ABSTRACT:

**Aim:** to compare micromorphological changes in primary and permanent dentin after etching with phosphoric acid (20% and 37.5%) for 7 and 15 sec. by SEM.

**Material and methods:** The study included 42 primary and permanent teeth, divided into 8 groups by etching time and acid concentration. Enamel and dentin were removed from the vestibular area and after the expiration of etching time samples were washed with water-air stream and dried with light airflow. From each sample 10 magnified images were made from central vestibular area. The cleaning effect was measured in percentage, as a ratio between the number of uncleaned tubules to the total tubules. Results were analyzed with One-way and MANOVA. Post hoc Multiple Comparisons test – SPSS 19 was applied.

**Results:** The proportion of uncleaned tubules in primary teeth was higher than that of permanent teeth at acid concentration of 20%. At a concentration of 37.5% this relationship is reversed. At 7 sec there was a bigger difference between the share of uncleaned tubules for primary and permanent teeth, while at 15 sec this difference virtually disappears. The difference in the proportion of uncleaned tubules between the two acid concentrations at 7 seconds etching is significantly greater compared to the same difference between the two acid concentrations by etching for 15 sec.

**Conclusion:** Effectively removed smear layer and no precipitate was observed in primary teeth even at 7 seconds etching with 37.5% acid.

**Key words:** etching time, primary dentin, permanent dentin, smear layer, SEM, peritubular dentin

The etching is a key moment in the preparation of the tooth for application of adhesive systems which are applied with the Total-etch approach [1]. Considering that, the etching of the dentin is of fundamental importance and at the same time is a problematic area for achieving sufficient bond strength [2, 3].

In dental practice enamel and dentin are both etched. The goal is to create a chemically clean surface and microretentions [3, 4]. Therefore, a micromechanical bond is created - via the formation of “resin tags” of the adhesive into the dentin tubules as well as a nano mechanical bond - via the penetration of the adhesive in the demineralized space between the collagen fibers of the intertubular dentin [3, 5].

At this stage, the data for a better and a long lasting bond with the dentin after the removal of the smear layer, which is achieved by total etching, prevails [1, 6, 7].

The adhesive dentine bond strength is a function of its morphology and the etching agents [5, 8]. The morphology of the dentin substrate can be changed as a consequence of age-related changes, the presence of carious and non-carious lesions, as well as the type of dentition – primary or permanent [4, 6, 7, 9-13]. The effect of the etching agent depends on the type and concentration of the acid, time and the manner of its application [14-18].

The comparison of the composition and the morphology of the dentin of the primary and permanent teeth shows some differences [8, 10, 16, 19-22]. In a study of the hardness of the coronal dentin’s central zone it was found that the dentin in permanent teeth is significantly harder than the one of the corresponding areas in the primary teeth [20, 22-26]. The dentin of permanent teeth is with higher mineralization [27], based on the fact that the hardness is directly related to the degree of mineralization [16, 20, 23]. The primary teeth dentin is characterized by a lower hardness, and hence it is with a lower degree of mineralization in comparison with the one in permanent teeth. A lower calcium and phosphorous concentration in the peritubular and intertubular dentin is found as well as lower micromechanical features [21, 23, 25, 26, 28, 29].

Furthermore, there is a difference in the tubular density and the size of the dentin tubules - characteristics which define the dentin permeability. These differences lead to a different amount of intertubular dentin, which is the largest and most significant component of the dentin in terms of bonding procedures [10, 21, 26, 30].

Studies on the hybride layer, performed on primary and permanent teeth, also indicate for differences in the thickness of this layer. The formed hybrid layer in primary teeth is much thicker than the one in permanent teeth when the same protocol of adhesive application is performed [21, 23, 31].

All of this gives grounds to suggest that the probable reason for these results are the different dentin reactions of deciduous teeth to acid used for etching before the application of the adhesive system [8, 22, 25, 28, 32, 33].

All established parameters to achieve adequate dentin...
MATERIAL AND METHODS

Selection and preparation of experimental samples: The study used intact extracted teeth from both dentitions. The primary teeth were collected from healthy children between the age of 7 and 9 after their parents signed informed consent for the use of the teeth in the experiment. The permanent teeth were also collected from healthy patients aged 55-65 years who also signed informed consent. The deciduous teeth were extracted due to physiological exfoliation or because of orthodontic treatment and the permanent - due to periodontal problems. After extraction, the teeth were placed in 10% formalin solution for 10 minutes, then until the time of execution of the task were stored in saline.

