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ORIGINAL RESEARCH

Morphological Changes in the Caries Excavated Dentin Prepared by Rotary, Carisolv™ and Er, Cr: YSGG Laser (Biolase™) Instruments: A Scanning Electron Microscopic Evaluation

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ABSTRACT

The *in vitro* study presented in this article was performed to evaluate the morphological changes in caries excavated dentin after using three different caries removal methods. These methods include mechanical, chemomechanical (Carisolv™) and Er, Cr: YSGG Laser (Biolase™) by means of scanning electron microscope. Extracted carious human molars were ground to flat surfaces to expose caries surrounded by sound dentin. The caries were then removed with tungsten carbide bur and airotor handpiece (G1), by chemomechanical (Carisolv™) method (G2), and by irradiation with an Er, Cr: YSGG Laser (Biolase™) unit (G3). The samples were then observed under a scanning electron microscope (SEM) with different magnification powers (×800, ×2000 and ×5000). SEM study revealed a considerable difference in the surface characteristics of the dental tissue. The surface which was treated with Er, Cr: YSGG laser (Erbium, Chromium: Yttrium – Scandium – Gallium – Garnet) showed the best result with no residual smear layer followed by surfaces which were chemomechanically excavated with Carisolv™ gel. The treatment with mechanical method resulted in a surface with a smear layer without any micro retention. Thus, from the results obtained, it was concluded that Er, Cr: YSGG laser produced a scaly, irregular and rugged tooth surface without smear layer, which can be of highly retentive nature. Carisolv™ treated dentin showed uneven surfaces with many undermined areas. There were partially patented dentinal tubules and residues of contaminant smear layer. The dentin surfaces prepared using tungsten carbide bur and spoon excavator produced smearing and smear plugs in the tubular orifices.

Keywords: Er, Cr: YSGG Laser, Carisolv, Scanning electron microscope, Caries-affected dentin.

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INTRODUCTION

Dental caries is ubiquitous in all population and is the key factor responsible for dental pain and tooth loss throughout the world.[1] It is a process that may take place on any tooth surface in the oral cavity, where dental plaque is allowed to develop over a period of time.[2]

Caries dentin removal by mechanical means is a non-specific nature of excavation that may result in excessive loss of tissue thus, affecting the prognosis of the treated tooth.[3] Other inherent fundamental drawbacks of this approach are unpleasantness to patient, need for local anesthesia and potential effect to the pulp due to heat and pressure.[4]

The advent of adhesive dentistry has enormously simplified the guidelines for cavity preparation. The design and extent of the current preparations are basically defined by the extent and shape of the caries lesion extended by slightly beveling the cavity margins in order to meet the modern concept of minimally invasive dentistry.[5] The superficial necrotic zone of caries-infected dentin that harbors the core bacterial biomass should be excavated, leaving only residual caries-affected dentin lining the cavity with sound enamel margins and dentin adjacent to the enamel dentin junction. This process enables the best peripheral seal to be achieved with the current adhesive dentin bonding system.[6]

New caries excavation techniques have been introduced as plastic and ceramic burs, caries-disclosing dyes, enzymatic caries dissolving agents, sono-abrasion, air abrasion, laser ablation, photoactive disinfection (PAD), ozone therapy and caries infiltration of low viscosity resin. All the techniques aim toward the removal of caries-infected tissue as selectively as possible by being minimally invasive through maximum preservation of caries-affected tissue. Minimal invasive preparation technique claims to achieve controlled removal of infected and softened dentin while preserving healthy and hard dental tissues and causes minimal discomfort to the patient.[7]

Chemomechanical caries removal system uproots only the softened carious dentin preserving the healthy tissue. The Carisolv™ system is based on the nonspecific proteolytic effect of sodium hypochlorite into which three naturally occurring amino acids; glutamic acid, leucin and lysine are incorporated to form a gel. These amino acids are responsible for the chlorination of partially degraded collagen in carious dentin after which they initiate the disruption of the altered collagen fibers in carious dentin.[8]

