Visualization of Resin Penetration into Enamel Caries Lesions of Temporary Teeth – a Confocal Microscopic Study

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Carious enamel infiltration with a low viscous light cured resin (Infiltrant) is an innovative and promising approach to arrest these lesions. The confocal laser scanning microscopy (CLSM) can be used to analyze the penetration of the Infiltrant into the enamel. Most studies were performed on permanent teeth but given the structural differences to temporary teeth several aspects regarding their enamel infiltration must be elucidated. In additional it should be borne in mind that children have limited patience, so reducing the treatment time is desirable. Purpose of this study was to visualize the penetration of a low viscosity resin (Icon Infiltrant, DMG, Hamburg, Germany) into natural caries lesions of temporary teeth after 2 min of application time. Exfoliated human deciduous teeth with proximal non-cavitated enamel caries lesions were infiltrated for 2 min and then light-cured, respecting the classic therapeutic procedures proposed by the German company. The visualization of the resin infiltration was accomplished using the indirect staining protocol described by Sebastian Paris et al in 2009. Lesion depths (LD) and penetration depths (PD) were assessed using dual fluorescence confocal microscopy. This technique appears to be feasible in the case of deciduous teeth, can be used in further studies and an application time of 2 minutes was sufficient in most cases.

Key-Words: temporary teeth, infiltration of carious enamel, infiltrant, CLSM, white spot lesions

Tooth decay remains a major problem in Romania and the chronic lack of specialists in Paedodontics cause the temporary teeth to be frequently affected by this pathology.

For many years, dentistry was influenced by a mechanical approach characterized by the use of high-speed rotary cutting instruments and radical removal of diseased portions of the tooth, along with material-driven geometric extensions to areas that were assumed to be caries-resistant [1].

All these aggressive approaches had a negative effect on cooperation with the pediatric patients leading often to failures and requiring advanced techniques for behavioral management.

Development of new chemical substances and especially of more efficient resins and the better understanding of their kinetics have led to a new paradigm called "minimally invasive dentistry" which implies maximum preservation of healthy dental structures over a lifetime and is required for children and adolescents.

The DMG German Company introduced a new and innovative way to approach the treating of incipient caries – Icon, a caries infiltrant to be introduced in early lesions, able to achieve the maximum preservation of dental structures over. Anyhow the product and the technique are very new on the market and still some aspects are not fully understood [2].

The aim of resin infiltration is to soak up the porous lesion body with a low-viscosity resin (infiltrant) that is subsequently hardened with blue light [3].

Since the porosities of enamel caries lesions act as diffusion pathways for acids and dissolved minerals, infiltration of these pores with resin occludes the pathways and thus lesion progression is hampered or even arrested [4].

In recent years we observe an increase in the addressability of adolescents and adults with malocclusions to therapy with fixed orthodontic appliances. Most of the adult patients require an interdisciplinary therapy with prolonged treatment times involving good oral hygiene. In the absence of good oral hygiene new white spot lesions (WSL) develop on the teeth. We undertook a series of studies on the occurrence of WSL in correlation with fixed orthodontic appliances and the infiltration therapy proved to be effective both aesthetically by masking the whitish appearance and also regarding the stability over the time of the treated lesions [5 - 7].

Non cavitated enamel caries lesions in primary teeth can nearly be completely infiltrated in vitro. Infiltrants with high penetration coefficients (PK) seem to be better suitable than those with low PK [8].

Most studies were performed on permanent teeth but given the structural differences to temporary teeth several aspects regarding their enamel infiltration must be elucidated.

The mean thickness measurements observed in the deciduous teeth enamel was 1.14 mm and in the permanent teeth enamel was 2.58 mm. The primary enamel structure showed a lower level of Ca and P, thinner thickness and higher numerical density of rods [9]. Additionally it should be borne in mind that children have limited patience so reducing the treatment time is desirable.

Purpose of this study was to visualize the penetration of a low viscosity resin (Icon Infiltrant, DMG, Hamburg, Germany) into natural caries lesions of temporary teeth after 2 min of application time.

