Comparative studies of Products for Prevention of Demineralization: *A Clinical study*

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Introduction

Enamel demineralization associated with orthodontic treatment can and does occur when the patient is unable to adequately remove plaque adhering to the teeth adjacent to the orthodontic appliances [1-5,7-10]. The areas of demineralization are early carious lesions referred to as white spot lesions. The lesions appear clinically as white chalky areas on the facial surface of the tooth, often ending abruptly at the orthodontic bracket bonding area [5]. Treatment of the lesions includes remineralization with high levels of fluoride [1-5,7,9-10], but they will remain esthetically unpleasing. Camouflage with enamel bleaching[11] or esthetic veneering is often required for an acceptable appearance.

Prevention of enamel demineralization would certainly be a better choice than treatment of the lesion. Numerous preventive measures have been employed in the past [1-5,7-10]. These include: oral hygiene instructions [8], diet modification [10], fluoride releasing orthodontic products [5-6,9], fluoride varnishes [1-2,4], filled resin sealants[5], and chlorhexidine varnishes[4]. Efficacy varies with each technique, as some require patient compliance and others require continued application by the practitioner. An ideal preventive measure would be one that is implemented by the practitioner at the time of bonding with little or no change in the bonding protocol and requiring no patient compliance. Fluoride releasing bonding agents and sealants attempt this, but the fluoride ion concentration becomes deficient in a matter of weeks after bonding and requires recharging with high concentration fluoride gels thereafter [3-4,9].

A recent development of note is the use of organo-diselenide molecules as antimicrobials. The element selenium is known in medicine as a powerful antioxidant and has been linked with reduction in rates of inflammation and has potential uses as a chemotherapeutic drug[12]. Organic-diselenides also catalyze the formation of superoxide radicals (fig.1) resulting in an environment toxic to microbes. The half-life of the selenium containing free radical produced in the reaction is an extremely short 60 nanoseconds with an equally short path length of 35 nm, much shorter than an average bacterium. Selenium toxicity in humans has been studied and amounts as high as 400μg per day have been shown to be safe[13]. Due to these properties, the diselenide radical is only toxic to cells in close vicinity to their concentration. Coating a surface with these molecules creates an environment hostile to microbes due to the production of the diselenide radicals, and therefore formation of a biofilm on the coated surfaces is inhibited. Recently a line of orthodontic products was introduced (SeLECT Defense™, Element34 Technology Inc., Lubbock, TX) that contains organo-diselenide coated brackets, elastomeric ligatures, adhesives and bonding agents.

Potentially this technology would allow the practitioner to bond cases with no change in his or her protocol and reduce the need for additional preventive treatments during the course of treatment, saving both time and

money. For selenium technology to be effective, and efficient, at preventing white spot lesions it must either inhibit bacterial plaque on the enamel surface or act as a physical barrier between enamel and plaque, and it must do so for the duration of the orthodontic treatment. The system must demonstrate durability to the extreme pH of the oral environment, resistance to abrasion from mastication and daily oral hygiene, and maintain a continually effective concentration of the organo-diselenide compounds. The objective of this study was to investigate the effectiveness of SeLECT Defense Primer and SeLECT Defense Enamel Surface Sealant in preventing enamel demineralization in vivo.

METHODOLOGY

Teeth Preparation and *in situ* appliance fabrication

Following consent from the donors, freshly extracted human molar teeth was collected from various clinics of the Dental School of the University of Texas Health Science Center at San Antonio (UTHSCSA). The teeth were cleaned of debris/stains, and examined with transilluminator to eliminate teeth caries, cracks or enamel malformations. Selected teeth were cleansed with pumice to remove the remnants of pellicle from the buccal surface. 180 enamel blocks, each measuring approximately 3 mm x 2 mm, were produced from the selected teeth. A tooth slice (~150 μm thick) was cut from each tooth block using water-cooled diamond wire saw. This slice was used as control slice (Pre-test slice) for confirmation of absence of any demineralization in these blocks. Following this, each block was used to fabricate an *in situ* appliance, which was used to carry the enamel tooth block on a tooth surface in a human subject. The appliance is based on the design of brackets used in orthodontics, and consists of an orthodontic molar pad with retentive mesh backing, which has a rectangular stainless steel band welded to it to form a box within which the test block is retained using Intermediate Restorative Material (IRM) cement (fluoride–free). The surface of the enamel block was covered by Dacron gauze to encourage plaque growth and accumulation. All appliances were sterilized with ethylene oxide prior to intra-oral application.

