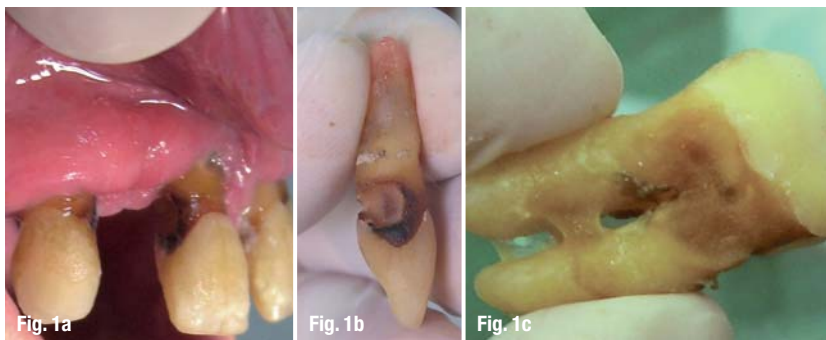


Morphological changes in hard dental tissue prepared using the Er:YAG laser

Author Drs Snejana Ts. Tsanova & Georgi T. Tomov, Bulgaria



Figs. 1a–c _Extracted teeth with carious lesions.

_In recent years, prevention and early caries detection, as well as shifts in the understanding of the chemical and biological basis of the demineralisation process in hard dental tissue and the possibility of carious lesions undergoing remineralisation, have superseded the classical operative approach to caries treatment postulated by Black and promoted minimally invasive preparation (MIP). The main categories of MIP techniques include rotary handpieces and burs, chemomechanical cleaning with Carisolv gel, air abrasion and dental lasers.^{1,2} The trend for alternatives to the conventional method of preparation led to a focus on the impact of alternative techniques on hard dental tissue and underlying dental pulp. MIP techniques claim to be able to achieve controlled removal of infected and softened dentine while preserving the healthy, hard dental tissue and do so with minimal discomfort to the patient. However, current data provides contradictory evidence of the impact of MIP techniques on hard dental tissue compared with conventional preparation. Possible reasons for this are the variety of experimental studies and difficulties in standardising the results of clinical

research. It is worth noting that the studies that have given the most positive evaluation of the alternative methods of preparation (Carisolv, laser) use mainly clinical criteria for evaluation (patient's perception and tolerance, noise, atraumatic work, colour and texture of the dentine when probing, etc.), which are all rather subjective. While new, improved versions of alternative systems for preparation on the market claim to be highly clinically efficient, there is still little information about them (the modified Carisolv colourless gel, multi-frequency, high-energy lasers, air-abrasion). This makes it necessary for research in this rapidly developing, promising field of dentistry to be updated periodically. The objective of the present *in vitro* study was to evaluate by SEM the ultrastructural changes in hard dental tissue treated with several alternative systems for caries removal and preparation.

_Materials and methods

The study used 20 human teeth, freshly extracted because of advanced periodontal disease. The preparations involved natural carious lesions on tooth surface (Figs. 1a–c). The teeth were divided into four groups of five teeth ($n = 5$) according to the preparation technique:

- Group 1:** Mechanical rotary preparation with steel burs/micromotor;
- Group 2:** Mechanical rotary preparation with diamond burs/air turbine;
- Group 3:** Chemomechanical preparation with Carisolv colourless gel (MediTeam AB; Figs. 2a–c);
- Group 4:** Laser preparation by Er:YAG laser (Lite-

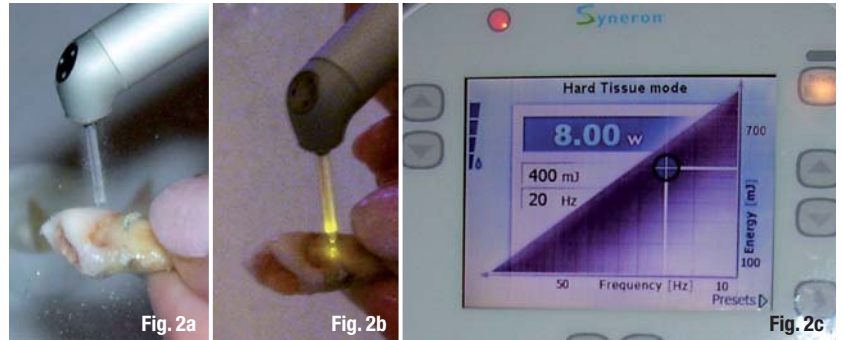
Touch, Syneron; Figs. 3a–c). Preparation was done strictly according to the manufacturers' instructions. The removal of caries was confirmed clinically through observation and probing. After preparation, the teeth were immersed in a 4% buffered glutaraldehyde fixative solution (0.075 M, pH 7.3) for one hour. They were then rinsed in distilled water and placed in a cold sodium cacodylate buffer (0.02 M, pH 7.2, 660 mosm) for 90 minutes for fixation of the organic matter. Subsequent dehydration was carried out through an ascending series of ethanol concentrations (30, 50, 70, 80, 95 and 100%) for one hour per series. The teeth were critical point dried in a desiccator. The dried specimens were then mounted on a metal stand and gold-coated (200–250 nm) by cathode atomisation under vacuum.