Grouping of the experimental samples. The study included 42 intact teeth (primary and permanent incisors and canines). The teeth were divided randomly into 8 groups of 5 teeth in each group (only primary and only permanent), depending on the etching duration and phosphoric acid concentration (table 1).

<table>
<thead>
<tr>
<th>Group/tooth type</th>
<th>Etching</th>
<th>20% phosphoric acid</th>
<th>37,5% phosphoric acid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>7 sec</td>
<td>15 sec</td>
<td>7 sec</td>
</tr>
<tr>
<td>Group/primary teeth</td>
<td>Group 1</td>
<td>n= 5</td>
<td>Group 3</td>
</tr>
<tr>
<td></td>
<td>Group 2</td>
<td>n=5</td>
<td>Group 4</td>
</tr>
<tr>
<td></td>
<td>Group 6</td>
<td>n= 5</td>
<td>Group 8</td>
</tr>
</tbody>
</table>

n = number of samples

Preparation the tooth surface. With a turbine round bur (ISO 806 314 001 534 012 for primary teeth and ISO 806 314 001 534 014 for permanent teeth) and water-air cooling a cut in medio-distal direction along the vestibular surface is made. The purpose was pre-marking the depth of removal of enamel and dentine. With a turbine fissure diamond bur (ISO 806204108524835010) and the water-air cooling the enamel and dentine parallel to the long axis of the tooth, at the depth of the round bur marking, are removed. Diamond burs were changed after every three teeth. The surface after drilling was polished with an abrasive disk (ISO 625900372523) each used only for one tooth.

All prepared samples were observed with an optical microscope OLYMPUS VANOX-T under magnification of 25x to 100x, to establish whether the enamel was completely removed from the vestibular surface.

Dentin etching. The etching agents 20% (Pekaetch 20, Heraeus Kulzer GmbH) and 37,5% (Esticid - Gel, Heraeus Kulzer GmbH) phosphoric acid were applied for 7 or 15 sec.

After the etching time expired each sample was washed with water-air stream for 15 sec and dried with gentle scattered airflow at a distance of 20-25 cm for 5 sec. The prepared samples were left at room temperature for 24 hours in separate sterile petri dishes for each group to avoid contamination before the SEM observation.

Control samples. One tooth from each dentition was not etched after the removal of enamel and dentin from the vestibular surface in order to be used as a control sample.

Development of SEM images. Ten SEM images were taken of each sample at magnifications of 1,500 from a zone with dimensions of 114µm \ 35.2µm in the central part of the vestibular surface of the researched object. The analysis of all 420 SEM images served for evaluation of the etching effect for each sample.

The criteria for assessing the cleaning effect of the acids are:
- Degree of removal of the smear layer by comparing the number of dentin tubules’ orifices without plugs and those which are partially or completely obstructed from smear layer on each image;
- Presence of a smear layer within the intertubular dentin - presence of precipitates and deposits on the surface of the intertubular dentin.

The cleaning effect is measured in percentages – as a ratio between the number of uncleaned (fully or partially obstructed by a smear layer) tubules and the whole amount of tubules in each image.

For a statistical measuring of the results single-variate (One-way ANOVA) and multivariate (MANOVA) dispersion analysis for comparison of quantifiable indicators in more than two groups and assessment of the combined impact of several factors were used. PostHocMultipleComparison test after establishing a statistically significant difference between the groups for analyzing the differences in pairs - packet SPSS 19 was also used.
RESULTS

Control samples
Dentin control samples without etching indicated a smear layer in both types of teeth – primary and permanent (figure 1).

![Fig. 1. Representative SEM images of a smear layer on the dentin surface of a primary (A) and a permanent (B) tooth.](image1)

The non-etched dentin surface was covered with a smear layer with identical characteristics in both types of samples. The smear layer could be seen as a veil that covers the treated dentin surface. The inherent microcanal structure of the dentin cannot be seen. In some places the smear layer was cracked. Visible cracks, which probably correspond to the entrances of the dentin tubules, could be observed. The surface of the smear layer was scattered with particles with irregular shapes and different sizes which were visibly not well attached to it.