Study protocol

Thirty fit and well adults (12 males, 18 females), aged l8-50 years, from different ethnic origins and from varied socioeconomic status, took part in this study. The sample size calculation and justification is described under data handling. The subjects were identified with code numbers (SD01 to SD30). The approval of the Institutional Review Board (IRB) of University of Texas Health Science Center at San Antonio, was obtained. Subjects were recruited based on the following criteria.

To be included in the study, subject must:

1. read and sign a written informed consent form that explains the study.
2. have at least 20 teeth exposed to the oral environment
3. have experienced caries, but will exhibit no unrestored cavitated caries.
4. have unrestored enamel on the buccal surface of at least one lower first or second permanent molar, which will be serve to carry the *in situ* appliance since they are at a suitable distance from the major salivary glands, and will minimize the possibility of irritation to the surrounding mucosa.

5. accept to give a full medical and drug history
6. have normal salivary flow rate (stimulated and unstimulated flow of $\geq$0.7 ml/min and $\geq$ 0.2 ml/min respectively) ascertained from a preliminary sialometry.

The following category of subjects were excluded:

1. Taking any antibiotics or medication that could adversely affect the salivary flow rate.
2. Patients with intermittent swelling of salivary glands, local disease (oral candidosis, lichen planus, etc), and Sjögren's syndrome patients.
3. Without sound mandibular molars to carry the appliance

Intra-oral procedure: The experiment comprised of two distinct phases during which the subjects wore two enamel blocks and were exposed to the following treatments in a double blind randomized crossover design as shown in table 1. That is, while some subjects are in G1 phase, others will be in G2.

G1: one block coated with SeLECT Defense enamel Sealant and the other non-coated.

G2: one block coated with SeLECT Defense Primer and the other coated with chlorhexidine varnish.

Subject Codes	Phase 1 (28 days)	Phase 2 (28 days)
SD01 - SD15	G1	G2
SD16 - SD30	G2	G1

Table 1

In each phase the enamel block in the appliance was subjected to the demineralization process for 28 days by exposure in oral cavity. The appliance was fitted by a qualified dentist licensed in the state of Texas, who was different from the laboratory assistant that processed and analyzed the samples to produce the final data. In order to fit the appliance in the mouth of a subject, the buccal surface of the mandibular molar tooth (first or second) chosen to carry the appliance was carefully etched for 30 seconds, in accordance with current principles of dental practice, washed and dried for a further 30 seconds, and isolated using cotton rolls. The bottom of the appliance was loaded with SeLECT Defense light cure adhesive paste, and carefully positioned to avoid causing occlusal interference and to avoid soft tissue irritation. The excess composite material that spilled out from the sides of the appliance was used to cover the sides, beveling it to present a comfortable streamlined (non-catching) surface, when the slab comes in contact with a soft tissue surface (*e.g.* tongue). The adhesive paste was cured using visible Light Curing Unit applied for 20 s.

Following the fitting of the appliance, each subject was provided with fluoride toothpaste and a toothbrush to be used for routine oral hygiene. The subjects brushed their teeth two times daily for 2 minutes on each occasion, preferably morning and last thing before bed. In order to monitor this brushing, a diary will be provided to each subject to record the number of toothbrushing performed each day and the time it was done. Further, subjects were instructed to return the remaining toothpaste after each study phase; the weight of toothpaste was measured before and after each study phase. All subjects were asked to maintain their normal dietary habits; however, the use of any other oral hygiene product was prohibited. At the end of each 28 days period, the appliance was detached and sent to the laboratory for analysis. The next appliances (G2) were cemented in place on the same tooth and on the same dental arch as the first appliance and were worn for 28 days.

Post-Treatment Processing: In the laboratory the blocks were removed from their respective appliances, and an enamel slice (Post-test slice) was cut from each block. Both the pre-test and post-test slices were processed for microradiography as follows. Using a multiprep grinding/polishing system (Allied high tech, CA, USA), both sides of each slice were polished to achieve planoparallel surfaces as well as reduce the thickness of the slice to 80 µm (the appropriate thickness for TMR). Copious amount of water was used during the polishing process both for lubrication and to wash away the slurry created.

