Early detection of white spot lesions with digital camera and remineralization therapy

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ABSTRACT

Background. In this paper , the characteristics of early stage of dental caries is discussed and the methods we use to treat early stage of dental caries to increase the number of caries-free patients is presented. Studies from in vitro to in situ experiments and a clinical study were carried out to support clinical remineralization therapy.

Methods & Results To clarify the effect of time for remineralization, the degree of remineralization was assessed at 2 days, 6 days, and 10 days after 2-day demineralization in 0.01mol/L lactic acid buffer (pH 4.0 at 37 °C). The remineralization solution contained 3.0 mmol/L Ca, 1.8 mmol/L P, and 3 ppm fluoride adjusted to pH 7.0. A 10-day continuous remineralization with a 3 ppm fluoride, resulted in a high fluoride concentration. To evaluate mineral loss from sound tooth structure and white spot lesions, thin sections (about 90 µm) including white spots (WS) were prepared and exposed to oral conditions for 2 weeks continuously. The mineral loss from sound tooth structure was found to be twice that from WS. In another experiment during the remineralization period, enamel samples were immersed in three different bicarbonate solutions; 0.5, 5.0 and 50 mmol/L for 30min, two times per day. Both the bicarbonate and fluoride applied groups showed higher improvement in acid resistance and the amount of remaining mineral was almost two times higher than the controls ($p < 0.01$). In a clinical study we demonstrated remineralization, in patients who following professional mechanical tooth cleaning and fluoride prophylaxis paste. Using this regime, in patients with deciduous caries present at baseline, over 80% of permanent teeth were caries free at the age of 12 years. In these studies the digital camera with CasMaTCH™ and image analysis system showed several advantages for monitoring in de- and remineralization.

Conclusions White spot lesions, rather than intact tooth surfaces, can be mineralized through the daily clinical procedures described in this paper.

KEY WORDS: remineralization therapy, bicarbonate, fluoride, acid resistance, early caries lesion, monitoring, digital camera.

INTRODUCTION

Clinically, the caries process occurs as an interaction between the dental plaque, as a part of a pathological biofilm, and the tooth surface and/or subsurface. Caries progresses when the equilibrium of demineralization and remineralization is out of balance, leading to net mineral loss and discoloration. Because of clearly differentiated discoloration from adjacent sound enamel, the clinical appearance of the demineralized area may vary from matt opaque to demarcated opacity (so called white spot) after careful tooth drying. The presence of white demineralization areas parallel to the gingival margin covered with plaque is evidence that carious demineralization has taken place. Recently these signs and symptoms, based on discoloration, were adopted in the clinical caries detection criteria, known as ICDAS; International Caries Detection and Assessment System (Pitts 2004).

In this paper, the characteristics of the early stage of dental caries discussed together with presentation of the method we use to treat the early stages of dental caries to increase the number of caries-free patients.

MATERIALS & METHODS

Effect of time for remineralization

To clarify the effect of time for remineralization, bovine enamel samples were used and the degree of remineralization was assessed at 2 days, 6 days, and 10 days after 2-day demineralization in 0.01mol/L lactic acid buffer (pH 4.0 at 37°C). Remineralization solution contains 3.0 mmol/L Ca, 1.8 mmol/L P, and 3 ppm fluoride adjusted to pH 7.0. (Iijima and Koulouride, 1988, 1989).

In situ acid resistance of white spot lesions

To study in situ acid resistance of white spot lesions, 16 human premolars were used. The teeth had in vivo formed white spot (WS) lesions in proximal surfaces. These premolars had been exposed to a fluoride mouthrinse (0.05% NaF solution) and fluoride dentifrice (1,000 ppm F) on a daily basis. Thin sections (about 90 μ m) including the WS lesions were prepared and exposed to oral conditions for 2 weeks continuously (Iijima and Takagi, 2000).

Acid resistance of human enamel after bicarbonate application during remineralization

To determine the effect of bicarbonate application on acid resistance of human enamel during remineralization, samples were prepared for demineralization and remineralization similar to that stated above except for preparation of the bicarbonate solution. Three different bicarbonate solutions; 0.5, 5.0 and 50 mmol/L were prepared just before the application. During remineralization period, enamel samples were immersed in each bicarbonate solution for 30min, 2 times per day (Tanaka and Iijima, 2001).

