Changes in Mechanical and Microstructural Characteristics of Demineralized Tooth Enamel after Conventional and Modified Infiltration Techniques

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Abstract. The research carried out a comparative assessment of the physical and mechanical properties of tooth enamel: healthy; demineralized; demineralized and subsequently infiltrated with a flowable composite according to a standard or modified technique. An original in vitro model of artificial caries of human tooth enamel was used for the study, the clinical-topographic, color-textural and physical-mechanical properties of which correspond to the characteristics of enamel caries in vivo. Comparative analysis of the results of kinetic microindentation of enamel samples allows to characterize the biomaterial from the standpoint of physical materials science, to determine the advantages and disadvantages of different regimens of resin infiltration. The advantages of the modified infiltration technique are illustrated by significantly greater, as compare with classic method, increase in microhardness and elasticity against background of a decrease in a creep index of the infiltrated enamel in its deep zones. The results reflect the fact of incomplete obturation of microporous in the deep layers of enamel after classical treatment.

1. Introduction

In recent years, the early detection, prevention and treatment of enamel demineralization (ED) or enamel caries (EC) has become an increasingly important problem of modern cariology and orthodontics [1-14]. High quality of EC treatment can be provide by microinvasive resin infiltration technique (RIT), carried out as monotherapy or in combination with other methods [15-18]. This technique is based on the properties of liquid-flow composite (LFC) to diffuse into demineralized area with subsequent obturation of enamel microporosities [16, 19]. The problematic issues associated with the late complications of RIT (more often caries progression), indicate the need for more in-depth research of conventional, modified or combined infiltration techniques in in vitro studies [18, 20, 21]. The highly controversial issue of the usefulness (completeness, depth) of resin obturation of microporous enamel with standard infiltration regimen [22-27]. The mechanisms of EC progression before and after different infiltration techniques are mainly due to changes in the physical and mechanical properties of enamel (microhardness, elasticity, etc.) under the influence of cariogenic risk factors (unsatisfactory oral hygiene, constant consumption of carbohydrate and sugar-containing foods, etc.), as well as due to the emergence and spread of a stress-strain state at the boundaries of the lesion and intact enamel [28-34].

Methods of micro- or nanoindentation are used to assess the physical and mechanical properties of intact or affected enamel, as well as their dynamics during or after performing various therapeutic and prophylactic treatments [18, 35]. Certification of the physical and mechanical properties of enamel with an assessment of the possibility and features of its remodeling after various treatment modalities requires the use of optimal models of artificial enamel caries (AEC), the development of adequate methods for their
reproduction in vitro and the creation of appropriate hardware and technological support, since existing methodological approaches are not always universal.

The aim of this in vitro study was to analyze in a comparative aspect the physical and mechanical properties of tooth enamel: healthy; demineralized; demineralized and subsequently treated according to standard and modified infiltration techniques.

2. Materials and methods
The study was approved by the Ethic Committee of Perm State Medical University and executed at the support of RFBR (grant № 17-48-590562-r-ural). The material for the in vitro study was 18 intact (without visible defects of the crown) first and second premolars of the upper and lower jaws, removed according to orthodontic indications, with patients consent. The teeth were subjected to atraumatic mechanochemical cleaning according to an original technique (Gileva O.S. et al., 2001). Artificial enamel caries lesion was created on the vestibular surface of the crown according original protocol, using special lab equipment, instruments and solutions: “A device for modeling experimental caries in the stain stage using the enamel window method” (utility model patent № 172561 from 17.07.2017) and “Separator for storing dental biopreparations for artificial caries modeling” (utility model patent № 171409 from 05.30.2017), as well as demineralizing gel of the original composition (rationalization proposal № 2757 from 05.17.2018) (see figure 1). The development of AEC was confirmed using computed tomography, stereomicroscopy, scanning electron and atomic force microscopy (AFM).

The method of kinetic continuous microindentation was used to certify the physical and mechanical properties of enamel using a high-precision Micro-combi tester (MCT, CSM Instruments SA, Switzerland) equipped with a combined microindentation module, an automated sample stage (along the X, Y, Z axes), precision depth and acoustic emission sensors, a video microscope with three accessory lenses and a progressive scan CCD camera (figure 2). The tests were carried out in accordance with the International Standard ISO 14577, which regulates the indentation of the material with the simultaneous registration of three parameters: the load on the indenter, the movement of the indenter, and the indentation time during the elastic and plastic deformation of the material.