Identification of demineralization

Using TMR: The polished control slices were placed in a specially fabricated radiographic plate-holding cassette, incorporating an aluminium step wedge (10 steps of 24.5 µm thickness). The cassette was loaded with type lA high resolution glass X-ray plates (Microchrome Technology, CA, USA) and placed into a Phillips x-ray generator system set up for this purpose. This apparatus is equipped with a copper target and nickel filter, producing monochromatic radiation of wavelength appropriate for hydroxyapatite (l84 Angstroms). The plates were exposed for 10 minutes at an anode voltage of 20kV and a tube current of 10 mA, and then processed. Processing consists of a 5 minute development in Kodak HR developer and 15 min fixation in Kodak Rapid-fixer before a final 30 minute wash period. After drying, the microradiographs were subjected to visualization and image analysis using a computer program (TMR2006 version 3.0.0.6). The enhanced image of the microradiograph was analyzed under standard conditions of light intensity and magnification and processed, along with data from the image of the step wedge, by the TMR program. By this method, the parameters of integrated mineral loss (Δz, vol%.µm) and lesion depth (LD, µm) were quantified for any demineralized surface. This process yielded the following information:

1. The post-test TMR parameters (Δz_2 and LD_2) of any demineralization.
2. The pre-test and post-test TMR images of the lesions.

Using Polarized Light Microscopy (PLM): The tooth slices were further visualized using PLM, which is a histological technique that detects the earliest stage of demineralization.

Results and conclusions

The results of the study as observed with PLM and TMR are shown in figures 1-4 and figures 5-8 respectively. Both devices showed demineralization on tooth blocks that were not protected (control) and those coated with chlorhexidine but not on those with SD primer or sealant coating. Thus SeLECT Defense Primer or Sealant applied alone on tooth surface was effectiveness in preventing enamel demineralization in vivo.

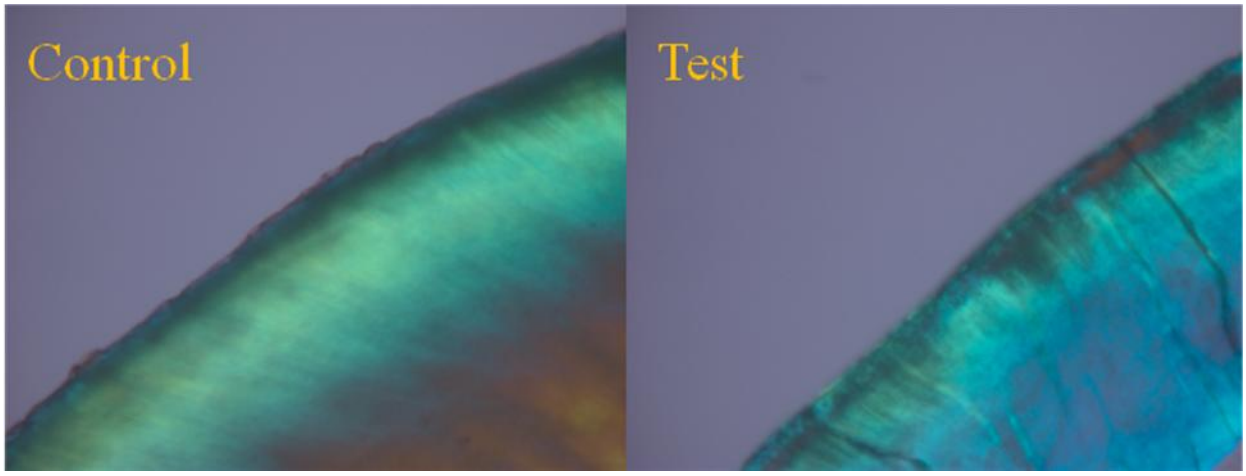

Figure 1: PLM image of tooth slice from the control not protected with any material. Note the subsurface demineralization in test slice, typical histology of whitespot lesion.

