Selenium Distribution in Human Soft Tissue Determined by Using X-ray Scanning Analytical Microscope and X-ray Absorption Fine Structure Analysis

Motohiro Uo,*[1] Kiyotaka Asakura,[2] Takao Kohgo,[3] and Fumio Watari[1]
[1]*Department of Biomedica Materials and Engineering, Graduate School of Dental Medicine, Hokkaido University, North 13, West 7, Kita-ku, Sapporo 060-8586*
[2]*Catalyst Research Center, Hokkaido University, North 21, West 10, Kita-ku, Sapporo 001-0021*
[3]*Department of Oral Pathology, Graduate School of Dental Medicine, Hokkaido University, North 13, West 7, Kita-ku, Sapporo 060-8586*

(Received October 5, 2005; CL-051270; E-mail: uo@den.hokudai.ac.jp)

Se localilzation in the human oral mucosae which contain dental amalgam or dental silver alloy particles was analyzed by X-ray scanning analytical microscopy (XSAM). Se distribution was visualized, and the localization in the neighbor of amalgam or silver particles was confirmed. The chemical state of Se was analyzed by X-ray absorption fine structure (XAFS) analysis. Se was estimated as the low valency state. The possibility of using XSAM and XAFS for the analysis of the distribution and chemical state of rarely contained elements in biological tissue was suggested.

Selenium (Se) is an essential element in humans. It is important not only as a cofactor in enzymes but also in detoxification of heavy metals such as mercury. The relationship between the level of Se and heavy metals, especially mercury (Hg) and silver, had been studied.[1] A positive correlation between Se and Hg in blood was reported. As the detoxification mechanism, the formation of Hg–selenoprotein complex was suggested. However, the current study of the interaction of Se and heavy metals employed macroscopic analysis. The distribution of Se in the areas surrounding heavy metals in the human body has not yet been studied, because the concentration of Se in human body is very low, making it diffucult to measure the elemental distribution.

The authors employed the X-ray scanning analytical microscopy (XSAM) for the analysis of metallic elements in soft and hard tissues.[2] XSAM enables elemental distribution analysis from Na to U by energy-dispersive spectroscopy of fluorescent X-rays in air without pretreatment even if the sample contains water. This feature is desirable for the analysis of biological specimens. XSAM also has higher sensitivity for heavier elements than elemental distribution analysis with electron microscopic methods. The authors also employed the X-ray absorption fine structure (XAFS) analysis for human tissue containing metallic elements in low concentrations. XAFS analysis using synchrotron radiation makes it possible to analyze the state of eroded metal in the human body at low concentrations of around 1 to 100 ppm by using fluorescence XAFS.[3] In this study, XSAM and fluorescence XAFS were employed to determine the selenium distribution in the human oral mucosa containing metallic dental restoratives.

Two oral mucosa specimens excised from two different patients, which contained particle-like foreign bodies, were subjected to XSAM and XAFS analyses. These specimens were excised for pathological diagnosis for pigmentation. The specimens were fixed in 10% neutral buffered formalin and embedded in paraffin by the conventional method. The embedded tissue blocks were sliced. The residual blocks of tissue embedded in paraffin were subjected to elemental distribution analysis using XSAM (XGT-2000V, Horiba Co., Ltd., Kyoto, Japan). The XSAM observation was carried with the incident X-rays generated from an Rh anode under conditions of 50 kV and 1 mA. The incident X-ray was passed through the X-ray guide tube (XGT) 100 μm in diameter. During the XSAM observation, the specimen stage was scanned in the air. The mapping images were integrated 100 times (scan speed was 3000 seconds per scan).

The XAFS spectra were measured at BL-9A of the Photon Factory, Institute of Material Structure Science, High-Energy Accelerator Organization (KEK-PF). The electron storage ring was operated at 2.5 GeV with 300–500 mA. The synchrotron radiation was monochromatized with an Si(111) double-crystal monochromator. The incident X-ray was focused using two bent conical mirrors into 1 mm in diameter and the specified area of the specimen where Se was enriched was irradiated. The XAFS spectra of the Se K-edge were measured in a fluorescent mode using a multielement solid-state detector (Camberra). I_0 signals were monitored using an N_2 filled ionization chamber.

