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Electron microscopy characterization of king scallop (Pecten maximus) shells from low-voltage SEM to 3D-EBSD reconstruction

Dr Lise Guichaoua[1], Dr Benazir Khurshid[3], M Arnaud Benchetrite[3], Ms Stéphanie Bessette[1], Dr Bryce Stewart[2], Pr Natalie Reznikov[3], Pr Roland Kröger[4], Pr Raynald Gauvin[1]
[1]McGill Electron Microscopy Research Group, Department of Mining and Materials Engineering, McGill University , Montreal, Canada, [2]Marine Biological Association, Plymouth, United Kingdom, [3]Department of Bioengineering, Faculty of Engineering, McGill University, Montreal, Canada, [4]School of Physics, Engineering and Technology, University of York, York, United Kingdom

Background

The shell of the bivalve Pecten maximus, also called the king scallop, was previously found to be detrimentally affected by the presence of metal contamination, in particular Cu, Pb, and Zn originating from mining activities on the Isle of Man [1]. In addition to a reduction of shell thickness, scallop shells from the contaminated area exhibit a sharp break line in the mineralization within the foliated region of both the top and bottom valves. Our data suggest that these mineralization break line caused reduced fracture strength compared to pollution-free scallops, which results in increased mortality due to predation and during the process of dredging. These break lines have already been referred to as being of aragonitic prismatic structure [2].

To shed light on the possible impact of metal contaminations on the growth and strength of scallop shells and in particular on the 3D morphology and microstructure of the scallop, from contaminated and uncontaminated sites, we used characterization tools at different length scales (from cm to µm) using electron and X-ray probes to determine areas of interest in contaminated and healthy (uncontaminated) shells to finally realize crystallographic orientation map using EBSD technique and localized 3D reconstruction by focused ion beam techniques (FIB). The aim of this study was to combine these techniques to produce a full 3D EBSD map of the crystallographic orientations of the critical zones.

Methods

The scallop samples were cut using a diamond cut-off wheel (South Bay Technology Low Speed Diamond Wheel Saw MODEL 650) to extract a large cross-section of contaminated and healthy scallop to study the whole shell section. The cross-sectioned samples were then polished with lapping pads to a felt disk with a 50 nm colloidal silica suspension, to obtain a perfectly flat surface for future SEM observations and EBSD analysis.

We first applied 3D X-ray microscopy using micro-computed tomography (microCT) scanning with a ZEISS Xradia 520 Versa X-Ray microscope, at different resolutions to locate potential structural defects or the mineralization break line on contaminated shell and healthy shell. The same samples were then analyzed by scanning electron microscopy (SEM) coupled with elemental analysis by energy dispersive X-ray spectroscopy (EDS) to detect differences of microstructure or orientation on the shell cross section. The equipment used for this purpose is a Hitachi field emission gun (FEG)-SEM SU8230 with low voltage analysis (3KeV) by secondary and backscattered electrons (PD-BSE) imaging, Bruker XFlash EDS detector for elemental quantification point analysis, and Bruker Flat Quad detector for EDS mapping analysis. A 10 nm layer of carbon coating was necessary to be able to run long EDS mapping acquisition.

Electron Backscattered Diffraction analyses were conducted for both types of samples on a SU3500 SEM from Hitachi under VP-SEM mode at 20keV on main areas detected by the previous SEM analyses.

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The FIB tomography is performed using a Hitachi Ethos NX5000 FIB-SEM to obtain a stack of images and the Dragonfly software from Comet [3] to build the 3D volume samples from each type. In addition, the FIB-SEM NX5000 is equipped with a Symmetry S2 EBSD camera from Oxford Instruments that can allow us to obtain stacks of EBSD maps to build 3D volumes with crystallographic orientation information.

Results

MicroCT analyses carried out at different resolutions (from 30 µm to 2.5 nm pixel size) have shown that the break line visible in the contaminated shell is also present in healthy shells, but less marked and finer than that observed in the contaminated shell. These lines extend across the entire shell surface, although their point of initiation remains a mystery.
SEM analysis enabled us to observe the variation in microstructure across the entire cross-sectional area and to determine the zones of interest for the different microstructures observed in each type of shell. The zone we call the "break line" presents a prismatic microstructure as expected, different from the microstructure observed in the main parts of the shell, largely composed of elongated and foliated grains. In addition, a detailed analysis of the samples revealed the presence of a main prismatic layer, which was found to be thicker in the contaminated samples, and we were also able to observe several thinner prismatic layer in both types of sample.

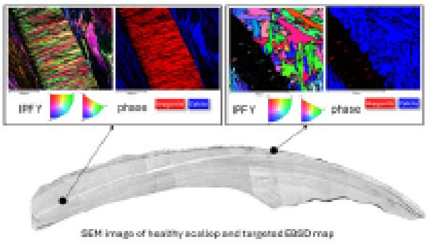
EBSD analyses, carried out on various zones of interest selected from SEM observation, confirmed that the foliated microstructure is calcite. We detected that the prismatic layer was indeed clearly well-crystallized aragonite for the disruptive line. EBSD maps obtained in regions containing thinner disruptive layers reveal that there is no detection of specific crystallographic orientation.

Conclusion

The combination of microscopy techniques using electron probes and X-rays at different length scales has enabled us to gain a better understanding of the structure of scallop shells and the impact of metal contamination from mining on the Isle of Man.
After a detailed study using microCT and low voltage SEM, targeted EBSD analyses revealed that the break lines present over the entire shell surface in all shells are prismatic in structure and are associated with the aragonitic myostracial layer, while the rest of the shell is predominantly composed of calcite with a foliated microstructure. This aragonitic layer, well crystallized in its thickest zone according to EBSD results, shows no detectable crystallization in its thinnest zone. A more in-depth study of these areas would help determine the cause of this lack of identification.

EBSD mapping acquired with the FIB-SEM NX5000 will enable us to obtain 3D volumes with crystallographic orientation information for healthy and contaminated shells, allowing us to compare growth and development of each type of scallop and detect defects in shell crystallization depending on their environment.

Graphic:



SEM image of healthy scallop and targeted EBSD map

Keywords:

Scallops, SEM-FEG, EBSD, 3D-FIB, 3D-EBSD

Reference:

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