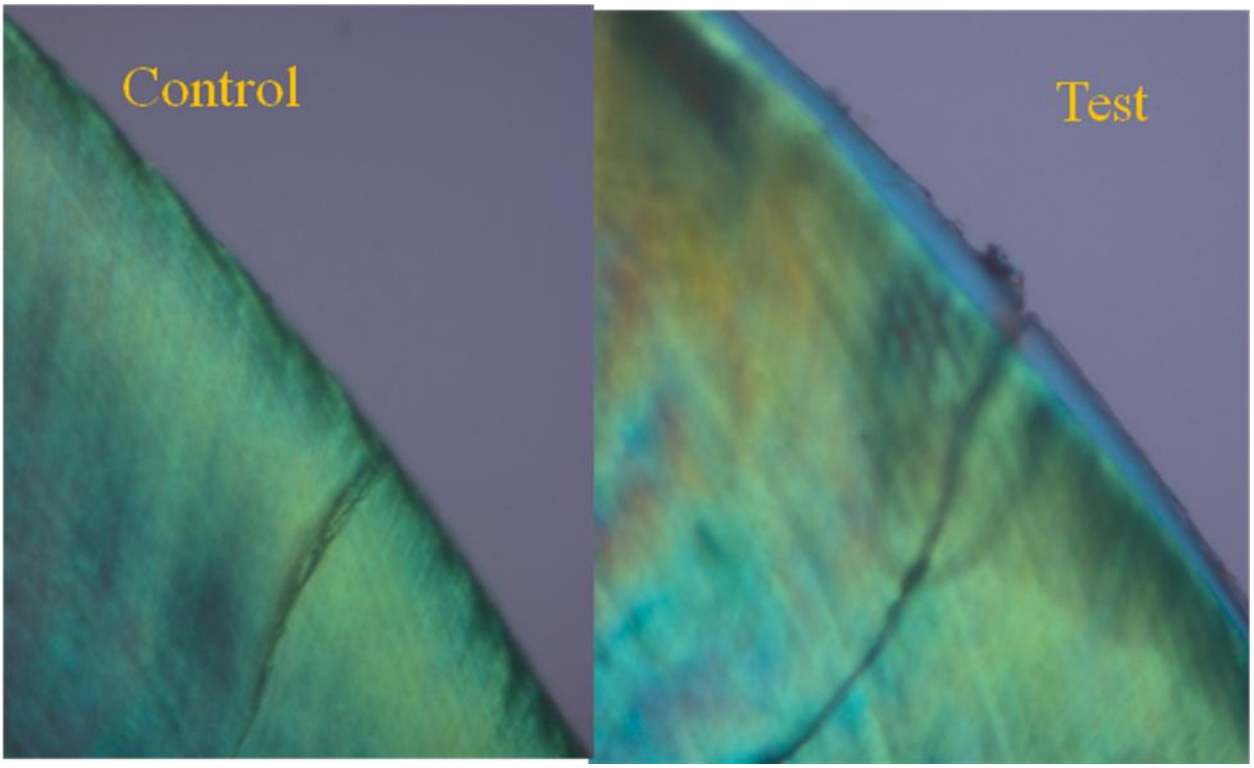

Figure 2: Blocks coated with SeLECT Defense Enamel Sealant. Note the layer of the SD sealant that protected the tooth surface against demineralization.