When 20% phosphoric acid was applied the following results were observed:

- **Groups 1 and 2 - 20% phosphoric acid for 7 seconds**
  The etching of the dentin surface in the two groups - primary and permanent teeth, breaks, but does not completely remove the smear layer (figure 2).

![Fig. 2. Representative SEM images of dentin surface of a primary (A) and permanent (B) tooth etched with 20% phosphoric acid for 7 seconds. A preserved smear layer on the intertubular dentin can be observed.](image2)

The smear layer was removed primarily over the dentin tubules and preserved over the intertubular dentin (pins, fig. 2A and B). In some places some of the dentin tubules orifices were not exposed. That’s why the number of opened dentin tubules was smaller than their real quantity. Many of them remain obscured from the smear layer (pointer fig. 2A and B).

- **Groups 3 and 4 - 20% phosphoric acid for 15 seconds**
  The results in group 3 primary and group 4 permanent teeth with increased etching time of 15 sec are similar to the previous ones. Some of dentin tubules remain by par-
Dentin tubules in Group 3 which were obscured with smear plugs were rarely observed when comparing the results between Group 1 and Group 3a (figure 2 and figure 3), but remnants of the smear layer and precipitates on the intertubular dentin were preserved. The results are similar in the groups of permanent teeth - 2 and 4. Uncleaned dentin tubules in group 4 were more rarely observed, but still remnants of smear layer on the intertubular dentin in both groups (fig. 2B and fig. 3B) could be seen.

The following results when applying 37,5% phosphoric acid were observed:

Groups 5 and 6 – 37,5% phosphoric acid for 7 seconds

A fully removed smear layer of the intertubular dentin and of the dentin tubules’ orifices can be observed in the tested primary teeth from group 5 (fig. 4A). In the permanent teeth from Group 6 precipitates and residues of the smear layer on the intertubular dentin surface can still be observed (pins, fig. 4B). There are also remnants of plugs in the orifices of the dentin tubules (fig. 4B pointer).

Groups 7 and 8 – 37,5% phosphoric acid for 15 seconds

In the group of permanent teeth (Group 8), we observe a clean of a smear layer and plugs dentin surface as well as a lack of precipitates after 15 seconds of etching with 37,5% phosphoric acid (fig. 5B).
In primary teeth (group 7) there is no difference in the quality of cleaning of the dentin surface as compared to the samples from group 5 (fig. 4A). It is again observed that the smear layer is completely removed from the intertubular dentine and the dentin tubules orifices are opened. It is noteworthy that the result of cleaning of the primary teeth which were etched with 37.5% acid for 7 seconds is the same as the one with the extended time for etching (fig. 5).

**Statistical analysis**

Table 2 presents the total number of tubules and the uncleaned ones of all samples from the different groups. Statistical analysis was made for the evaluation of the cleaning effect of the two acids concentrations in both dentitions.

**Table 2.** Number of present uncleaned tubules after etching with a different percentage of phosphoric acid.

<table>
<thead>
<tr>
<th>Time</th>
<th>Tooth type</th>
<th>Number of samples</th>
<th>Number of SEM images</th>
<th>20% phosphoric acid</th>
<th>37.5% phosphoric acid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Total number of tubules</td>
<td>Number of uncleaned tubules</td>
</tr>
<tr>
<td>7 sec.</td>
<td>Primary tooth</td>
<td>n = 5</td>
<td>50</td>
<td>8699</td>
<td>905</td>
</tr>
<tr>
<td>7 sec.</td>
<td>Permanent tooth</td>
<td>n = 5</td>
<td>50</td>
<td>10095</td>
<td>884</td>
</tr>
<tr>
<td>15 sec.</td>
<td>Primary tooth</td>
<td>n = 5</td>
<td>50</td>
<td>8894</td>
<td>131</td>
</tr>
<tr>
<td>15 sec.</td>
<td>Permanent tooth</td>
<td>n = 5</td>
<td>50</td>
<td>10194</td>
<td>154</td>
</tr>
</tbody>
</table>

The cleaning effect is measured in percentage as the ratio between the number of uncleaned (fully or partially obscured of smear layer) tubules to the total number of tubules on the respective SEM images.

