Influence of Photo-activated Toluidine Blue on the Interface Between a Caries Infiltrant and White Spot Lesions

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White spot lesions represent the first stage in the evolution of the caries process and are heavily populated with cariogenic bacteria. The photo-activated disinfection therapy can be used as an adjunctive form of treatment to the classic infiltration technology. The purpose of this study is to assess the influence of the photo-activated toluidine blue on the infiltration kinetics.

Keywords: white spot lesions, interfaces, toluidine blue, photo-activated disinfection, infiltration of carious enamel

White spot lesions are clinical entites increasingly common in the current generation of children and adolescents. The major aesthetic, prophylactic and therapeutic implications of this relatively new pathology in dentistry made us to grant it a special attention.

The presence of clinically detectable, localized areas of enamel demineralization, observed as white spot lesions of different opacity, is a sign that the caries process has begun. Dental caries results in the dissolution of apatite crystals and the loss of calcium, phosphate and other ions, which eventually leads to demineralization of the tooth substrate [1].

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. [2]

Proximal caries diagnosis and staging assessment is still a challenge in many industrialized countries and the lack of compliance with preventive behavior by patients is still a major problem, so it is important to diagnose proximal caries in early stages (limited to enamel) in order to arrest and control them so they can benefit of a much more tissue-preserving approach similar to preventive treatment methods [3]

Currently, the diagnostic of carious lesions includes visual, tactile and radiographic examination. Often, these traditional methods are not sensitive enough to detect carious lesions limited to the enamel. Digital imaging fiber-optic transillumination (DIFOTI) allows an early diagnosis in order to approach a remineralisation treatment for the carious process to become reversible [3, 4].

The mouth provides a large number of diverse surfaces on wich a wide variety of complex biofilms are able to form. These biofilms consist of a complex microbial community embedded in a matrix of polymers of bacterial and salivar origin. They are inherently more resistant to antibiotics, antimicrobials and antifungical agents wich make the use of novel antimicrobials such as antimicrobial Photodynamic Therapy (aPDT) more important [5].

The use of photosensitizers for microbial eradication can be traced back to before the age of chemotherapy, it involves a light-sensitive photosensitizer, light, and molecular oxygen. Photodynamic treatment (PDT) is a process in which microorganisms are treated with a photosensitizing drug and then irradiated with low-intensity visible light of the appropriate wavelength. The transfer of energy from the activated photosensitizer to available oxygen gives rise to the formation of highly reactive oxygen species, such as singlet oxygen and free radicals, which can kill microorganisms by damaging essential cellular molecules, including proteins, membrane lipids, and nucleic acids. The technique has been shown to have effects against a range of oral pathogens and also against drug-resistant bacteria [6-8].

Toluidine blue O is a solution that is blue–violet in color. It can stain granules within mast cells, and proteoglycans and glycosaminoglycans within connective tissues. Toluidine blue is a basic thiazine metachromatic dye with high affinity for acidic tissue components, thereby staining tissues rich in DNA and RNA. It has found wide applications both as vital staining in living tissues and as a special stain owing to its metachromatic property [9].

Experimental part

For this experiment 12 superior and inferior premolars, extracted for orthodontic purposes, were selected, which presented one or more white spot lesions on the proximal sides. Teethes were cleaned after extraction and kept in saline solution and constant temperature until they were used. The 12 premolars presented 18 white spot lesions which were divided in two groups: group A with 9 lesions in which the infiltration was done after the photodynamic therapy with toluidine blue (FotoSan, CMS Dental, Copenhagen, Denmark) (fig.1) and group B, as control, with 9 lesions in which the infiltration was done according to manufacturer instructions.

Lesions from group A were treated for 2 min with clorhidric acid (Icon-Etch), followed by a 20 s washing with water, air dried for 20 s, then toluidine blue was applied for 20 seconds (0.1mg/mL) (Fotosan agent medium viscosity, Fotosan; CMS Dental, Copenhagen, Denmark) as a photosensitizer. The light source was a light-emitting diode (LED) in red spectrum (wavelength 625-635 nm;

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Fig.1 The complete FotoSan System

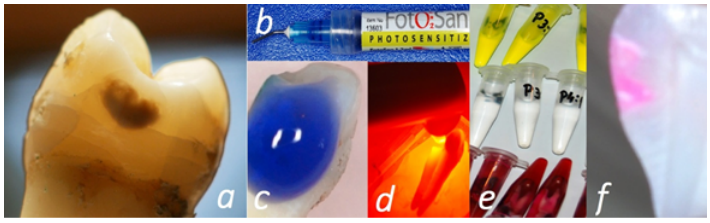

Fig.2 Steps during the experimental part: a –white spot lesion, b-toluidine blue, c,d- the photodynamic therapy with toluidine blue, e-staining, f-specimen prepared for confocal laser scanning microscope