Clinical remineralization therapy and Digital Imaging

Clinical remineralization therapy includes professional mechanical tooth cleaning (PMTC), digital imaging capturing and analysis. PMTC is a part of routine clinical practice by dental hygienists in Nagasaki University Preventive Dentistry Clinic. As a part of professional oral health care to promote remineralization, patients who have WS lesions received PMTC to remove plaque from all tooth surfaces. PMTC is carried out using mechanically driven instruments (specially designed contra-angle hand piece and reciprocating tips) and fluoride prophylaxis paste. (Axelsson, 1999).

Image capture and analysis

A commercially available color chart (color and size matching sticker; CasMaTCH™ ; <http://www.bearmedic.co.jp>) taken simultaneously with the dentition was used to adjust the color values of displayed images so as to reproduce the same color as the original chart is introduced. Using this standard color scale and digital image analysis technique, enamel demineralization and remineralization were monitored and evaluated. In a clinical study, plaque induced demineralization on the enamel surface was captured with a digital camera (baseline image and data). At subsequent visits, further digital images were also captured, analyzed, and compared with baseline data.

Since WS are usually associated with plaque deposits, the dental plaque deposit has to be removed prior to taking the photographs. After carefully cleaning and drying the teeth, WS are usually well demarcated and suitable for photographic capture. To capture the images in a clinical setting, the distance between subject and camera needs to be about 45 cm. The digital image of WS can be captured with a digital camera (Nikon D1, Tokyo, Japan) held almost perpendicular to the buccal surface with CasMaTCH. Light is supplied by a ring flash mounted around

120 mm lens (F4; Medical Nikkor, Tokyo, Japan). The camera system setup was adjusted as follows; the quality of image is Fine, ISO is 200, and white balance is speed light mode.

The color chart contains 9 color chips including black, gray, and white within a square cm. The RGB signals generated by a digital are device-dependent i.e., different digital cameras produce different RGB responses for the same scene. Eight-bit RGB data were used for characterization of the tooth color. To reproduce the same color as the original color chart, white, gray, and black values were adjusted to its L in L a b values such as, 93, 58, and 22, respectively, with clicking a color picker in the ADOBE PHOTOSHOP™ (7.0J; Adobe Systems incorporated, San Jose, Cal., U.S.A.). The contrast and brightness can be adjusted through this manipulation. Subsequently, the image was converted to gray scale image (8-bit range) with NIH Image (version 1.60, <http://rsb.info.nih.gov/nih-image/>) and saved as Tagged Image File Format (TIFF).

After 2:1 magnification of the image, the Region of Interest (ROI) was drawn to define the buccal surface of the enamel samples. Each pixel within ROI records a numeric value corresponding to the brightness of that point on the original image. The brightness values for 8-bit range gray scale images range from 0 to 255. NIH Image displays zero pixels as white and those with a value of 255 as black. The gray values recorded by the NIH Image (as a single column of text) were exported into a spreadsheet (Kaleida Graph 3.5, Hulinks, Tokyo, Japan) and distribution of gray values, so called Histogram, Histogram related data values mean and S.D. were calculated and analyzed.

Clinical examination

Clinical examination was based on the WHO criteria (WHO, 1987) and performed by calibrated dentists using a dental mirror and explorer with gentle tactile pressure. After careful cleaning and drying the teeth, WS

lesions were recorded as early caries and cavities with remarkably softened wall or floors were also recorded as dental caries by dfs and DMFS.

RESULTS

Effect of time for remineralization: Remineralization of the lesions appeared to be complete after 10 days of demineralization. Although there is a subsurface lesion in the remineralized region, almost 80% of the original density levels were

recovered. The mean fluoride gradient are shown in Fig.1. A 10-day continuous remineralization with a 3 ppm fluoride, resulted in a high fluoride concentration. Fluoride profiles of remineralized samples exposed to the intra-oral environment for 24 hours or to 1.0 mmol/L KOH solution did not show appreciable loss of fluoride. Samples exposed to the 0.01 mol/L lactic acid buffer showed rather lower fluoride profile, however, there was no statistically significant differences of fluoride concentration with any of the three test treatments.