In all groups tested for etching with phosphoric acid 20%, a statistically significant difference (p < 0.0001) was detected. When etched for 7 sec (group 1) the share of uncleaned dentine tubules is 10.43%. By increasing the etching time to 15 sec (group 3) the share of uncleaned tubules is 1.48%. The smaller value of the share of uncleaned tubules means higher cleaning effect (table 3). In the group of permanent teeth that were etched for 7 sec the share of uncleaned dentin tubules from all SEM images from group 2 is 8.76%. When etched for 15 sec the share of uncleaned tubules in group 4 is 1.51%. Better cleaning effect is achieved when the etching duration was 15 sec (table 3).
The analysis of the influence of the type of tooth shows statistically significant difference in the proportion of uncleaned tubules between primary and permanent teeth, but only when etched for 7 sec (p <0.001). Etching for 15 sec shows no statistically significant difference (p> 0.05) (table 4).

A statistically significant difference was found in evaluating the combined impact of these two factors – etching time and type of teeth (permanent or primary) when 20% acid was used (table 5) (p <0.001). For this purpose a multivariate analysis (MANOVA) was used.

The statistical processing of the data on the effect of cleaning of the 37.5% phosphoric acid for different times of exposure - 7 and 15 sec in the group of primary teeth (groups 5 and 7) showed no statistically significant difference (p> 0.05) (table 6). The increased etching time did not lead to better cleaning effect in the group of primary teeth - the results showed that 100% of the dentin tubules are cleaned with a 7 sec etching (table 6). The statistical analysis of the results under the same conditions for the group of permanent teeth (groups 6 and 8) showed a statistically significant difference in the effect of cleaning for 7 sec and 15 sec (p <0.0001) (table 6).
The results of the influence of the tooth type factor show a statistically significant difference (p = 0.000). In permanent teeth a weaker cleaning effect was observed. The effect of the etching time was also statistically significant. When etching for 7 sec was applied a weaker cleaning effect in the permanent teeth is observed. The co-influence of the two factors – etching time and tooth type was also assessed as statistically significant – the differences in the cleaning effect between primary and permanent teeth during etching for 7 sec had different levels compared to these which were etched for 15 sec. The results show a significant difference in cleaning effect on the teeth types that were etched for 7 sec and show insignificant difference when etching for 15 sec was performed (table 7).

A multivariate analysis (MANOVA) was also performed in order to assess the combined impact of all the above mentioned factors - tooth type, etching time, etching agent concentration (table 8). Therefore a model is applied including all factors and studying their impact on the share of uncleaned tubules.

### Table 6. Uncleaned tubules in samples of primary and permanent teeth after etching with 37,5% phosphoric acid.

<table>
<thead>
<tr>
<th>Group</th>
<th>Time for etching</th>
<th>Number of SEM images</th>
<th>Uncleaned tubules (%) mean±SE</th>
<th>T</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 5 – 7 sec</td>
<td>50</td>
<td>0.04 ± 0.02</td>
<td>Mann Whitney</td>
<td>22.2</td>
<td>&lt; 0.0001*</td>
</tr>
<tr>
<td>Group 7 – 15 sec</td>
<td>50</td>
<td>0.00 ± 0.00</td>
<td>U test</td>
<td></td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Group 6 – 7 sec</td>
<td>50</td>
<td>3.22 ± 0.14</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 8 – 15 sec</td>
<td>50</td>
<td>0.00 ± 0.00</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*The difference is statistically significant

When a 7 sec etching is applied, the share of uncleaned dentin tubules of all SEM images of Group 6 is 3.22%. When etching for 15 sec is applied the share of uncleaned tubules in group 8 is 0%. Better cleaning effect in the groups of permanent teeth was found at the 15 sec etching time.

### Table 7. Co-influence of the factors etching time and tooth type when etching with 37,5% phosphoric acid is applied in terms of the relative share of uncleaned tubules (results of a two-factor analysis).

