviromental impact in the original trace elements and isotope composition as well as primary crystallographic structures.

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