Carbon dioxide, Holmium, Neodymium and Erbium lasers were used for the removal of carious dentin.[9] Among these, Erbium lasers are more commonly used because they can more effectively ablate enamel and dentin due to their highly efficient absorption power in both water and hydroxyapatite. The Erbium frequency of 2.78 and 2.94 μm are primarily absorbed by water as the target chromophore. Ablation of tooth structure is primarily driven by absorption of laser energy by the interstitial water in enamel and dentin. Explosive expansion of the interstitial water disrupts the hard tissue structure along their weakest planes of cleavage. Er, Cr: YSGG laser would be favorable for caries removal because it does not damage dental pulp tissue.[10]

During cavity preparation, the surface of bond will be covered by a smear layer, which is not attached firmly to the tooth surface. Bonding effectiveness may be impaired by thick smear layers. The recognition of the role of smear layer in dentin bond strength highlights the importance of the cavity preparation method.[11]

This *in vitro* investigation was undertaken by means of scanning electron microscope to evaluate the morphological changes in the caries excavated tissue after using the three different caries excavation techniques namely mechanical, chemomechanical (Carisolv™) and laser (Er, Cr: YSGG).

MATERIALS AND METHODS

Sampling Procedure

Thirty extracted cavitated human permanent molars were used in this study. Teeth with caries lesions limited to the occlusal surface and extending at least half of the distance from the enamel-dentin junction to the pulp chamber were included. Only central dentin portion that is located directly above the pulp was used in order to minimize any regional variation between the periphery and the central dentin substrate.[12] These characters were determined by visual and radiographical inspections.

Sample Preparation

The specimens were ground perpendicular to the long axis to expose a flat surface containing a central zone of caries-infected dentin surrounded by sound dentin using a low speed diamond saw under running water. The exposed flat surfaces were then polished with a wet 600-grit silicon carbide paper (Fig. 1).

Experimental Groups

The teeth were randomly divided into three groups (n = 10), according to the caries removal methods. The groups were as follows:

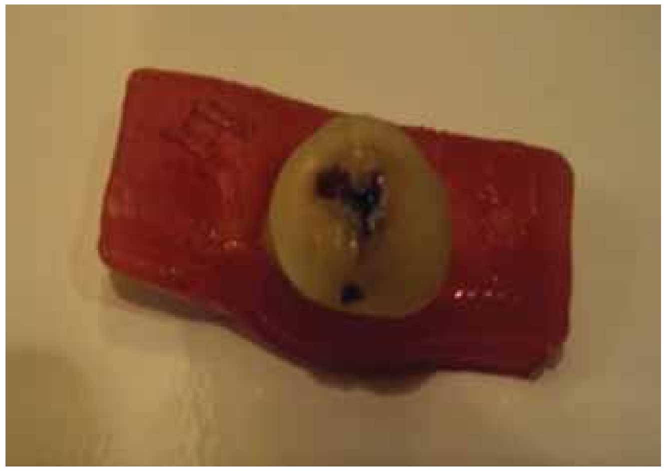


Fig. 1: Extracted carious tooth

Group I: Mechanical rotary preparation by diamond bur/ air turbine

Group II: Chemomechanical preparation with Carisolv™ gel

Group III: Laser preparation by Er,Cr:YSGG laser (Biolase™ Waterlase MD).

The protocol for preparations was strictly followed according to the manufacturer's instructions. The infected dentin was removed till clinically detectable hardness of the dentin was felt.

Mechanical Rotary Excavation (GI)

A round tungsten carbide bur was used in airotor handpiece to remove carious tissue. The full extent of carious dentin including a periphery of sound dentin was excavated. A straight spoon excavator was used for softened caries removal. Tissue removal was terminated when the soft dentin has been removed from the cavity surface as clinically detected by using a probe to check the firmness.