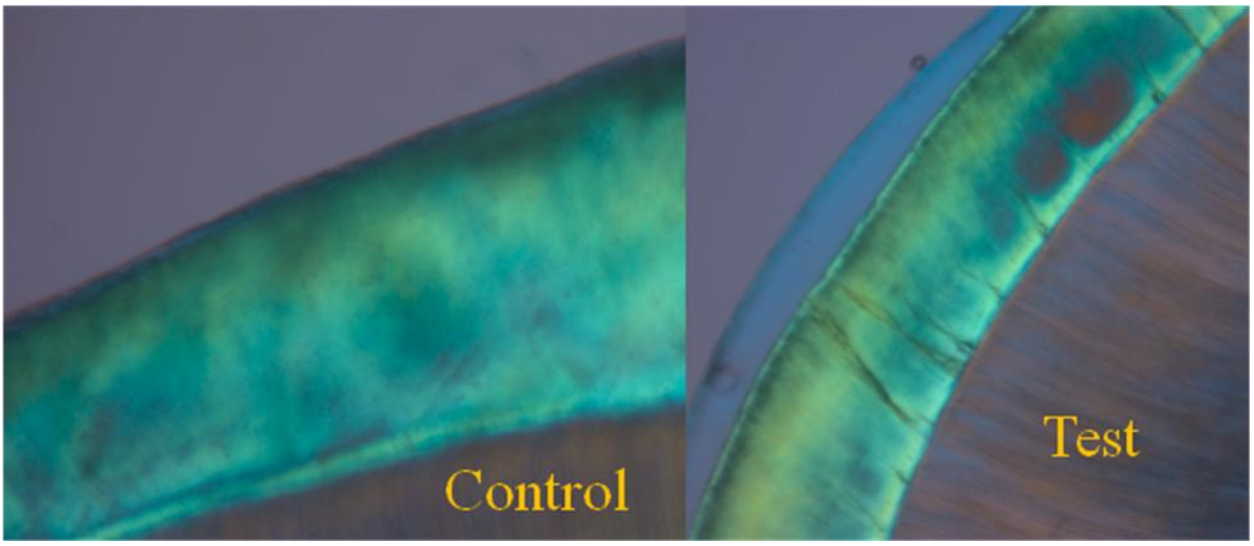

Figure 3: Blocks coated with SeLECT Defense Primer. Note the layer of protective primer in test slice.

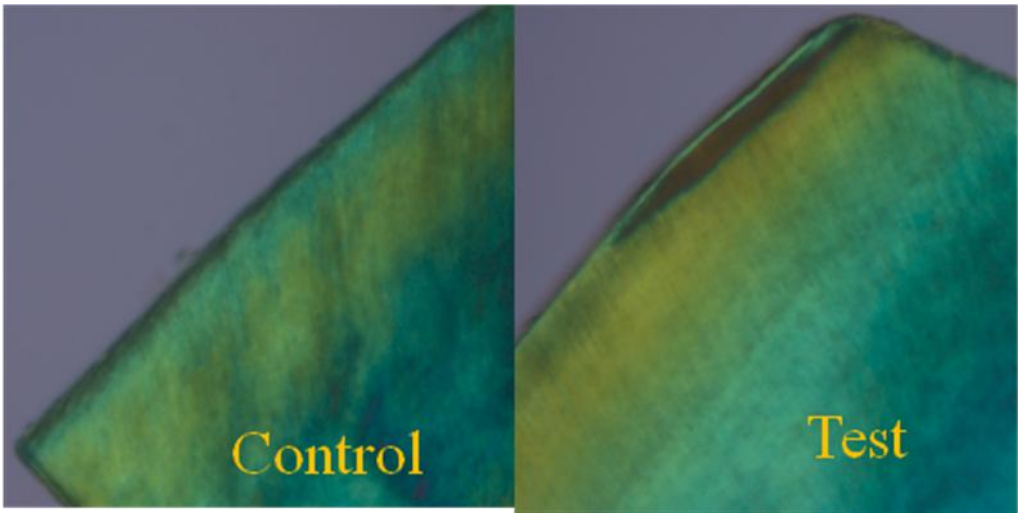

Figure 4: Blocks coated with Chlorhexidine Varnish. Note the subsurface demineralization in test slice, typical histology of whitespot lesion.