Figure 1 shows the transmission X-ray image and S, Hg, Ag, and Se distribution images of specimen A with XSAM. The S distribution image shows the shapes of specimens. In the transmission X-ray image, spotlike untransparent parts were found. In these untransparent spots, Hg and Ag were observed. Se was existed in the vicinity of the Hg and Ag localized spots. Usually, Se

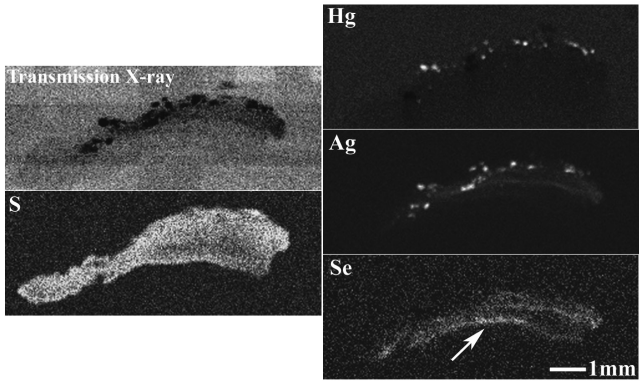

Figure 1. Elemental distribution images of dental amalgam-like particles in human oral mucosa (specimen A).

is not detected in the tissue by XSAM image because of its low concentration. Therefore, Se localization would be caused by the existence of Hg and Ag. Dental amalgam consists of Ag–Sn–Cu alloy powder and mercury. The foreign bodies in specimen A were considered to be dental amalgam. After trituration of the alloy powder and mercury, the mixture is applied in the oral cavity. In the initial stage of trituration, there are large amounts of unreacted mercury and alloy powder, and the mixture shows little fluidity. The mechanical strength of the dental amalgam in the initial state is low. After 24 to 48 h of trituration, most of the mercury is amalgamated. In Figure 1, Ag and Hg show different distributions. This suggests that the debris of dental amalgam formed in the filling or polishing process in the earlier stage was dispersed into the oral mucosa. Selenium was concentrated in the tissue surrounding the dispersed foreign bodies.

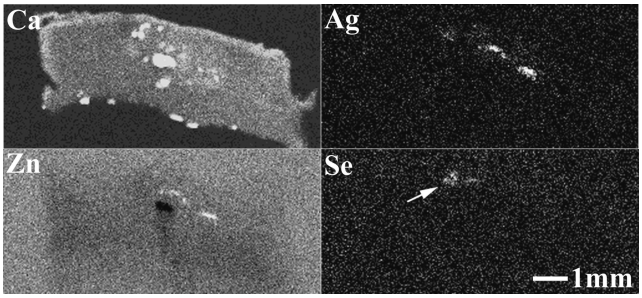

Figure 2. Elemental distribution images of dental silver alloy-like particles in human oral mucosa (specimen B).

Figure 2 shows the Ca, Zn, Ag, and Se distribution images of specimen B obtained by XSAM. Ca localization caused by the calcification was observed. Ag and Zn were localized in the same positions. This means the foreign body consists of homogeneous materials such as alloys. In the fluorescent X-ray spectrum from the particle indicated by the arrow in Figure 2, Ag, Zn, and Cu were detected. These are typical components of dental silver alloy. Se was localized in the vicinity of the silver alloy particles, but the Se concentration was lower than that of specimen A. In Figures 1 and 2, the Se was localized in the vicinity of Hg or Ag, but their distributions were not same. The reason of the difference in the distribution was assumed as follows. The XSAM images mainly show the distribution of alloy particles, which were encapsulated by granulation tissue. The Se would be reacted with the dissolved Hg or Ag outside of the granulation tissue, the Se distribution would be differ from those of Hg or Ag.