Chemomechanical Preparation with Carisolv™ (G2)

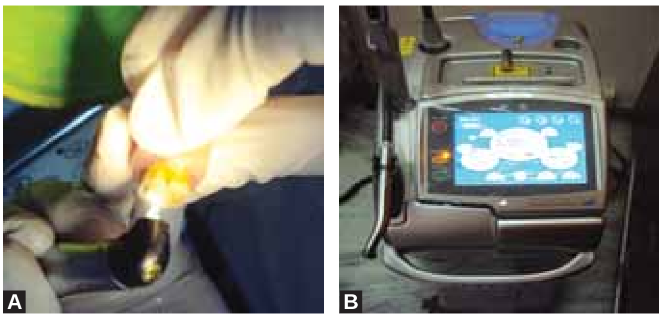
Caries excavation using Carisolv™ was done as per manufacturer's instructions. Premixed gel was introduced into the cavity for 40s. The mixture was agitated against the dentin using a metal mace tip instrument. Once the gel became cloudy with a muddy consistency, it was rinsed away. A second fresh mix of gel was then applied and the mixture was further agitated. Excavation was deemed complete when the gel failed to become cloudy. Finally, the cavity was checked with a dental probe for hardness (Figs 2A to C).

Laser Device Biolase™ (G3)

Er, Cr: YSGG laser (Biolase™ Waterlase MD) with a power output of 3.5 W, pulse duration of 140 μs, frequency of

Figs 2A to C: Chemomechanical preparation with Carisolv™ and special instruments

20 Hz, energy density of 175 mj, and a Turbo handpiece with MX700 micron focusing lens were used. The non-contact mode was used at a distance of 3 to 5 mm. Cooling was obtained by setting the equipment at the 80% air and 70% water level (Figs 3A and B).



Figs 3A and B: Laser preparation with Er,Cr:YSGG laser (Biolase™ Waterlase-MD)

Scanning Electron Microscopy (SEM)

After caries excavation, the samples were prepared for scanning electron microscopy (JEOL JSM—5600 LV) (Fig. 4). These specimens were immersed in 4% glutaraldehyde solution for 1 hour at room temperature which were then rinsed with distilled water. The samples were then placed in cold buffer solution of sodium cacodylate for 90 minutes to fix the organic matter after which, specimens were dehydrated in ascending grades of ethanol (30, 50, 70, 80, 95 and 100%) for 1 hour in each series and then dried in a critical point drier-based desiccator. The dried specimens were mounted on a metal stand and gold sputter coated (200-250 nm) by cathode atomization under vacuum. SEM images of the caries excavated surfaces were obtained. For each specimen, three microphotographs with different magnifications (×800, ×2000 and ×5000) were made. Each SEM photomicrograph was evaluated, described and the morphological findings were compared.

Fig. 4: Scanning electron microscope (JEOL. JSM-5600LV)