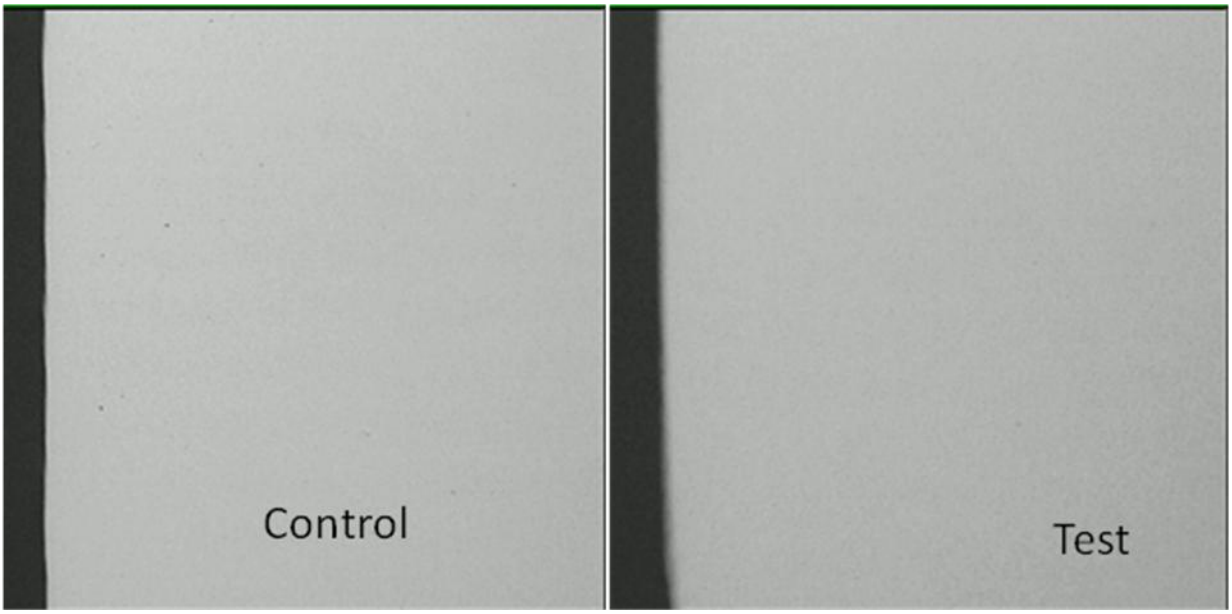

Figure 5: TMR image of tooth slice from the control not protected with any material. Note the surface demineralization in test slice.

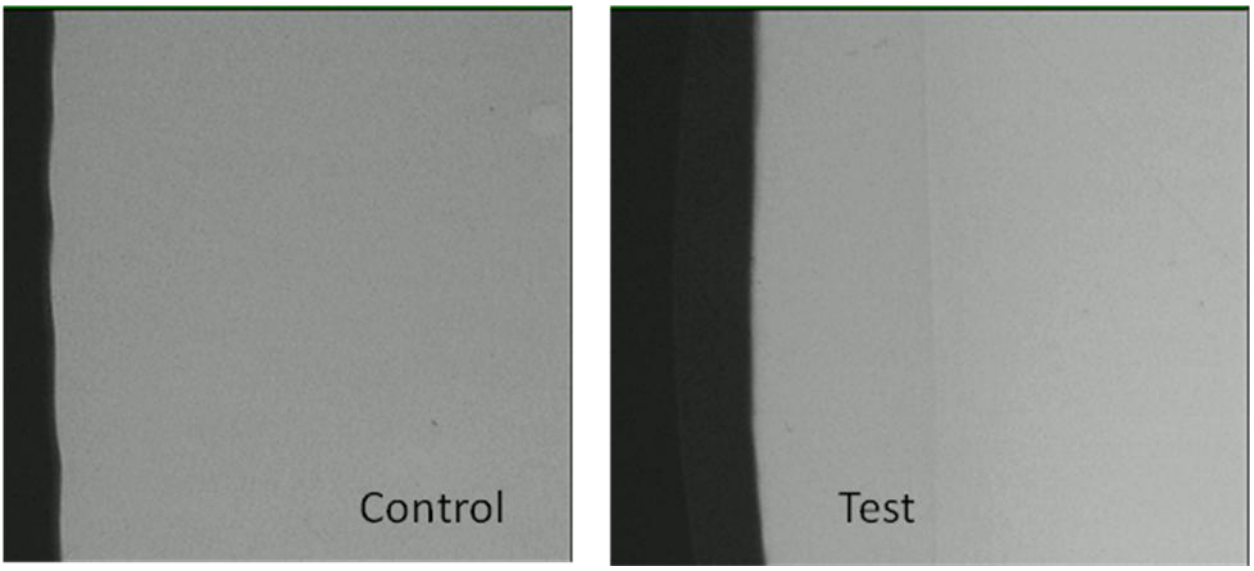

Figure 6: TMR image of tooth slice protected with SeLECT Defense Primer. Note the layer of protective primer in test slice.