In situ acid resistance of WS lesions: Fig.2 shows a microradiographic example of WS lesion before and after exposure to cariogenic conditions for 2 weeks which advanced to approximately 460 μm from outer enamel surface to the deepest part of the lesions and areas of the sound enamel adjacent to the WS lesion. Comparison of the mineral loss of sound area with WS, showed that the mean ratio of sound/WS was approximately a factor 2.

Acid resistance of human enamel after bicarbonate application during remineralization: After applying bicarbonate solution during remineralization, remaining mineral of both bicarbonate and fluoride applied groups after the second acid challenge was compared with fluoride applied groups. The former groups showed higher improvement in the acid resistance and the amount of remaining mineral was almost two times higher level (Fig.3, $p < 0.01$).

Clinical remineralization therapy: The long-term clinical treatments with PMTC and fluoride application (mainly APF solution, 9,000 ppm F as NaF and fluoridated toothpaste, 1,000 ppm F), three times per year to the patients who have WS lesions in deciduous or permanent teeth was assessed. Despite the presence of deciduous caries at baseline, over 80% of permanent teeth were caries free at the age of 12 years (Fig.4). It was possible to monitor de- and remineralization of WS lesions using CasMaTCH by the change of distribution pattern of gray value and also by numerical data (Fig.5).

DISCUSSION

From these studies, it is obvious that remineralized lesions are chemically stable and the surface of WS lesions were much more acid-resistance than sound enamel. Considering that both in vitro and in vivo formed WS samples were

remineralized under fluoride conditions, a fluoride-rich acid resistant mineral must have formed in the microspaces of the demineralized enamel. The reduction of lesion porosity with this mineral depositon will greatly increase the enamel resistance against subsequent acid challenges. Furthermore, bicarbonate-treated enamel lesions during remineralization were more acid resistant. It was suggested that these ions may penetrate into the subsurface lesions and react as buffering agents against the acid challenge. As a result, with the help of fluoride-rich mineral they apparently improved the decrease of pH in subsurface microspaces.

Using a standard color scale and digital image analysis technique, monitoring of enamel demineralization and remineralization was possible. In a clinical situation, there are some cases of WS lesions that show evidence of arrest, progression, and regression. The digital camera with CasMaTCH™ and image analysis system used in this study shows several advantages for monitoring de- and remineralization. Although this method is limited to assessment of anterior teeth, one of the most important features of this method is that the frequency distribution of gray values from sound, demineralized, and remineralized enamel showed a normal distribution. Therefore, statistical evaluation is possible. Judging from the change of the distribution pattern, one can speculate whether the caries process is in the stage of arrestment, progression or regression by viewing the pattern.

Detection of early caries lesions

Today, clinicians and researchers collaborate to investigate more sensitive methods to enable early detection and quantification of caries lesions (Bjerkhagen et al. 1982; Sundström et al. 1985). Caries can be detected by quantitative laser or light induced fluorescence (QLF), because the fluorescence radiance at the site of caries lesions is decreased and thus appear as dark spots. There has been extensive in vitro study of QLF for quantification and longitudinal monitoring of mineral changes in smooth surface caries (Hafström-Björkman et al. 1992; al-Khateeb et al. 1997; ten Cate et al. 2000; Ando et al. 2001). However, there are few in vivo studies of the application and properties of QLF (de Josseline de Jong, et al. 1995; van der Veen et al. 2000; Tranæus et al. 2001). Based on the restricted data available, it appears that QLF is suitable for in vivo monitoring of mineral changes, as well as for caries preventive programs except the cost of the

equipment. Although the QLF method consists of several steps, both the inter- and intra-observer reliabilities are excellent (Tranæus et al. 2001).

There is another noninvasive method suitable for occlusal lesions. The DIAGNOdent seems to be most useful for early non - cavitated occlusal lesions and a promising alternative tool in contrast to the probe (Lussi et al.,1999; Lussi et al.,2001). The latter study reported that excellent intra-observer agreement for in vivo measurements, but the inter-observer reliability was not always very good (Tranæus et al. 2004).

Chemical intervention - Imitation and complement of saliva function - The basis of remineralization therapy is utilization of saliva functions. If early caries lesions are found under the plaque, firstly we have to imitate the saliva buffering function to improve pH inside the lesions. Secondly, fluoride has to be supplied to enhance and promote the remineralization. Although saliva contains fluoride, its concentration is not high enough to empower crystal growth with calcium and phosphate ions..