RESULTS

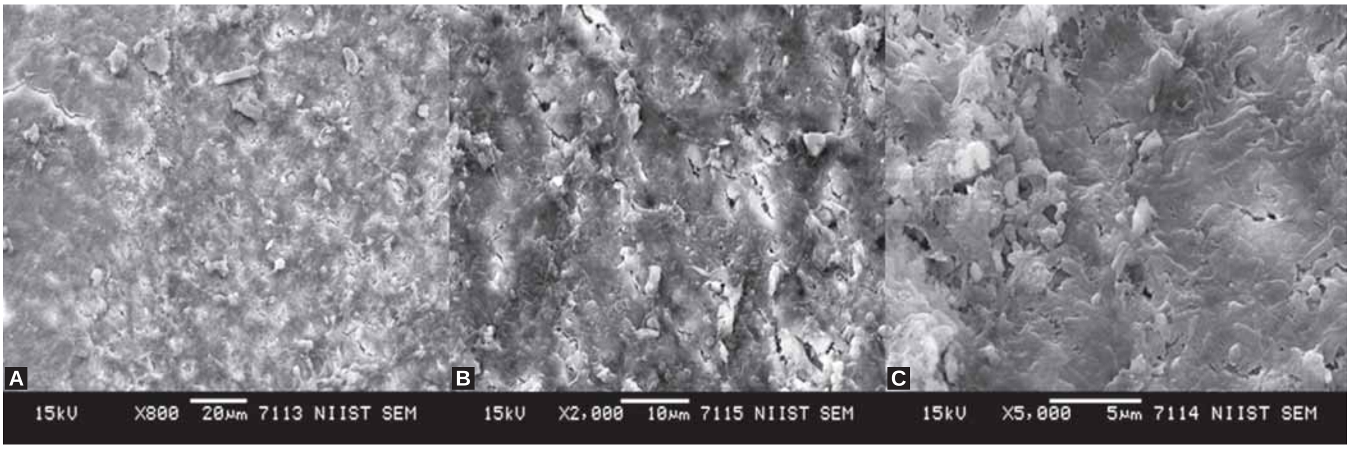
Morphological analysis of the cavity floor prepared with mechanical method appeared rougher with irregular particles scattered throughout the surface area. A well-defined smear layer was detected and, in some areas, missing smear layer was observed (Figs 5A to C). In the areas of water turbulence; there were patent dentinal tubule orifices but they were without a clear outline of both tubule lumens and peritubular and intertubular dentin.

Carisolv™ produced a reduced and more inconsistent smear layer with large areas of open tubular orifices. The dentinal tubule orifices were visible and there were almost no smear layer (Figs 6A to C). Preparing the organic matrix using chemomechanical preparation with Carisolv™ and protecting mineralized dental tissue at the same time resulted in rough appearance of the treated surface and considerable microretention development. Denatured collagen fibers and surface contamination occurred in few areas, blocking the dentinal tubule orifices.

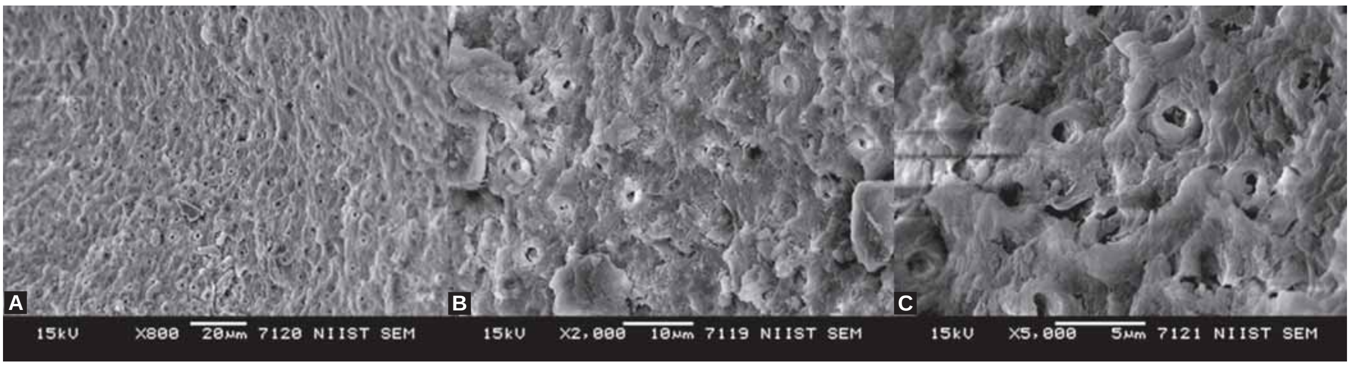
The dentin surfaces that received Er, Cr: YSGG laser irradiation showed a scaly, irregular and rugged appearance (Figs 7A to C). There were rough and irregular surfaces with no smear layer and dentinal tubule orifices were open without widening. The peritubular dentin protruded slightly from the surrounding intertubular dentin.