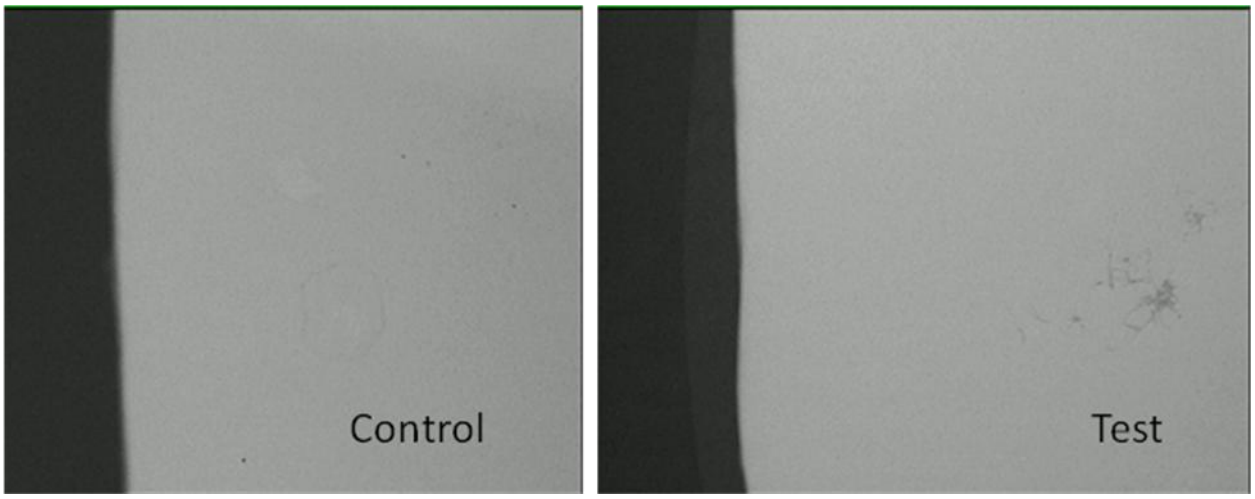

Figure 7: TMR image of tooth slice coated with SeLECT Defense enamel surface sealant. Note the layer of protective sealant in test slice.

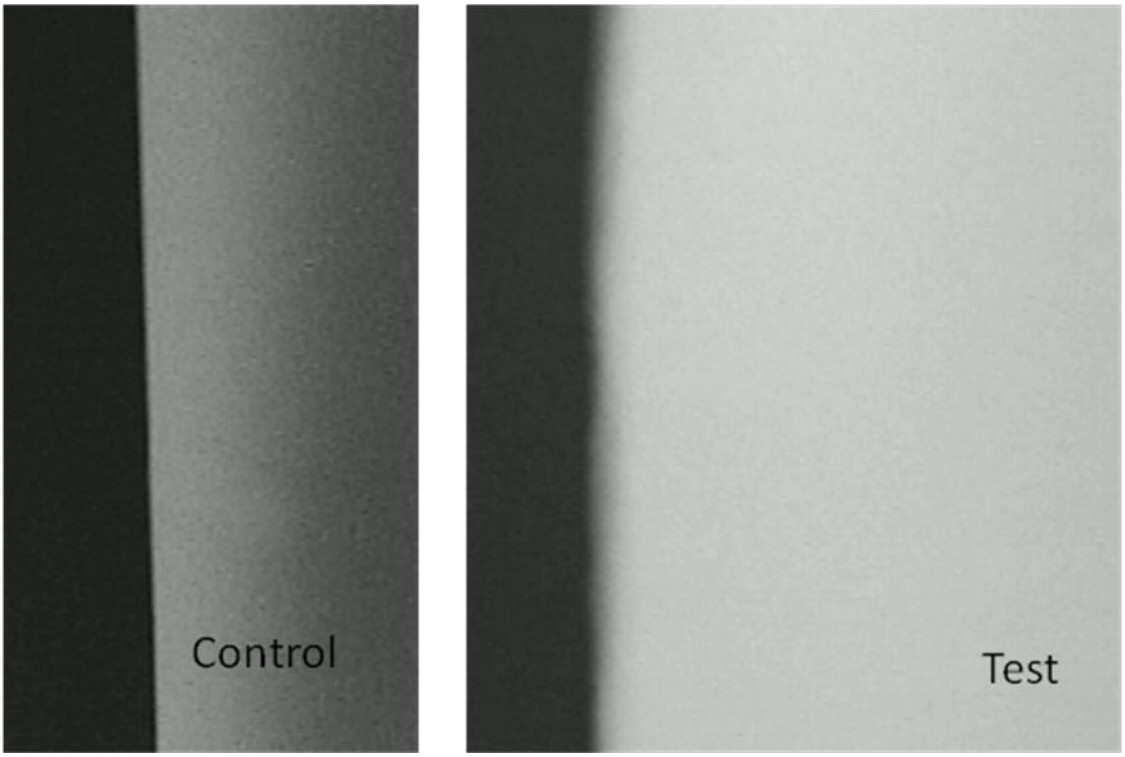

Figure 8: TMR blocks coated with Chlorhexidine Varnish. Note the demineralization on tooth surface in test slice.

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