Distribution analyses of trace metallic elements in oral mucosal tissues using high-energy SR-XRF

Various noble and base metal alloys are used for dental restoration. Even dental alloys, which have high corrosion resistance, undergo slight dissolution of the alloy elements. The eroded metallic elements cause the acute, chronic, and allergic reactions. Therefore, the detection and analysis of the distribution and chemical state of trace metallic elements in the oral mucosa or surrounding tissues are necessary to reveal the relationship between the dissolved metallic elements and histopathological changes. However, because the concentration of such eroded metallic elements in the oral mucosa is quite low, a highly sensitive analytical method is required. In addition, biopsy specimens have limited availability and mainly are required for histopathological diagnosis. Therefore, analysis of the elements should be carried out nondestructively. The author's group have applied the synchrotron radiation X-ray fluorescence (SR-XRF) analysis to detect trace metallic elements in various biomedical specimens [1-5].

Silver-based alloys (Ag-Pd-Au-Cu alloy, Ag-In-Sn-Zn alloy) are widely used for metallic dental restoration in Japan. To detect the K lines of Pd, Ag, In, and Sn, high energy (>25 keV) incident X-rays are required. Beamline BL37XU in SPring-8 provides high energy X-rays with high flux, enabling the detection of trace alloy elements used in dental restoration. Oral mucosal tissue was fixed in formalin, embedded in paraffin and thinly sliced. The slice was mounted on polyimide film (Kapton®, 12.5 μm) and analyzed using SR-XRF at BL37XU. The incident X-rays (30 keV) were focused into 2×4μm² and the specimen was scanned in the X-Y direction to obtain two-dimensional elemental distribution images. The scanning steps were varied from 5 μm to 50 μm depending on the scanning area.

Figure 1 shows typical images of elemental distribution in the oral mucosa and histopathological images obtained with hematoxylin-eosin (H-E) staining. Accumulated spots of Zn, Cu, and Ag were observed inside the mucosa. Figure 2 shows the detailed distribution images of the white rectangular region in Fig. 1. Localization of Ag and Pd could be observed in narrow vicinal regions. The XRF spectrum at the localized spots of these elements (white arrow in Fig. 2) is shown in Fig. 3. Clear peaks due to Ag, Pd, Au, Zn, and Cu can be identified. Zn and Cu are essential trace elements in the human body, while Ag, Pd, and Au are not endogenous elements in the mucosa. This mucosa was derived from an oral lichenoid lesion (OLL). An OLL is one of the commonly observed oral mucosal diseases, and the eroded metallic elements from dental restoration are suspected to be one of the causal factors of OLL. Ag-Au-Pd-Cu alloy is most commonly used for dental metallic restoration in Japan. Therefore, the elements detected in the mucosa are suspected to derive from the dental metallic restoration. Including this case, the authors analyzed six OLL tissues using SR-XRF, and the accumulation of dental alloy elements was detected in all the tissues [3,4].

Fig. 1. H-E stained histopathological image and SR-XRF elemental distribution images of OLL specimen.
In current diagnosis of eroded metal related symptoms (e.g., metal allergy and OLL), causal metal species are predicted by the skin patch test using various metal salt solutions. However, patch tests are not reliable because of their false positive/negative reactions. The identification of causal metallic elements by SR-XRF analysis would provide more reliable information and could be used to prescribe the removal of the causative restoration; this is a simple and innovative treatment. In addition, the author’s group applied SR-XRF to the estimation of metallic ion penetration into skin and the development of a novel metal allergy patch test using metal nanoparticles [1]. Thus, SR-XRF analysis of biomedical specimens will provide useful information concerning the effects of trace metallic elements in various tissues.

Motohiro Uoa,b

a Advanced Biomaterials Department, Tokyo Medical and Dental University
b Department of Materials Engineering, The University of Tokyo

E-mail: uo.abm@tmd.ac.jp

References