Two New Methods for Evaluation of Subsurface Enamel Lesions

K. FUJITA¹, H. KAWAMATA¹, T. ISHIZAKI¹, R.E. HAYMAN¹, T. UCHIYAMA², M. KIMURA², H. KIBA², and T. IKEMI²
¹SANGI Co., Ltd, Saitama, Japan, ²Nihon University School of Dentistry at Matsudo, Chiba, Japan

Objective: In evaluating enamel remineralization, it is important to clarify the junction between sound enamel and the initial subsurface demineralized lesion. Contact microradiography, commonly used to evaluate remineralization, does not show this subsurface junction clearly. We applied a dye-permeation technique and an Argon-etching technique to cross-sectioned demineralized enamel, and examined the ability of these two methods to identify the subsurface lesion.

Method: Samples were prepared by immersing extracted human molars in a lactic acid buffer solution (3.0mM-Ca, 1.8mM-P, pH 4.5) to create a subsurface demineralized lesion. (1) Dye method: For stereomicroscopy, samples were dehydrated in ethanol, embedded in resin (Rigolac™), cross-sectioned perpendicular to the tooth axis using a diamond disk under a stream of water, and polished to a width of 90 mm. The samples were then dipped in acid red dye for one minute and rinsed in water before observation of the enamel surface. (2) Argon-etching method: For SEM observation, a sample sliced to a width of 300 mm was fine-polished using a Imperial™ Lapping Film (3M) under a stream of water, then dehydrated in ethanol and freeze-dried using t-butylalcohol. The surface of the sample was then etched by Argon beam and SEM observation of the enamel surface conducted.

Results: Both the dye-permeation and Argon-etching techniques allowed for clarification of the subsurface junction. This was due to the difference in permeation of the dye between the subsurface demineralized enamel and sound enamel and to removal of the smear layer by the Argon beam.

Conclusion: In contrast to CMR, both the dye-permeation and Argon-etching techniques appear useful in clearly identifying the subsurface junction.

Fig.1 Stereomicroscopy observation of the subsurface demineralized lesion (control) and remineralized phase. Fig.2 SEM observation of the subsurface lesion (control) and remineralized phase.