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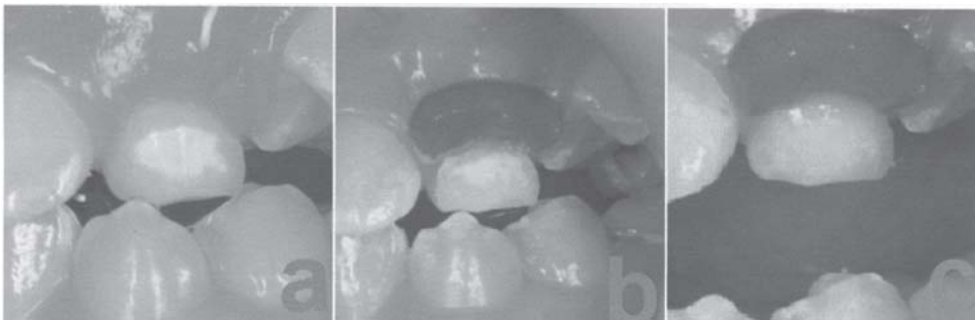

Fig. 1(a-c).. Clinical steps in the treatment of non-cavitated enamel caries lesions on temporary teeth

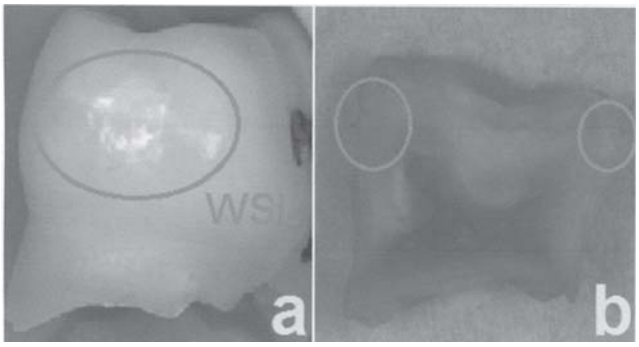

Fig.2a). The WSL on the proximal face of the exfoliated temporary molar b) the section through the temporary molar after staining with red fluorophore and protecting it by the infiltrant

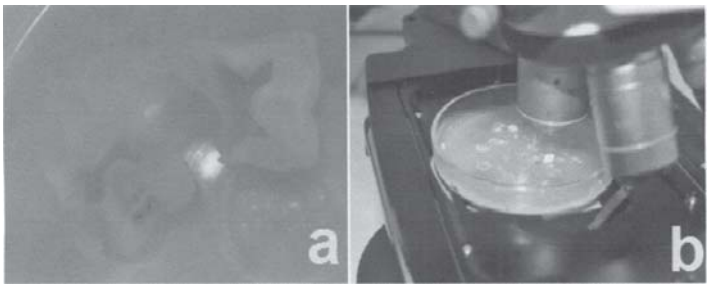

Fig.3. To stain the porous non-infiltrated structures the specimens were immersed in fluorescein (a) and after observed by CLSM (b)

Experimental part

Materials and method

The clinical steps for the therapy of non-cavitated enamel caries lesions applied to temporary teeth assume (fig.1): cleaning of the dental surfaces and isolation of the oral soft tissue. After this, the lesions were etched for 2 min using 15% hydrochloric acid gel (ICON®-Etch; DMG, Hamburg, Germany) to expose the layer of the lesion then the etching gel is washed away for 30 s and the tooth surface is dried for 10 s using compressed air (fig.1b). For the desication of the lesion we used ethanol (ICON®-Dry; DMG) for 30 s followed by air drying. A low viscosity resin infiltrant (ICON®-Infiltrant; DMG) was applied on the surface and allowed to penetrate the WSL for 5 min. Excessive material was wiped away with a cotton roll and the resin was light cured for 60 s. After we polished the roughened enamel surface using polishing discs (Sof-lex disk; 3M ESPE, Saint Paul MN, USA).

For our in vitro study exfoliated human deciduous teeth with proximal non-cavitated enamel caries lesions were infiltrated for 2 min and then light-cured, respecting the classic therapeutic procedures proposed by the German company (fig.2a).

The visualization of the resin infiltration was accomplished using the indirect staining protocol described [10].