The parts indicated by arrows in the Se distribution images in Figures 1 and 2 were measured with the fluorescence XAFS method. The Se K-edge XANES spectra of the above specimens and those of Se compounds are shown in Figure 3. Yamamoto et al. reported that the absorption edge of Se shifts toward higher energy with the increase of valence of Se.[4] The absorption edges of the spectra of specimens A and B were slightly shifted to higher energy than that of metallic Se, and there is shoulder structure at 12.66 keV in both specimen A and B. This suggests that the Se in those specimens would be mostly in the low-valence state

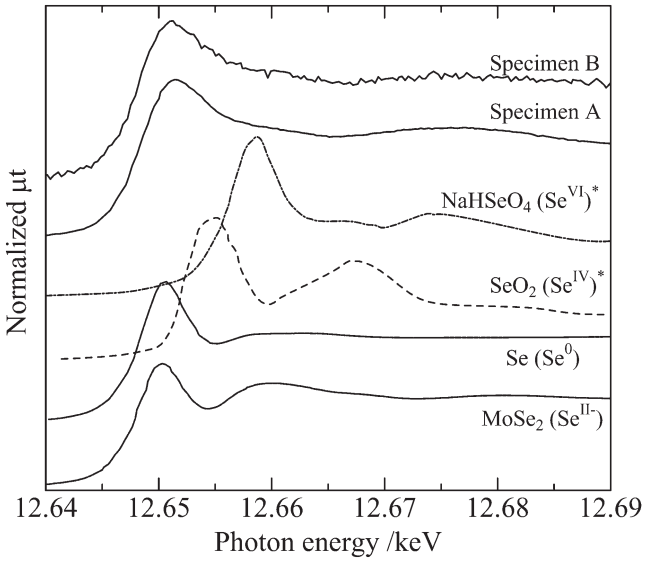

Figure 3. The Se K-edge XAFS spectra of oral mucosa specimens A and B and standard specimens (* after Yamamoto et al.[4]).

(nearly zero) and there would be some variety in the valency of Se.

Previously, the relationship between the Se and Hg concentrations in blood was reported.[1] The detoxification of Hg by selenoprotein was suggested as a possible reason of this phenomenon. In this study, the localization in the vicinity of dental amalgam which contained Hg and Ag and dental silver alloy was clearly visualized using XSAM and their chemical states were estimated using XAFS. Areas with high concentrations of Se were observed within 1 mm from the amalgam or silver alloy particles. Thus, the Se localization towards the Hg and Ag was revealed.

The XAFS measurements were done with the approval of the Photon Factory Advisory Committee (Proposal No. 2004G084). Part of this study was supported by Northern Advancement Center for Science and Technology, Japan.

References

1 M. Molin, B. Bergman, S. L. Marklund, A. Schütz, S. Skerfving, *Acta Odontol. Scand.* **1990**, *48*, 189; E. Johansson, *J. Trace Elem. Electrolytes Health Dis.* **1991**, *5*, 273; M. Kauppi, *Heavy Met. Bull.* **1995**, *2*, 16; G. Drasch, S. Mailänder, C. Schlosser, G. Roider, *J. Trace Elem. Med. Biol.* **2003**, *17*, 165.

2 M. Uo, F. Watari, A. Yokoyama, H. Matsuno, T. Kawasaki, *J. Biomed. Mater. Res. Part B: Appl. Biomater.* **2004**, *70B*, 146.

3 M. Uo, K. Asakura, A. Yokoyama, K. Tamura, Y. Totsuka, T. Akasaka, F. Watari, *Chem. Lett.* **2005**, *34*, 776.

4 T. Yamamoto, S. Itoh, Y. Tochihara, N. Noda, H. Akiho, M. Kobayashi, S. Noguchi, *Yokosuka Research Report*, **2004**, Vol. W03034, p. 1.

Published on the web (Advance View) December 10, 2005; DOI 10.1246/cl.2006.66