DISCUSSION

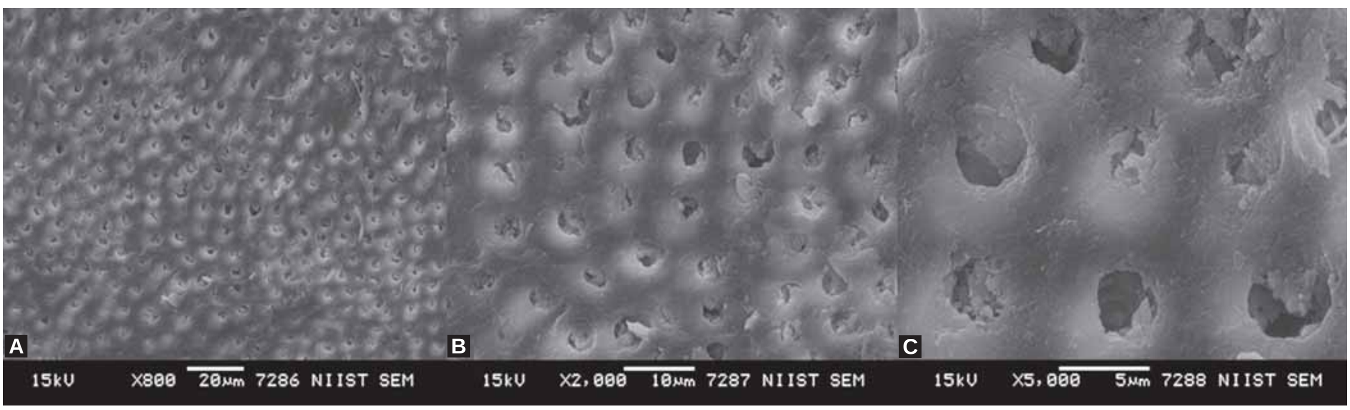
Minimal invasive dentistry has gained popularity with the developments of new adhesive systems and technological



Figs 5A to C: SEM images of tooth prepared with TC bur (×800, ×2000 and ×5000)



Figs 6A to C: SEM images of tooth prepared with Carisolv™ (×800, ×2000 and ×5000)



Figs 7A to C: SEM images of tooth prepared with Er,Cr:YSGG laser (Biolase™) (×800, ×2000 and ×5000)

improvements in tooth preparation.[13] Minimal invasive techniques claim to be able to achieve controlled removal of infected and softened dentin, while preserving healthy and hard dental tissues and perform with a minimal discomfort to the patient.[7] Fusayama (1966) claimed that 'cariogenic bacteria were never found beyond the softened front of dentin'. Elimination of the heavily infected dentin and preservation of the residual affected dentin were thus, defined as prerequisites for effectively arresting the carious process without harming the long-term survival of the pulp and the restoration.[5] This will enable the best peripheral seal to be achieved with the current adhesive dentin bonding agents.[6] The superficial necrotic zone of caries-infected dentin which harbors the core bacterial biomass should be excavated leaving only residual caries-affected dentin lining the cavity with sound enamel margins and dentin adjacent to the enamel-dentin junction. The transition zone between infected and affected dentin is difficult to assess.[14]

Clinically determined hardness of a dentin lesion is a sufficient prerequisite for the success of a restoration.[15] Change in color is not a good indicator as gradual pathologic changes are not consistently color dependent.[6] Defining the actual end point of caries excavation is the start point of restoration and is often clinically challenging.[15]

EAM Kidd (2004) suggested that carious dentin should be removed up to the level of firm.[14] The adhesives that are available today, bind effectively with sound dentin through hybridization but bonding to the caries-affected dentin is less predictable and durable. This is due to the presence of wider zones of unprotected collagen as well as cracks and pores.[16]

The smear layer in caries-affected dentin may be more resistant to the action of self-etching primers, as they include acid resistant crystals and extrinsic proteins that have permeated into the mineral phase during demineralization cycles. If a residual smear layer is left on the surface, the adhesive resin will bind to crystals within it rather than to underlying dentin. Moreover, the acidity of the primer could also be buffered by the mineral content of the smear layer. DH Pashley (1995) found that the presence of mineral casts within the tubules of caries-affected dentin would prevent the formation of resin tags in dentinal tubules and it is believed that tags are thought to contribute to bond strength.[10]