<table>
<thead>
<tr>
<th>Type of tooth</th>
<th>Etching time</th>
<th>Uncleaned tubules (%) mean±SE</th>
<th>Numbers of SEM images</th>
<th>Factor</th>
<th>F</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary teeth</td>
<td>7 sec</td>
<td>0.04</td>
<td>50</td>
<td>Tooth type</td>
<td>468.94</td>
<td>= 0.000</td>
</tr>
<tr>
<td></td>
<td>15 sec</td>
<td>0.00</td>
<td>50</td>
<td>Etching time</td>
<td>495.85</td>
<td>= 0.000</td>
</tr>
<tr>
<td>Permanent teeth</td>
<td>7 sec</td>
<td>3.22</td>
<td>50</td>
<td>Tooth type</td>
<td>468.94</td>
<td>= 0.000</td>
</tr>
<tr>
<td></td>
<td>15 sec</td>
<td>0.00</td>
<td>50</td>
<td>*Etching time</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Empirical level of statistical significance (MANOVA)

The results of the influence of the tooth type factor show a statistically significant difference (p = 0.000). In permanent teeth a weaker cleaning effect was observed. The effect of the etching time was also statistically significant. When etching for 7 sec was applied a weaker cleaning effect in the permanent teeth is observed. The co-influence of the two factors – etching time and tooth type was also assessed as statistically significant – the differences in the cleaning effect between primary and permanent teeth during etching for 7 sec had different levels compared to these which were etched for 15 sec. The results show a significant difference in cleaning effect on the teeth types that were etched for 7 sec and show insignificant difference when etching for 15 sec was performed (table 7).
Table 8. Co-influence of the factors etching time, tooth type and acid concentration in reference to the relative share of uncleaned tubules (results of MANOVA).

<table>
<thead>
<tr>
<th>Type of tooth</th>
<th>Concentration</th>
<th>Etching time</th>
<th>Uncleaned tubules (%)</th>
<th>Number of SEM images</th>
<th>Factor</th>
<th>F</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary teeth</td>
<td>20%</td>
<td>7 sec.</td>
<td>10.43</td>
<td>50</td>
<td>Type of tooth</td>
<td>31.42</td>
<td>≤ 0.000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15 sec.</td>
<td>1.48</td>
<td>50</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Average value for 20% concentration</td>
<td></td>
<td>5.95</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>37.5%</td>
<td>7 sec.</td>
<td>0.04</td>
<td>50</td>
<td>Etching time</td>
<td>4972.10</td>
<td>≤ 0.000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15 sec.</td>
<td>0.00</td>
<td>50</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Average value for 37.5% concentration</td>
<td></td>
<td>0.02</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total for 20% and 37.5% concentration</td>
<td></td>
<td>5.24</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Average value for primary teeth</td>
<td></td>
<td>2.99</td>
<td>200</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Permanent Teeth</td>
<td>20%</td>
<td>7 sec.</td>
<td>8.76</td>
<td>50</td>
<td>Tooth type - Concentration</td>
<td>311.89</td>
<td>≤ 0.000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15 sec.</td>
<td>1.51</td>
<td>50</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Average value for 20% concentration</td>
<td></td>
<td>5.14</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>37.5%</td>
<td>7 sec.</td>
<td>3.22</td>
<td>50</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>15 sec.</td>
<td>0.00</td>
<td>50</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Average value for 37.5% concentration</td>
<td></td>
<td>1.61</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total for 20% and 37.5% concentration</td>
<td></td>
<td>5.99</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Average value for permanent teeth</td>
<td></td>
<td>3.37</td>
<td>200</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>20%</td>
<td>7 sec.</td>
<td>9.59</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>15 sec.</td>
<td>1.49</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Average value for 20% concentration</td>
<td></td>
<td>5.54</td>
<td>200</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>37.5%</td>
<td>7 sec.</td>
<td>1.63</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>15 sec.</td>
<td>0.00</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Average value for 37.5% concentration</td>
<td></td>
<td>0.82</td>
<td>200</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total for 20% and 37.5% concentration</td>
<td></td>
<td>5.61</td>
<td>200</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Average value for primary teeth + perm teeth.</td>
<td></td>
<td>3.18</td>
<td>400</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Empirical level of statistical significance (MANOVA)
The following statistically significant effects were obtained (p <0.0001, table 8):

1. One-dimensional effect:
   a. Type of tooth – the share of uncleaned tubules is statistically significantly higher in permanent teeth - 3.37% compared to 2.99% for primary teeth;
   b. Concentration – the share of uncleaned tubules is statistically significantly higher at 20% vs. 37.5% - respectively 5.54% compared to 0.82%;
   c. Etching - the share of uncleaned tubules is statistically significantly higher at 7 sec than 15 sec - respectively 5.61% compared to 0.75%.

