A comparative histologic study of the pulp–dentinal interface in undemineralized and demineralized tooth sections

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This study examines cellular differences between undemineralized and demineralized tooth sections. The material comprised unerupted and partly erupted third molars. After fixation for 24 h, 10- to 15-μm ground sections were prepared from half of the tooth, using a cutting–grinding system. The sections were compared with demineralized routine sections from the other half of the tooth. Undemineralized sections showed a better capacity for preserving the tissue integration. Especially between the odontoblast layer and the predentin a low frequency of disjunctions or tears were noted. At this level of examination fixation quality and vacuole frequency (intra-cellular spaces) did not show any differences between the two methods. □ *Dental pulp; dentin; odontoblast*

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Current understanding of the cellular reactions in the pulp–dentinal organ in relation to caries is mainly based on studies using demineralized sections (1–3). The demineralization procedure leads, however, to elimination of the enamel, whereas the classical method of producing undemineralized ground sections destroys the pulp. Thus, early cellular reactions in relation to enamel caries lesions have never been demonstrated in the same section (2). Combined information of the cellular and structural interrelation is therefore conventionally obtained by indirect methods (4, 5), which may lead to misinterpretations (6, 7).

A histologic technique, the cutting–grinding technique (8), was developed to overcome the problems associated with obtaining thin sections of the interface between soft and mineralized tissues and other hard materials. A slight modification of this technique was recently used for preparation of undemineralized tooth sections (9). The usefulness of the method for examining cellular responses to exogenic injuries is, however, strongly related to its capacity to reproduce histologic features with the same consistency as routine methods.

The present study was carried out to evaluate the fixation quality and preservation of the cells in the pulp–dentinal interface of undemineralized and demineralized tooth sections.

Materials and methods

The material comprised nine unerupted and six partly erupted third molars that had been surgically removed. To optimize pulp fixation, the teeth were either 1) longitudinally divided with a sharp chisel in the occlusal fossae, or 2) transversally divided by cutting a groove around the tooth below the cervical enamel with a water-cooled tungsten–carbide bur in a high-speed handpiece. A chisel was placed in this groove and tapped with a hammer, and the root

removed. The divided teeth were then placed in diluted Karnovsky (2% paraformaldehyde 1.25% glutaraldehyde, and 3% Macrodex), pH 7.2. After 24 h at 5°C the specimens were rinsed in phosphate buffer, pH 7.2. Each specimen was divided in two parts on a Mikro-trenn (Hofer, Aathal-Seegräben, Switzerland). One part underwent an undemineralization method and the second part a routine demineralization method.

Undemineralization method

The specimens were dehydrated with increasing alcohol concentrations, and plastic infiltration was done with hydroxyethylmethacrylate (Technovit 7200 VLC, Kulzer, Norderstedt, Germany). The infiltrated tissue was mounted in embedding molds and placed in a light-polymerization oven (Histolux, Kulzer). The polymerization was done by fluorescent light with 450-nm wavelength. Sections were prepared corresponding to the cutting plane made by the Mikro-trenn. A more detailed description of the method has recently been published (9).

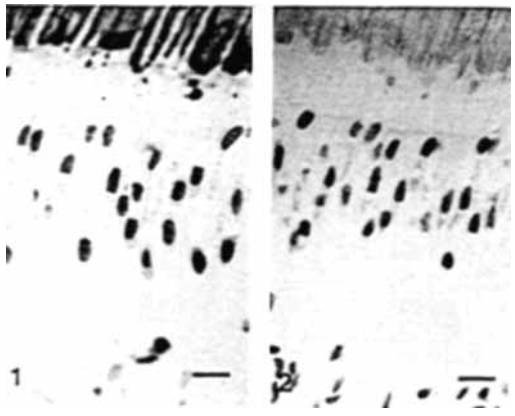
Demineralization method

The tissue blocks were demineralized for 3–4 weeks in 10% ethylenediaminetetraacetic acid (EDTA), dehydrated, and embedded in paraffin wax. Serial sections of 3–4 μm were prepared through the central part of the pulp corresponding to the cutting plane made by the Mikro-trenn.

The sections were stained with hematoxylin and eosin and toluidine blue. Within each tooth, comparisons were made between one routine demineralized section randomly selected from 10 serially cut sections and one undemineralized section. The two methods were evaluated with regard to four conditions:

1. Integration between the pulp tissue and the odontoblast layer: a) Optimal integration—that is, no disjunctions or tears; b) 50% or more of the pulp tissue was optimally integrated with the odontoblast layer; and c) less than 50% of the pulp tissue was optimally integrated with the odontoblast layer.