Briefly, all accessible porosities were stained with a red fluorophore by storing the specimens in ethanolic solution of 0.1% RITC (Rhodamine B isotiocyanate mixture of isomers – 283924, Sigma Aldrich, Steinheim, Germany) for 12h. Subsequently, specimens were dried with compressed air and low viscosity resin infiltrant (ICON®-Infiltrant; DMG) was applied on the surface and allowed to penetrate the WSL for 2 min. The excessive material was removed after using cotton rolls and the material was light cured for 60 s with Astralis5 polymerization light with an output intensity of 530Mw/cm^2 (Ivoclar Vivadent AG, Lichtenstein).

The next step was to bleach all the red stain that has not been covered (protected, enclosed) by the infiltrant. The bleaching was done using 30% hydrogen peroxide (95302 – Sigma Aldrich, Steinheim, Germany) solution for 12h at 37°C. After bleaching the specimens were washed with water for 60s. After this the primary teeth were cut perpendicular to the lesion surface resulting two halves (fig.2b).

To visualize porous structures that were not infiltrated, specimens were stained using a green fluorescent staining. Specimens were immersed in 50% ethanolic solution of 100µM NaFl (fluorescein sodium salt – 46960, Fluka, Sigma Aldrich, Steinheim, Germany) for 3 min, specimens were washed in deionized for 10 s, dried and observed using a confocal laser scanning microscope (fig.3).

Confocal laser scanning microscopy is an optical imaging technique which increases the optical resolution and contrast of a micrograph by point illumination using lasers and a spatial pinhole to eliminate the out-of-focus light that are not in the focal plane and will not enter into the final image.

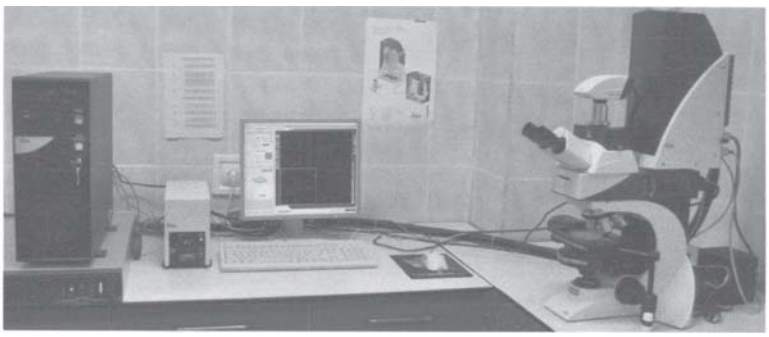

Fig.4. Leica confocal laser scanning microscope model DM 2500 used in this study

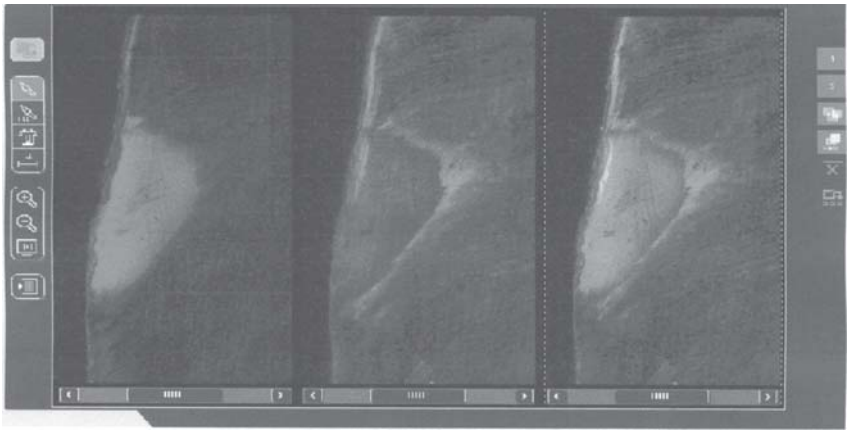

Fig.5. Left is the red image obtained after detecting the RITC staining of the specimen, in the middle is NaFl (green) and right is the composed image by superimposing the two fluorescents