Figure 1. Immersion of the teeth samples in melted wax, installation in a separator, subsequent immersion in a demineralizing gel.

Figure 2. Micro-combi tester (CSM Instruments SA, Switzerland) for the study of physical and mechanical properties of the tooth sample.

The objects of the study – 12 teeth samples with artificially induced EC were randomized into two groups depending on the type of RIT reproduced in vitro: standard technique (ICON technology) or modified version. The standard resin infiltration (SRI) treatment reproduced in vitro involves the stage of 2 min. conditioning of the enamel lesion before its actual infiltration with a composite. For the second group samples, a modified resin infiltration (MRI) technique, with 4 min. enamel conditioning was applied. In addition, in a separate series of studies (6 samples) the microhardness of intact and demineralized enamel was determined. Cross sections of teeth were prepared for testing and then subjected to gentle mechanically and ultrasonically cleaning. Optical microscopic images of demineralized enamel with indenter’s traces presented on figure 3 and figure 4.
The following physical and mechanical parameters of enamel slices: microhardness (HIT, GPa) – by the Oliver-Farr technique; elastic modulus (EIT, GPa); creep (CIT, %); relaxation (RIT, %) and elastic component of indentation work (ηIT, %) were studied for the area of intact enamel (undamaged enamel zone that does not have direct contact with the focus of demineralization) – zone I, for the perifocal area of the lesion, at the border of intact and demineralized enamel – zone II, as well as for enamel in the center of demineralization infiltrated according to SRI or MRI technique.

Microindentation of demineralized enamel, infiltrated with a light composite according to the SRI and MRI methods, was carried out in two zones on transverse slices (figure 5): 1) in the subsurface layer of the enamel; 2) at a distance of 85% from the vestibular surface towards the enamel-dentin border, which ensured the penetration of the indenter into the transitional zone, not densely obturated with the infiltrant after SRI. The depth of the zone was calculated using the formula (1):

$$z_1 = k \times 0.85 \text{ (micron)}$$

where $k$ is the depth of the lesion (determined by computed tomography); $z$ – the depth of filling the demineralized enamel with the infiltrant according to the standard technique (determined by computed tomography); $z_1$ – zone of indentation both for SRI and MRI techniques.

All teeth samples were examined on a computed tomography scanner to determine the depth of the AEC focus and the level of its filling with the infiltrant (Table 1).

The parameters of enamel: microhardness (HIT, GPa), elastic modulus (EIT, GPa), creep (CIT, %), relaxation (RIT, %) and elastic component of indentation work (ηIT, %) were analyzed by the microindentation method. At all stages, microindentation was carried out with a step of 10 to 30 μm in both directions (X, Y). At each object of the study, 20-40 microindentations were carried out in an automatic mode. The value of the force applied to the indenter was 0.5 N, which ensured microindentation in the surface layers of the enamel. The amount of microindentations performed was 480 measurements.
Comparative assessment of the depth of filling the AEC focus with a light composite according to the standard and modified infiltration treatment.

| Tooth No. | Depth of AEC zone, µm | Depth of AEC filling (Z, µm) | Thickness of non-infiltrated area, µm | Z = k × 0.85 (µm) |
|-----------|-----------------------|-----------------------------|-------------------------------------|------------------|
| SRI       |                       |                             |                                     |                  |
| 1         | 506.0                 | 405.8                       | 100.2                               | 430.1            |
| 2         | 461.1                 | 360.1                       | 101.0                               | 392.0            |
| 3         | 316.4                 | 191.1                       | 125.3                               | 269.0            |
| 4         | 423.8                 | 298.0                       | 125.8                               | 360.2            |
| 5         | 442.3                 | 319.3                       | 123.0                               | 376.0            |
| 6         | 352.1                 | 213.4                       | 138.7                               | 299.3            |
| MRI       |                       |                             |                                     |                  |
| 7         | 513.9                 | 513.9                       | —                                   | 436.8            |
| 8         | 460.3                 | 460.3                       | —                                   | 391.0            |
| 9         | 328.3                 | 328.3                       | —                                   | 279.1            |
| 10        | 409.5                 | 409.5                       | —                                   | 348.1            |
| 11        | 437.2                 | 437.2                       | —                                   | 371.6            |
| 12        | 349.9                 | 349.9                       | —                                   | 297.4            |