The ability of human saliva to neutralize acid has already been assessed and determined to be due primarily to bicarbonate (Helm et al. 1982).. From this study bicarbonate accounted for approximately 50% of capacity for acid neutralization for resting saliva, while about 80% of the capacity for acid neutralization for stimulated saliva was due to bicarbonate. However, the use of exogenous carbonate may be questioned since dentine contains about 5 vol% by weight of carbonate, which is double the amount of enamel and gives rise to a higher solubility (Featherstone, 1994). Distribution and chemical inter-conversion among carbon species such as H_2CO_3 , HCO_3^- , CO_3^{2-} in water is well explored using chemical equilibrium models to study the carbonic acid system (http://darwin.nmsu.edu/molb_resources/tutorials/env-engr/carbonate/carbonate.html). Distribution patterns depend on mainly pH in the water. Considering the normal pH range of saliva around pH 7, bicarbonate ions, HCO_3^- , are the main chemical form. For example at a pH = 7.0 the relative concentrations of H_2CO_3 , HCO_3^- , CO_3^{2-} are 17%, 83% and less than 1.0% respectively. It has been proposed that bicarbonate ions can improve the pH inside the lesions, by penetration into the subsurface lesions spaces and becoming loosely absorbed onto the crystal surfaces (Nelson et al.1983). This absorbed bicarbonate may work as a buffer and inhibit the pH from decreasing and improve the pH inside the lesions. Combined

application of bicarbonate and fluoride during remineralization made the enamel samples more acid resistant (Tanaka and Iijima, 2001).

A small change in the fluoride concentration in the oral fluids such as saliva and plaque has a significant effect on the progression of de- and remineralization of enamel and dentine. In vitro studies indicated that levels of fluoride in the range of 0.05-0.1 ppm in the mineralizing solution in pH-cycling model may be satisfactory at enhancing remineralization (Featherstone et al., 1986). Later the minimal level of fluoride concentration decreased at the level of 0.03 ppm (Featherstone et al., 1991). They speculated from these in vitro studies that low concentrations in the range of 0.1-0.2 ppm fluoride in the saliva for prolonged periods of time may be very efficacious for caries control. Indeed, fluoride concentrations persist at around 0.15 ppm levels after bedtime application (Zero et al., 1988) Thus, it has been proposed that periods during sleep may be a very important time for remineralization to occur. This is of particular relevance considering that the pH of dental plaque remains in the alkaline range during periods of prolonged abstinence from ingestion of fermentable carbohydrates.

In conclusion it is expected that the combination of in - office bicarbonate and fluoride application followed by oral health care products to promote remineralization would produce additional remineralization effects. Clinically, it is essential to realize that the WS lesions with rather an intact surface layer can be remineralized through daily clinical practices which introduced here.

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Fig.1 Fluoride incorporation in remineralized enamel at baseline (A) and after 24-hour treatments in intra-oral environment (B), 1.0 mol/L KOH (C) or 0.01 mol/L lactic acid buffer pH 4.0 (D). From Iijima and Koulourides with permission. J Dent Res 1989; 68: 1298- 1292.

Fig.2 In vivo formed WS lesion was derived from the patient who has an experience of fluoride application and exposed to the plaque acidity for 2 weeks. From before and after microradiogram, it was obvious that the original surface of WS lesion is still remaining even after acidic conditions. Furthermore, mineral profile showed that there was no change of mineral vol% at the surface layer ca. 200 μm. From Iijima et al. with permission of Caries Res. 2000; 34: 388- 394.

The amount of mineral loss

Fig.3 Remaining mineral of both bicarbonate and fluoride applied groups during remineralization showed higher improvement in the acid resistance and the amount of remaining mineral was almost two times higher level. From Tanaka and Iijima , J of Dentistry. 2001; 29: 421- 426.

Relationship between dfs at baseline and DMFS levels

Fig.4 Relationship between dfs at baseline and DMFS levels after professional care plus self care at the age of 12 years. Despite of initial caries level of dfs, person with caries free in permanent teeth was over 80%.

Fig.5 Both numerical data of gray values and its distribution pattern changes in the region of interest (lower central incisors) were sufficient enough to monitor de- and remineralization of WS lesions.