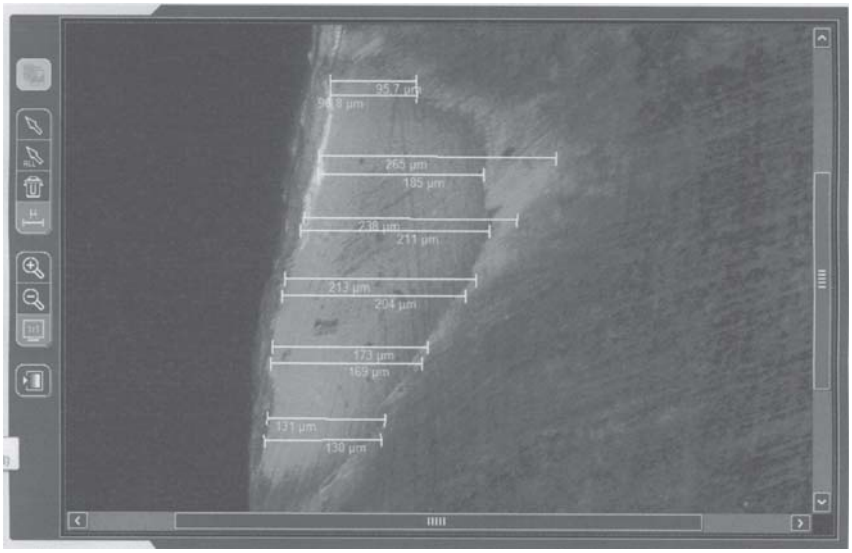

Fig.6. Measuring the lesion depths (green) and penetration depths (red) with a dedicated software

For the present study the specimens were observed using a *Leica* CLSM model DM 2500, using 10x objective (fig.4).

Dual fluorescent mode was used to simultaneously detect RITC and NaFl. For RITC the 532nm wavelength laser was used and the emitted light was recorded at wavelengths higher than 590 nm. For NaFl the 488 nm wavelength laser was used and the emitted light was recorded at 525 nm. The specimens were scanned and recorded with a lateral resolution of 1024x1024 pixels (733x733µm or 1010x1010µm dependent on lesion size) at 400 Hz speed (fig.5).

The measurements were made using LAS-AF software (Leica Application Suite – Advanced Fluorescence) (fig.6).

Results and discussions

The applied technology, though laborious, generated impressive images that clearly differentiate porous areas (affected by decays) from sound enamel and infiltrated areas.

The use of computers in generation and measurements of these images is essential. But are those measurements precise? Generally computers are very accurate in measuring things. Actually this is exactly what they do the best since they are infinitely precise, mathematically based beings. Computer science and analyze of biomedical imaging have a lot of applications and advantages in almost every aspect of the orthodontic and paedodontic practice [11-15].

In an excellent recently published study it was told that 3 minutes application of an infiltrant seems to be sufficient to achieve an almost complete penetration of natural caries lesions in vitro. Further studies should aim to evaluate, if the application time might be reduced to 2 min and, if these established resin layers are capable to inhibit lesion progression [16].

Since shorter application times are clinically more feasible, especially when treating children and given the structural characteristics of the enamel in deciduous teeth (thinner thickness) we tried to check if 2 min for infiltration time is enough. It seems to be so, especially in superficial non-cavitated proximal lesions in deciduous molars with a lesion depth (LD) < 300µm where 85.7 % of the lesion is infiltrated in 2 min . Further studies should be done in order to evaluate deeper lesions.

Rhodamine and fluorescein allow selective visualization due to well separated excitation and emission wavelengths [16, 17].

Most of the studies are performed in vitro on extracted teeth but given temporary teeth characteristic of having limited life they can be used in ex-vivo studies to achieve more conclusive results.

In dentistry many clinical and experimental contexts require the use of non-destructive methods such as metallographic microscopy to analyze surfaces [18, 19]. In infiltrated and exfoliated temporary teeth we have to develop the proper, little destructive method to better describe the interface between healthy, demineralized and infiltrated enamel.

Resin infiltration proved to be efficient not only in arresting of incipient caries but also in masking the developmental defects or post-orthodontic de-calcifications.

The masking effect of resin infiltration was dramatic in some cases but not in others. The long-term colour stability of this technique should be followed up through continuous

clinical and scientific studies [20]. The effect of the resin infiltration technology on more complex lesions like molar incisor hypomineralizations (MIH) should be investigated and also the use of ultrasound for the improvement of the penetration.

Conclusion

This technique appears to be feasible in the case of deciduous teeth, can be used in further studies and an application time of 2 min was sufficient in most cases.

Further studies are necessary to better characterize the infiltration of the resin into the enamel of the temporary teeth at different application times.

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