Scanning microscopy was performed using an electron microscope (515SEM model, Philips), with accelerating voltage of 25 kV in secondary emission mode. For each specimen, we took five photographs of randomly chosen areas with the same magnification (x 2,000) and various photographs at a different magnification. Using the SEM photomicrographs, we evaluated, described and compared the morphological findings and differences in the enamel and dentine tissues after treating the teeth using alternative methods for caries removal and cavity preparation.

Results

When analysing the SEM photomicrographs of the specimens examined, we found that the conventional method of cavity preparation with steel burs and micromotors at low speed without water-cooling (group 1) resulted in a contaminated surface with a thick smear layer of dentine debris without visible dentinal tubule orifices on all treated surfaces (Figs. 4a & b). The walls of the cavities were smooth and rounded and the border between enamel and dentine hardly noticeable.

Preparation with diamond burs, an air turbine and water-cooling (group 2) yielded a thin,



Figs. 2a–c Chemomechanical preparation with Carisolv colourless gel and hand excavators.

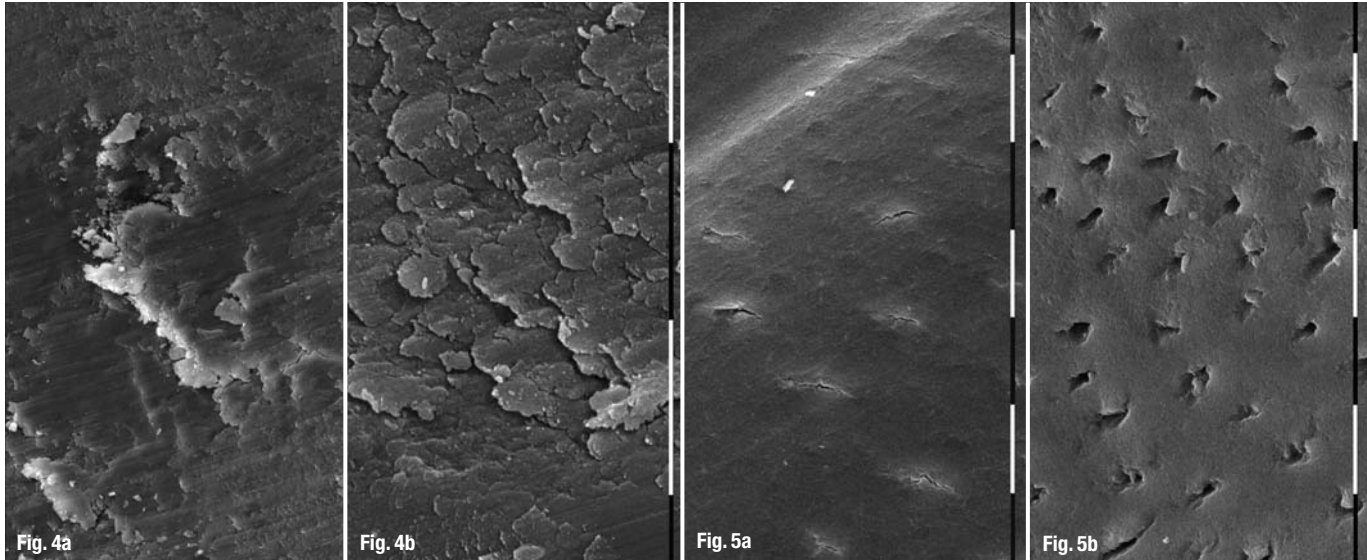
smooth, and in some places absent, smear layer (Fig. 5a). In the area of water turbulence, there were patent dentinal tubule orifices, but without a clear outline of tubule lumens or peri- and intertubular dentine (Fig. 5b). The boundary between enamel and dentine was unclear, and the cavity had smooth contours.

The dental surface topography after chemomechanical preparation with Carisolv gel (group 3) was clearly rougher compared with that of groups 1 and 2. The dentinal tubule orifices were visible and there was almost no smear layer (Fig. 6a). Preparation of the organic matrix using chemomechanical preparation with Carisolv while preserving mineralised dental tissue resulted in a rough appearance of the treated surfaces and considerable micro-retention (Figs. 6b & c). Denatured collagen fibres and surface contamination occurred in some places, blocking the dentinal tubule orifices (Fig. 6d). The cavity form in group 3 followed the initial carious lesions' forms without going beyond their boundaries.