2. Integration between the odontoblast layer and the predentin: a) Optimal integration—that is, no disjunctions or tears; b) 50% or more of the odontoblast layer was optimally integrated with the predentin; c) less than 50% of the odontoblast layer was optimally integrated with the predentin.



Figs. 1 and 2. The odontoblast–predentin region from comparable localizations in the same tooth, photographed in transmitted light. Fig. 1. Undemineralized section (toluidine blue). Fig. 2. The region from a demineralized section (toluidine blue). The bars correspond to 10 μm.

3. Occurrence of vacuoles in odontoblasts: The number of stained odontoblasts and the occurrence of vacuoles (intra-cellular spaces) were scored using a light microscope at a magnification of ×1000 (Olympus, AHBS, Tokyo, Japan). The vacuole percentage was estimated in relation to the number of stained odontoblasts, taking thickness and stain penetration depth into consideration. Three anatomically comparable localizations with longitudinally cut tissue were examined in each section. Comparisons were made within each of the three localizations (A, B, and C). A was located in the coronal part, C in the cervical region, and B between A and C. The data were evaluated by means of the Wilcoxon signed ranks test (10). Photomicrographs were used for duplicate examination.

4. Quality of fixation: The fixation quality was evaluated by the distinctness of the cells and the occurrence of pycnotic cells.

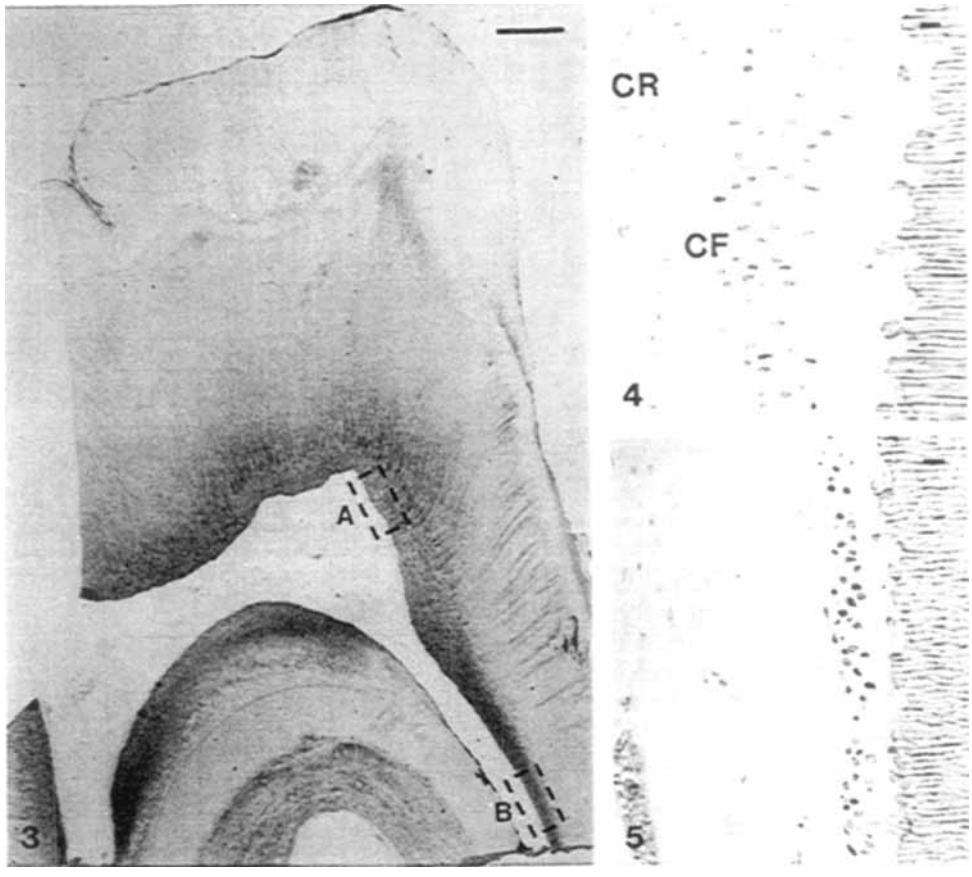


Fig. 3. Overview of an undemineralized tooth section. Framed areas A and B are shown in Figs. 4 and 5 (toluidine blue). The bar corresponds to 1000 μm.
Fig. 4. Detail of frame A in Fig. 3 demonstrates a pseudostratified, columnar appearance of the odontoblast layer. CR = cell-rich zone; CF = cell-free zone (toluidine blue). The bar corresponds to 10 μm.
Fig. 5. Detail of frame B in Fig. 3 shows a low-cuboidal odontoblast layer (toluidine blue). The bar corresponds to 10 μm.