2. Two-dimensional effects – co-influence of two factors
   a. Type of tooth and concentration - the share of uncleaned tubules in primary teeth is higher than the one in permanent teeth at a concentration of 20% (5.95% against 5.14%) and at a concentration of 37.5% this relation is reversed (0.02% for the primary against 1.61% for permanent teeth);
   b. Type of tooth and etching - in the 7 sec etching a more notable difference between the share of uncleaned tubules for primary teeth and permanent teeth is observed (5.24% vs. 5.99%), whereas for the 15 sec etching this difference virtually disappears (0.74% vs. 0.76%);
   c. Concentration and etching - the difference in the proportion of uncleaned tubules between the two concentrations with etching for 7 sec is significantly greater (9.59% with 20% acid as compared to 1.63% with the 37.5% acid) compared to the same difference between the two concentrations and etching for 15 sec (1.49% for 20% acid compared to 0.00% for 37.5% acid);

3. Three-dimensional effect – the co-influence of the three factors - it manifests itself by the absence of a statistically significant difference between etching 7 sec and 15 sec for primary teeth as cases of uncleaned tubules at 37.5% concentration (table 6) and the existence of such in permanent teeth (table 6). The corresponding difference at 20% concentration were confirmed as statistically significant (table 3).

The cleaning effect is increased with increasing of the concentration and the etching time as the manifestation is different depending on the type of tooth - primary or permanent.

DISCUSSION

Our study aimed to trace the micromorphological features of the etched dentine as a substrate for adhesion. There were significant differences in the effect of etching agents on the dentin in the process of removing the smear layer. They showed that the application of phosphoric acid of varying concentrations and for different time causes the receiving of dentine substrate which is different for the teeth from the two dentitions.

After any cutting of the tooth surface a smear layer is formed. This layer contains the basic components of the enamel, inter- and peritubular dentin, including dentin tubules’ content, mixed with water, microorganisms, toxins, enzymes, saliva. This layer may vary in composition, thickness, density and degree of binding to the underlying tooth structure, depending on the location of the preparation [37-43]. When a preparation of the dentin surface is performed, the dentin tubules are cut and therefore obscured by small plugs of this layer, which will lead to reduction of dentin permeability [44-46]. The smear layer can’t be removed by washing with water, but it is subject to dissolution in a total etching [3, 15].

The results of our study show that the reactivity of the dentin of the primary teeth against an acid impact is different from the one of the permanent teeth (Table. 3, 6, 8), which is in confirmation of the results obtained by other researchers [15, 16, 26, 31, 47]. This rejected our working assumption that there are no differences in the dentin in the teeth of both dentitions.

The removal of the smear layer is related to the acid concentration used and the time of contact with the dentin surface [14, 15, 18, 21, 26, 48]. It is what our results reflected in the tables 3 to 8 showed.

The conclusion that can be drawn on the basis of our study is that the 20% phosphoric acid applied for two different etching times did not create dentin surface cleaned from a smear layer. Better cleaning effect is found in samples from permanent teeth (table 4). In primary teeth (fig. 2A) this smear layer is slightly affected. This is probably due to the formation of a thicker smear layer which may be associated with the presence of a larger amount of organic matter in the primary teeth, which is more resistant to the acid etching. The presence of residues from the smear layer and precipitates on the dentin surface probably will lead to unsatisfactory adhesion of the interface between the dentin and the hybrid layer which is a mediator in adhesion. Obscuring of dentin tubules with smear plugs will not allow the entry of a bonding agent and the formation of adhesive tags that are also contributing to the bond strength. Overall, this can be a reason for an adhesion failure. Therefore, the existing smear layer will hinder the primer and the bond from the adhesive system to carry out their purpose.

The 37.5% phosphoric acid that is used for dentin surface etching removes the smear layer in primary teeth much faster than in permanent teeth (p <0.0001, table 6 and 7) [10, 16, 26, 31]. We found that the application of 37.5% phosphoric acid for 7 seconds on the primary teeth and for 15 seconds on the permanent leads to a complete removal of the smear layer, both from the orifices of the dentin tubules (table 6) and the areas of intertubular dentine in both groups of samples (figure 4A and fig. 5B). The results from the multi-factorial analysis for assessing the simultaneous effect of the etching time and acid concentration show that the cleaning effect rises with the increase of the concentration and the etching time as the manifestation varies depending on the type of tooth - primary or permanent (table 8). This led to the rejection of the second part of our working hypothesis - that the etching time and acid concentration has no influence on the degree of removal of the smear layer.