FotoSan©; CMSDental, Copenhagen, Denmark). The diameter of the tip of the LED device was 6.2 mm and the lamp was held at a distance of 1 mm from the tooth surface. This step was followed by washing for 30 s with water, air dried and then with ethanol (Icon-Dry). After these steps, the tooth was immersed in 0.1% RITC alcoholic solution (Rhodamine B isotiocyanate mixture of isomers – 283924, Sigma Aldrich, Steinheim, Germany) for 12h. RITC staining had the purpose to stain all the lesions. The first staining step was followed by air drying for 30 s and infiltation with ICON®- Infiltrant, DMG for 5 min and polymerization for 30 s. The tooth was then immersed in 30% hydrogen peroxide solution (95302 – Sigma Aldrich, Steinheim, Germany) for 12 h at 37°C. After bleaching the specimens were washed with water for 60 s. To visualize porous structures that were not infiltrated, specimens were stained with a 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.2).

Lesions in group B used as control were prepared in the same way except toluidine blue mediated photodynamic effect step.

Specimens (samples) were analyzed using a confocal laser scanning microscope (CLSM) model Leica DM2500. A 10X objective was used both in fluorescence and CLSM observations. The dual staining of the samples was detected using two different lasers as follows: for RITC the 532 nm (green) laser was used and for NaFl Ex 488 nm laser (blue). The emission was recorded at 590 nm for RITC and at 525 for NaFl. The infiltrated lesions were scanned and recorded with a lateral resolution of 1024x1024 pixels (1010x1010 μm) at 400 Hz speed. The measurements were made using the LAS-AF software (Leica Aplication Suite Advanced Fluorescence) (fig.3).

Results and discussions

After analyzing samples using LAS AF software (Leica Application Suite Advanced Fluorescence) we noticed that there are not significant differences on the infiltration capacity between the 2 groups (fig.4-6).

The use of photodynamic therapy for the disinfection of caries could be beneficial for reducing the amount of dental tissue removed during cavity preparation and promoting an effective decontamination of the treated area before

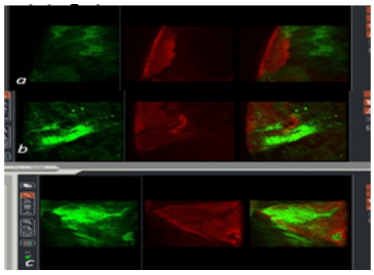

Fig.3 Specimens (samples) were analyzed using a confocal laser scanning microscope (CLSM) model Leica DM2500 and analyzed using the LAS-AF software (Leica Aplication Suite Advanced Fluorescence)

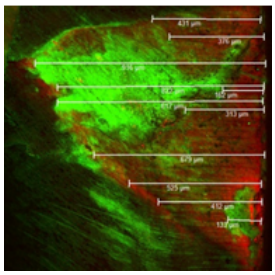

Fig.4 The measurements in a deep white spot lesion from group A (the toluidine group)

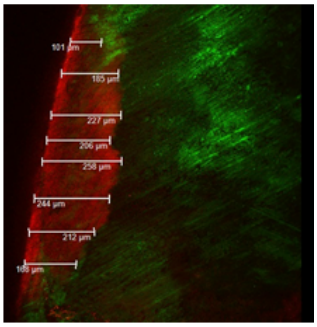

Fig.5 The measurements in a superficial white spot lesion from group A (the toluidine group)

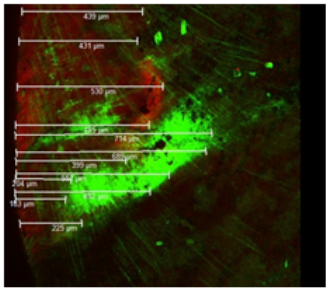

Fig.6 The measurements in a deep white spot lesion from group B

sealing the lesions. The treatment might become less traumatic for the patient and faster for the dentist besides improving the prognosis by decreasing the need for dental tissue cavity preparation [5].

Given the ability of photodynamic therapy to sterilize dental hard tissues affected by caries process and colonized with specific bacteria and the relatively equal ability of infiltration of white spot lesions we consider practical, for relapse prevention of caries and secondary caries, the introduction of this new therapeutic stage during the infiltration procedure.

Further studies are needed to see the effective antibacterial effects of the photodynamic therapy on white spot lesions, the extension of sterilization in the lesion and the possible interaction between the enamel porous structure and toluidine blue.

Conclusions

The infiltration technology, a classic in the treatment of white spot lesions, proves once again its effectiveness by almost complete sealing of the porous enamel. Application of a new step during infiltration, the disinfection of the lesion with photo-activated toluidine blue, does not seem to have

an influence on the interface between white spot lesions and the infiltrant. Its ability to infiltrate the lesion is not reduced.

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