Results

At this level of magnification there was no difference between the two methods with regard to fixation quality. The odontoblasts were well preserved (Figs. 1 and 2). The nuclei of the odontoblasts appeared distinct, with scattered chromatin structures. A few pycnotic cells were observed, however, and shrinkage of the cell bodies was evident as well. The intercellular spaces of the odontoblasts were noted but especially in transversally cut areas. In longitudinal aspects the spaces were difficult to define, and a more elaborated evaluation of the cell membranes and the cell-to-cell contacts was not carried out at this level of magnification.

In the coronal pulp part the odontoblast layer was pseudostratified and consisted of four to six rows. The individual odontoblast appeared columnar in the coronal part (Figs. 3 and 4), whereas it was more reduced and cuboidal in the apical region (Figs. 3 and 5). The width of the predentin zone was broadest in the coronal part (Fig. 4) and narrower in the apical region (Fig. 5). The cell-rich and cell-free zones of the pulp appeared less distinct in the apical region (Fig. 5), whereas the zones were well-defined in the coronal region (Fig. 4). Capillaries

Table 1. Classification of undemineralized and demineralized sections on the basis of tissue integration criteria along the odontoblast layer and the predentin

	Integration between pulp tissue and odontoblast layer				Integration between odontoblast layer and predentin			
	Optimal	≥50%	<50%	*n*	Optimal	≥50%	<50%	*n*
Histologic method								
Undemineralized section	8	6	1	15	13	2	—	15
Demineralized section	4	9	2	15	4	8	3	15

Table 2. Median values and ranges of vacuole percentages in the odontoblast layer in undemineralized and demineralized sections. A, B, and C represent counting localizations in the sections

	A*		B*		C*	
	Median	Range	Median	Range	Median	Range
Histologic method						
Undemineralized section	17	0–53	17	0–67	24	0–53
Demineralized section	26	14–71	19	6–36	35	7–59

* Wilcoxon signed ranks test: A, B, C, $p > 0.05$.

were frequently observed in the odontoblast layer and the cell-free zone, and occasionally accumulations of extravascular erythrocytes were seen as well.

The undemineralization method was more often than the demineralization method able to preserve an optimal tissue integration with no disjunctions or tears along the interface between the pulp tissue and the odontoblast layer, and between the odontoblast layer and the predentin. This was particularly evident with regard to the interface between the odontoblast layer and the predentin (Table 1). Table 2 shows the median values of the vacuole percentages in the odontoblasts in undemineralized and demineralized sections. In localizations A and C the frequency of vacuoles tended to be lowest in the undemineralized specimens, but the difference was not significant (A: $p = 0.11$; C: $p = 0.16$).

Discussion

The study showed that an undemineralization method recently described in relation to tooth sections (9) was comparable to a routine demineralization method in terms of fixation quality and cell preservation along the pulp–dentinal interface. The undemineralization method tended to maintain complete tissue integration, particularly between the odontoblast layer and the predentin, in contrast to the demineralization method, whereby only a small fraction of the hard tissue was preserved. For this reason loss of tissue integration in demineralized specimens is not considered a reliable variable for evaluation of exogenous injuries as previously believed (11).

In spite of the different preparation procedures, the two methods reproduced very similar histologic views of the pulp–dentinal organ. Our findings on the overall histologic features were therefore in agreement with previous studies (12–15). However, the undemineralization method apparently gave the impression of fewer cell rows in the odontoblast layer than previously reported (12, 15). The more correct picture provided

by the undemineralization method is presumably a result of staining technique as neutral-buffered histologic stains only affect the outermost surface (8).

It has previously been suggested that vacuole frequency varies as a result of different histologic preparation methods (1, 16). The similar vacuolization frequency observed by us irrespective of the different postfixation procedures suggested, however, that vacuole frequency was more likely related to the fixation technique. The occurrence of vacuoles was previously used as an indication of various exogenous injuries (17–22), but several studies have clearly demonstrated that vacuolization per se is an unreliable indicator of cellular responses to pathologic stimuli (1, 9, 15, 16). However, systematic assessment of vacuole frequency defined as both intra- and inter-cellular spaces, as used by Stenvik & Mjör (23), has proved to be a reliable method for evaluation of differences in experimental procedures involving the pulp–dentinal organ.

In summary, our study demonstrated that the undemineralization method, in spite of the more elaborated preparation technique, reproduced tissue and cell histology with a quality that was similar to previously used routine demineralization procedures. The improved integration between the soft and hard tissues obtained by the method therefore makes it possible to initiate studies on cellular reactions in the pulp–dentinal organ to initial stages of enamel caries.

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