The different dentin reactivity from the two dentitions can be due to two reasons. We assume that the reasons for this could be the more pronounced buffering capacity of the dentin of permanent teeth and the consequent
possibility of self-limitation of the action of acids. Another possible reason is the difference in the number of dentin tubules [26]. According to the authors who defend the views about the smaller tubular concentration and the smaller diameter of dentin tubules – the dentin of the primary teeth exhibits also a lower permeability [10, 26]. The forthcoming biomorphosis of the pulp of deciduous teeth determines its reduced turgor, which can also have an impact. This leads to the assumption that the dentin of the primary teeth has a low surface moisture content, which leads to a change in the agent’s etching effectiveness in the removal of the smear layer as they exhibit a more aggressive action on the dentin [18, 26].

The etching of the dentin surface even for a short time can lead to significant changes in the dentin structure. It must be efficient, not extensive [1, 5]. In extensive demineralization of the intertubular dentin the collagen fibers will collapse and the calcium phosphate crystals will precipitate and therefore a protective layer that may not be completely impregnated by the adhesive primer and monomer could be formed [5, 31]. The rate of adhesives infiltration in the demineralized dentin gradually reduces in the direction towards the base of the hybrid layer [5, 6, 49]. In this case the removed mineral matrix is not completely replaced by the primer as a more unstable area at the base of the hybrid layer is left, which becomes a potential route for micro- and nanoleakage, hydrolytic and enzymatic degradation and as a whole - a place of bonding failure [50, 51]. The dentin etching is of fundamental importance to the adhesion effectiveness [16, 21, 26]. The increased reactivity of the dentin of the primary teeth against etchants is the reason that some authors recommend the etching time for them to be cut in half, as compared to that of the permanent [11, 31, 52, 53] in order to avoid the possibility of a deeper demineralization and subsequent incomplete infiltration of the primer/adhesive to the collagen fibers, which will compromise the effectiveness of the adhesion [18, 25, 54, 55].

The results of the survey show that reducing the etching time for the primary teeth dentin to 7 sec instead of 15 sec, recommended by manufacturers for the impact to permanent teeth, leads to the creation of dentin substrate which is similar to that of permanent teeth in the case of using 37.5% phosphoric acid. This would reduce the possibility of unnecessary increase of demineralization, damage to the collagen fibers, concerns about their future impregnation with the adhesive system and therefore concerns about the bond strength with consequences such as micro- and nanoleakage and restoration failure.

The foregoing, together with the results of our study, gives a reason to assume that using the same clinical adhesive application protocol in primary and permanent teeth is probably the main reason for the primary teeth to show lower adhesive bond strength at laboratory tests and reduced durability of the aesthetic restorations in clinical conditions.

CONCLUSIONS:
When 20% phosphoric acid is applied a dentin substrate free of smear layer is not created for the etching time of 7 and 15 sec in both dentitions. Efficiently removed smear layer and no precipitates were observed in primary teeth after 7 seconds etching with 37.5% phosphoric acid. The time of application of the etching agent and its concentration determine the removal of the smear layer.

Clinical relevance:
The creation of dentin substrate in primary teeth with morphological characteristics similar to that of permanent teeth, can be achieved by reducing the etching time to 7 sec instead of 15 sec recommended by manufacturers and applied for permanent teeth when using the 37.5% phosphoric acid as etching agent.

REFERENCES:


Please cite this article as: Gateva N, Gusyiska A, Stanimirov P, Kabaktschieva R, Raichev I. Effect of etching time and acid concentration on micromorphological changes in dentin of both dentitions. *J of IMAB. 2016 Apr-Jun;22(2):1099-1110. DOI: http://dx.doi.org/10.5272/jimab.2016222.1099*

Received: 08/01/2016; Published online: 08/04/2016

Corresponding Author:
Assoc. prof. Natalia Gateva
Department of Pediatric Dentistry, Faculty of Dentistry, Medical University - Sofia, 1, St. George Sofiiski str., Sofia, Bulgaria
e-mail: nataliagateva@yahoo.de