3. Results

Multilevel analysis of the microstructure and surface topology of the enamel in the AEC focus, created according to the original technology, established its compliance with the characteristics of demineralized enamel in vivo according to the data of clinical examination, stereomicroscopy, computed tomography, scanning electron microscopy (SEM) and AFM: code K02.0 MKB-10; code 2 ICDAS; zones of destruction of enamel, with a heterogeneous, highly rough surface relief of typical triangular shape, with a surface layer thickness of 43.1±31.2 µm and a depth of 423.8±107.4 µm; X-ray transparent dark areas with uneven optical density and multilevel (zonal) decrease in mineral density from 1.47±0.17 g/cm³ in the dark, to 2.03±0.15 g/cm³ in the transparent zone (by 53.1% and 73.4% of the density of intact enamel) (figure 6). The developed AEC model corresponded to enamel caries in vivo characteristics, what predetermined the indications for infiltration treatment.

The average depth of filling demineralized enamel with an infiltrant according to the SRI technique was 298.0±44.5 µm (p>0.05), whereas the average thickness of non-infiltrated area was 125.8±62.9 µm (p<0.05). The demineralized enamel was filled with the LFC only by 70.4±9.8% (figure 7).

Measurements of microindentation of demineralized zones after resin infiltration according to SRI or MRI did not differ significantly (p>0.001) in the subsurface layer.

Figure 6. Axial tomogram of 1.4d with a focus of artificial enamel caries:
- surface zone (AB) – 1.56±0.12 g/cm³;
- “body” of the lesion (BC) – 1.86±0.17 g/cm³;
- dark zone (CD) – 1.47±0.17 g/cm³;
- transparent zone (DE) – 2.03±0.15 g/cm³.

Figure 7. Axial tomogram of 2.4d with an area of not filtered demineralized enamel.
The microhardness of intact enamel in the perifocal zone (II), when exposed to the indenter with a force of 0.5 N, was 5.01±0.54 GPa; the average penetration depth was 2.38 μm, which is significantly (p <0.05) lower in comparison with the indicators of intact enamel in areas (I) distant from the lesion (5.86±0.25 GPa; 1.92 μm). The microhardness of demineralized enamel 0.96±0.015 GPa, the average penetration value of the indenter – 6.2 μm, which is significantly (p<0.01) lower than the indices of intact enamel in both zones (I, II). The microhardness of demineralized enamel after SRI does not significantly (p<0.05) exceed the values of demineralized enamel before treatment (1.21±0.36 GPa). The average value of indenter penetration 5.0 μm, which is significantly (p<0.01) higher than the values of intact enamel in addition, the microhardness of enamel after SRI is significantly (p<0.01) 4.0-4.8 times lower than that of both zones of intact enamel (II, I). The microhardness of demineralized enamel after treatment with the use of MRI technique (4 min of conditioning) increased 2 times in comparison with the indicators of demineralized enamel before treatment (1.93±0.40 GPa). The average indenter penetration 3.8 μm; microhardness significantly (p<0.05) 1.6 times higher than the values of enamel after SRI, but at the same time significantly (p<0.01) 2.6-3.0 times lower than of both zones of intact enamel.

The modulus of elasticity of intact perifocal (II) enamel 83.8±14.4 GPa and is not significantly (p<0.05) reduced in comparison with the indices of intact enamel (85.4±6.6 GPa) in areas remote from the lesion. The modulus of elasticity of demineralized enamel 10.35±1.35 GPa, which is significantly (p<0.001) lower for both indicators of intact (I, II) enamel. The modulus of elasticity of the enamel after SRI is significantly (p<0.01) higher than that of demineralized enamel (20.26±4.12 GPa), but significantly (p<0.01) 4.2 times lower than the indicators of intact enamel (II, I). The modulus of elasticity of the enamel after MRI is significantly (p<0.05) 1.7 times higher than the parameters of the enamel after SRI (33.8±12.0 GPa), and in comparison with the indicators of intact enamel zones is 2.5 times reduced (p<0.05).

