Preparation of a High Quality Cross Section of a Bone Tissue for SEM --Application of the Cross-section Polisher to a Biological Specimen--

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1. Introduction

A bone tissue of a mouse tail, composed of hard and soft materials, was polished with the Cross-section Polisher (CP) for obtaining wide and smooth cross-section. The prepared specimen was observed with a SEM and analyzed with an EDS.

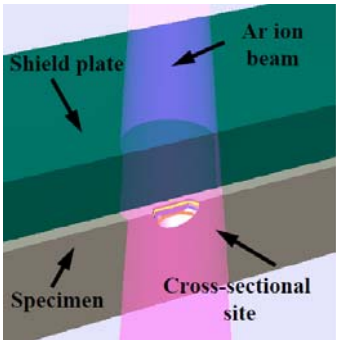
Cross-section Polisher (IB-09010CP)

2. Materials and Methods

(1) Specimen: mouse tail

Cross-section Polisher

It is a specimen preparation tool for cross-sectioning which uses an Ar ion beam. Usually a specimen is covered with a shielding plate and a broad Ar ion beam is irradiated on it. A cross section of the specimen is produced along to the edge of the shielding plate.



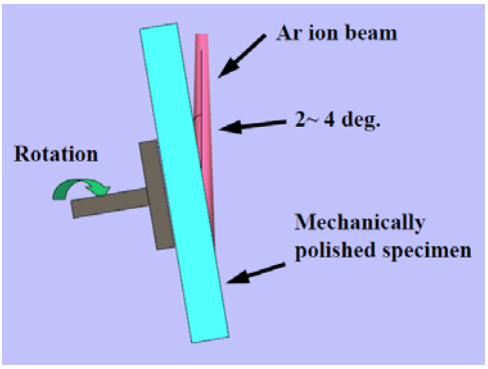
Principle of CP

Specimen preparation procedure

(1) Perfusion fixation: 2.5% glutaraldehyde / 0.1M PBS (pH 7.4)
(2) Washing: 0.1M PBS
(3) Post fixation: 2% osmium tetraoxide / 0.1M PBS (4℃/ 1.5h)
(4) Dehydration: ethanol
(5) Decantation: propyleneoxide
(6) Embedding: EPON812 (TAAB)
(7) Polishing: diamond lapping sheet (30 μm、10 μm、3 μm) → diamond paste (1 μm)
(8) CP polishing

CP polishing

Mechanically polished specimen was mounted on a rotation holder of CP.
An Ar ion beam was irradiated on the rotating specimen with grazing incidence of 2 to 4 degrees.
Accelerating voltage: 4 kV
Ion beam current: 150 μA
Processing time: 4h



Rotation holder for CP

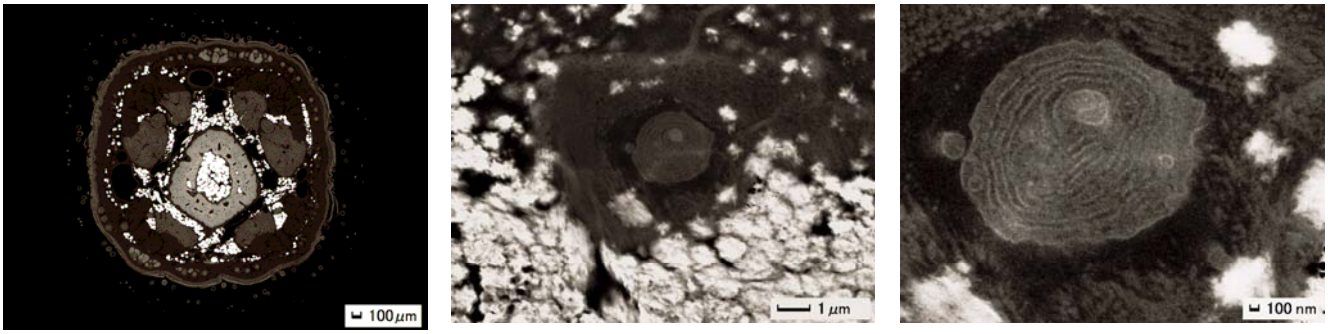
3. Result and Discussion

(1) SEM Observation

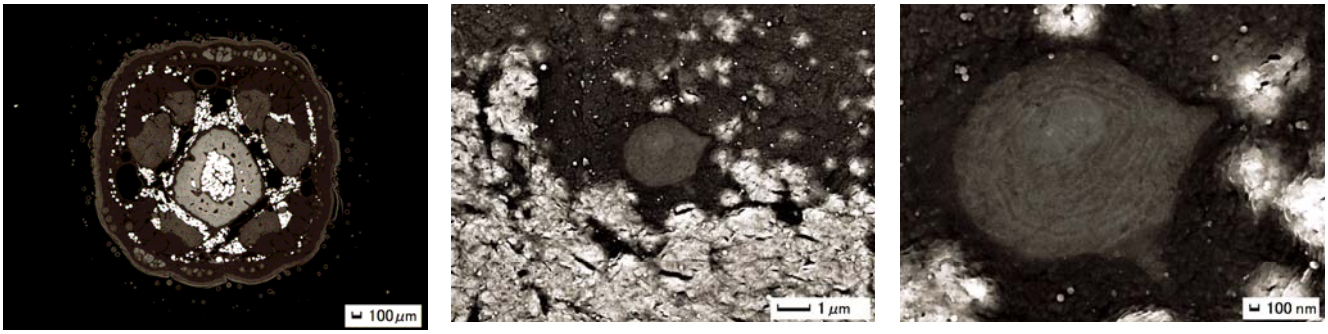
A CP-polished specimen and a mechanically polished specimen were observed at 10 kV in the backscattered electron mode with JSM-7001F Field Emission Scanning Electron Microscope.