Conventional excavation with burs and spoon excavators are the most popular caries excavation techniques. The selective removal of caries-infected dentin cannot be easily achieved through this method due to relative tactile insensitivity and operator variability; which leads to varying quantities of tissue removed. A major factor to consider in terms of restoring a cavity with current adhesives are the final dentin surface characteristics, such as surface roughness and the presence of smear layer, that can affect the final bond and the seal.[6]

The chemomechanical caries removing agent Carisolv™ was used in the study. It consists of a gel with amino acids and sodium hypochlorite, which contains 0.5% w/v sodium hypochlorite, 0.1 M of an amino acid mixture (glutamic acid, leucine and lysine) and water.[5] It is available in two tubes system and the contents of the tubes were mixed and introduced into the cavity using specific instruments provided by the manufacturer. The gel was applied in the cavity, after which the carious dentin was scrapped off using specially designed noncutting hand instrument.[4]

Lasers have been studied in recent years as a possible replacement for current caries excavation methods.[17] Erbium lasers have been pointed out as the most promising tool in the field of operative dentistry due to their specificity in ablating enamel and dentin without side effects to the pulp and surrounding tissue. The wavelength emitted by Er, Cr: YSGG laser (2.78 µm) coincides with the absorption peak of water and is well absorbed by all biological tissues including enamel and dentin, bone and soft tissue, causing a rapid explosive expansion of the water. This interaction of the laser with water and the subsequent effect is known as the hydrophotonic effect.[13]

Lee et al (2007) claimed that the dentin irradiation with laser power output above 3.5 W would exhibit microcracks under SEM examination.[18] Hence, in this study, dentin surfaces were irradiated with a laser power output of 3.5 W to avoid the microcracks. Frequency is fixed at 20 Hz. Air pressure level of 80% could create the roughest surface and the water pressure level was elevated to 70% to get a least charred or carbonate dentin surface.[10]

Er, Cr: YSGG laser irradiation produced a scaly, irregular and rugged appearance of dentin in SEM evaluation. Absence of smear layer covering and partially closed dentinal tubule orifices were observed. The peritubular dentin was found protruding from the intertubular dentin. It could be due to the higher mineral content and the lower water content of peritubular dentin. Signs of carbonation were not found. The marked surface irregularities and lack of smear layer provide a concrete evidence for the physical mechanism of bonding with composite material after laser treatment.

The dentin surface treated with Carisolv™ observed under SEM in the present study showed uneven surface with many undermined areas. There were partially patented dentinal tubules and residues of contaminant smear layer covering the dentinal surfaces. The dentin topography after Carisolv™ treatment was granular and rough comparable to laser prepared dentin. This surface roughness and structural changes may play a crucial role in adhesion to composite material.

The scanning electron microscopic examination of the dentin surfaces prepared using tungsten carbide bur and spoon excavator produced smearing and smear plugs in the tubular orifice. This method leaves a homogenous smear layer with more or less uniform roughness, and dentinal tubules visibly obstructed with smear plugs. With regard to bonding receptiveness, the smear covered surface does not interfere with the etch-and-rinse adhesive, but may reduce the bonding effectiveness of self-etching adhesives.

CONCLUSION

The morphological analysis of the caries excavated dentin observed in this study leads to a conclusion that cavity preparation with Er, Cr: YSGG laser and Carisolv™ are consistent with the principles of minimally invasive preparation, providing clean surfaces and strong micro retentions ideal for adhesive restorations. The laser treated samples showed no evidence of thermal damage or signs of carbonization or melting. As laser-prepared surfaces are cleaner without smear layer, it could be assumed that current adhesives could improve adhesion on laser-prepared surfaces.

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