In the course of the experiments the fundamentally new data were obtained on the physical and mechanical properties of enamel (intact and treated with various infiltration techniques), which are not presented in the available literature. Thus, the creep (CIT, %) of enamel – a slow deformation occurring over time under the influence of a constant mechanical load or stress – was determined. The creep index of perifocal intact (II) enamel was 2.08±1.18% and did not significantly (p<0.05) exceed the values of intact (I) enamel in areas remote from the lesion (1.92±1.03%). The creep index of demineralized enamel with a high degree of reliability (p<0.001) was 11.4-12.3 times higher than that of intact enamel, both perifocal and distant from the lesion (23.68±1.70%). The creep index of enamel treated with the SRI technique (6.58±0.82%) was significantly (p<0.05) 3.6 times lower than that of demineralized enamel, but 3.2 times higher (p<0.05) than indicators of intact enamel. The enamel creep after MRI was 1.6 times lower (p<0.05) than the same index after SRI (4.09±0.67%), but 2.0 times higher (p<0.05) than of intact enamel.

The values of the relaxation index (RIT, %) of enamel as a process of achieving static equilibrium under the influence of load in time were also analyzed. Thus, the relaxation index of intact (I) enamel (–0.07±0.31%) did not significantly (p<0.05) exceed that of perifocal, visually intact enamel (–0.09±0.02%). The relaxation index of demineralized enamel (–0.17±0.18%) was significantly (p<0.05) 1.8-2.3 times lower than that of intact enamel in both zones. The enamel relaxation index after SRI (–0.11±0.13%) was significantly (p<0.05) 1.5 times higher than the values of the demineralized enamel, but 1.2–1.5 times lower than the intact enamel (II, I). The relaxation index of demineralized enamel after MRI (–0.10±0.10%) did not significantly (p<0.05) exceed that after SRI, approaching the values of perifocal intact enamel.

Finally, the values of the elastic component of the indentation work (ηIT, %) of the enamel were analyzed as the ratio of the elastic deformation work to the total indentation work. The elastic component of the work of indentation of intact (I) enamel – 47.30±2.87%; intact perifocal enamel – 43.15±3.91%; demineralized enamel – 26.84±0.92%; enamel after SRI technique– 28.40±2.27%; enamel after MRI – 29.33±4.46%, which, respectively, was 1.6, 1.5 and 1.5 times less than that of perifocal intact enamel. Comparison of the ratio of the elastic component of the indentation work to the general indentation work in the AEC focus and in the affected enamel zones, treated according to SRI or MRI, did not reveal significant differences.
4. Conclusion

The results of the in vitro study of the physical and mechanical properties of intact enamel characterize it as a high-strength and highly elastic biomaterial (HIT$_1 = 5.86\pm0.25$ GPa, EIT$_1 = 85.43\pm6.61$ GPa; HIT$_2 = 5.01\pm0.54$ GPa, EIT$_2 = 83.82\pm14.42$ GPa, respectively), capable of withstanding the directional deformation loads during the chewing loads. The data analysis shows that intact enamel in terms of microhardness fits into the characteristics of structural steels with surface carburizing, and the modulus of elasticity is comparable to the indicators of deformational aluminum alloys. In the course of microindentation studies, new, updated and more broader data were obtained on microhardness (HIT, GPa), elastic modulus (EIT, GPa), creep (CIT, %), relaxation (RIT, %) and elastic component of indentation work (ηIT, %) of intact and demineralized enamel, as well as of demineralized enamel treated with conventional or modified resin infiltration techniques and both in its surface layer and at a depth of 85% towards the enamel-dentin border.

The data of a principle importance that microhardness indices in the subsurface layers of demineralized enamel treated with SRI (HIT = 1.95±0.17 GPa) and MRI (HIT = 1.98±0.15 GPa) techniques did not differ significantly (p<0.001), whereas at a depth of 85% towards the enamel-dentin border according to SRI (HIT = 1.21±0.36 GPa) and MRI (HIT = 1.93±0.40 GPa) methods were significantly (p<0.05) differed. This confirms the fact of incomplete obturation of micropores at a depth of $z_1 = k \times 0.85$ (μm) and below, indicates the absence of the formation of “strengthening matrix “ in the interprismal spaces of the enamel after resin infiltration using the SRI technique. The results of this in vitro study clearly objectify the advantages of the MRI technique over the classical mode of infiltration treatment.

The results of physical and mechanical studies indicate the need to protect the perifocal zones of treated enamel at the border of its infiltrated areas to reduce the risk of stress and strain during functioning of the dental-maxillofacial system. So, it may be useful to combine caries infiltration technology with appropriate remineralizing techniques to reduce the risk of the secondary caries appearance at the border of visually intact and infiltrated enamel.

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