At low magnification, there are no differences in both preparation methods. At high magnification, fine structures of a soft bone cell which seemed to be mitochondria, endoplasmic reticulum and collagen fibers are clearly observed in the CP-polished specimen. On the contrary, many small cracks are observed and fine structures are not observed clearly in the mechanically polished specimen.

CP polishing

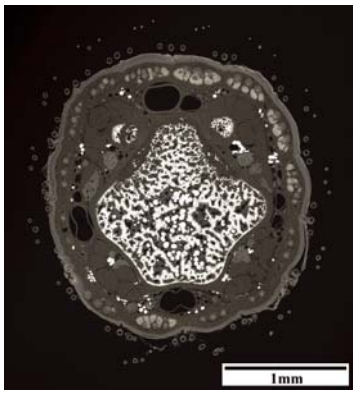


Mechanically polishing

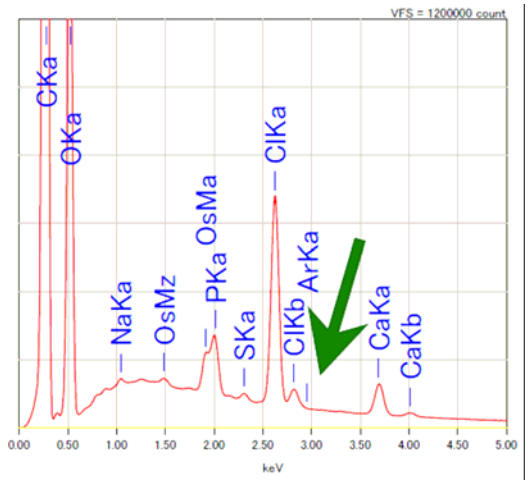


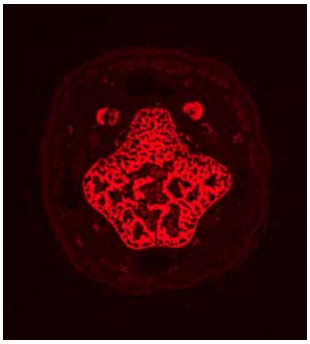
(2) EDS Mapping

A CP-polished specimen was analyzed at 15kV with JSM-6390LA Scanning Electron Microscope. The EDS spectrum was obtained from a whole field of view. Ca, P and S are detected but Ar is not detected (Arrow). It shows CP-polishing is excellent specimen preparation technique also for elemental analysis. X-ray maps show localization of Ca and P in a bone tissue and S in a skin and tail-hair. Ar map is formed with the background intensity at an energy of Ar and does not means localization of Ar.

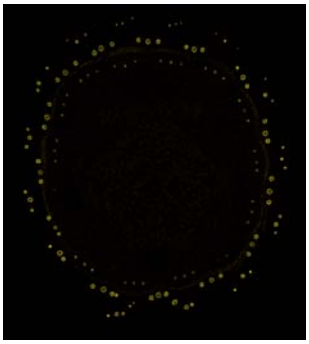


Backscattered Electron Image

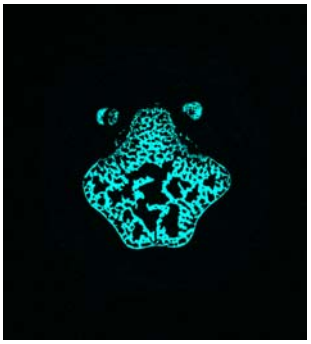




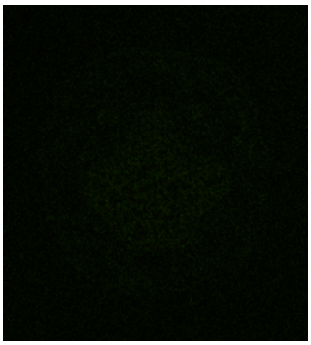
X-ray maps P K



S K



Ca K



Ar K

4. Conclusion

(1) Cross section of very wide area over 3 mm in diameter was polished.

(2) Very smooth cross-sectional surface was obtained in spite of composed material of hard and soft tissues.

(3) SEM observation ranging several ten to several ten thousand times was possible for one specimen. Fine structures of a cell were observed at high magnification.

(4) With EDS analysis, main elements from the tissue were detected but Ar from the CP polishing process was not detected.

5. References

[1] M. Shibata: JEOL News, [39] (2004) p.28

[2] M. Shibata. S. Asahina. T. Negishi. Proc. 8 APEM, (Kanazawa 2004) p.258