Cavity forms prepared with the Er:YAG laser (group 4) were characterised by a lack of definite geometric configuration and outlined cavity elements (Fig. 7a). There was a rough and irregular surface with no smear layer (Fig. 7b). Dentinal tubules were clearly exposed. Intertubular dentine was more ablated than peri-tubular dentine and this made the appearance of dentinal tubules more prominent (Fig. 7c). In the enamel, the typical architectonics of enamel prisms grouped in bundles

Figs. 3a–c Laser preparation with the LiteTouch Er:YAG laser in hard tissue mode (400 mJ/20 Hz, 8 W).





Figs. 4a & b SEM photomicrographs of tooth surfaces prepared with steel burs (x 500; 2,000 magnification). The surface is covered with a layer of debris and dental tubule orifices are not visible.

Figs. 5a & b A smooth, thin smear layer covers tooth surfaces prepared with diamond burs and an air turbine. In the area of water turbulence, partially removed contaminants and single dental tubule lumens were observed (x 500, 2000 magnification).

was observed. Laser ablation of part of the enamel rendered the surfaces highly retentive (Figs. 7d & e).

Discussion

The MIP approach is based on several principles: remove only irreversibly damaged dental tissue and avoid macro-retention preparation in healthy tissue.¹ Additionally, MIP techniques should protect the underlying pulp and leave the treated surface suitable for adhesive bonding.¹ The antibacterial effects of the alternative preparation techniques must not be lower than those of standard necrotomy with rotary instruments and should excel them rather.¹

Nowadays the laser devices available for clinical use are capable of effective, controlled ablation of hard dental tissue.² Some clinical trials have suggested that Carisolv gel is highly efficient in caries removal, leaving clean and retentive dental surfaces.² However, not all researchers agree with these conclusions. Therefore, such studies should be periodically updated owing to the constant introduction of new technologies.

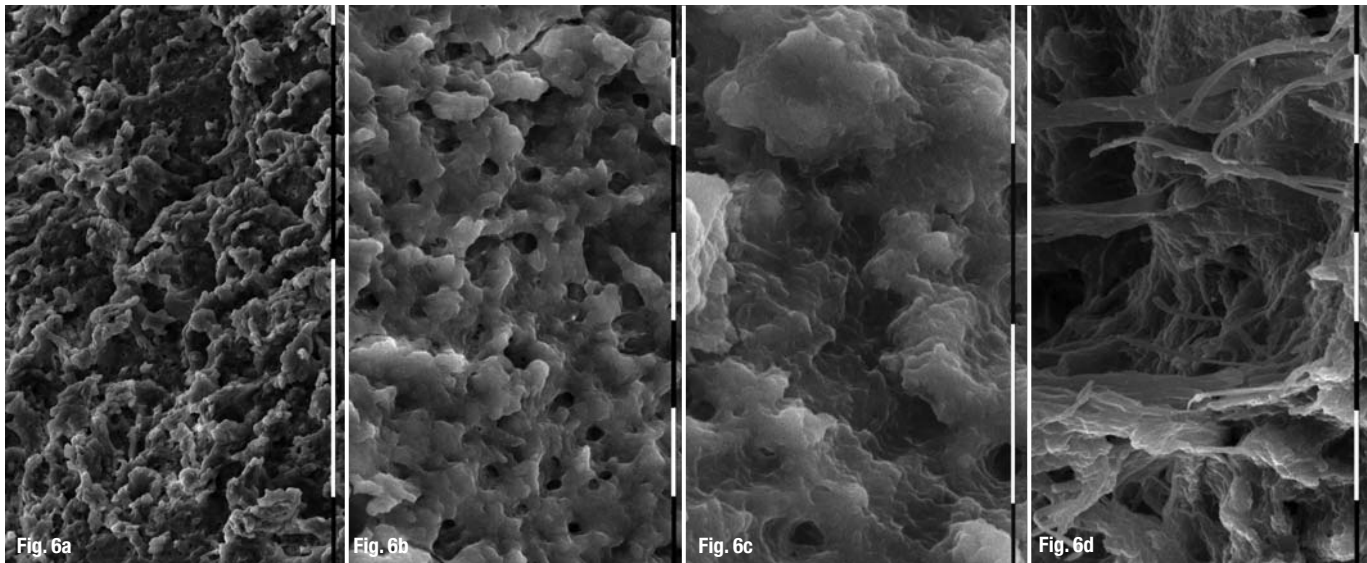
The experimental results of the present study revealed significant differences in the surface morphology of the samples studied, which would affect the ability to perform effective adhesive bonding. These morphological differences are highly dependent on the mechanism of action of the specific preparation systems.

Laser devices use a variety of physical media as sources for generating different wavelengths that are absorbed and interact with specific molecules in human tissues. The explanation for the hard tissue ablation is that the water content evaporates when exposed to laser irradiation, creating high in-

ternal pressure and subsequent micro-explosions. Inadequate water-cooling in this interaction of laser irradiation with tissue will lead to undesirable thermal effects.³ Depending on parameters such as pulse energy and frequency, CO₂ lasers, Nd:YAG and Er:YAG lasers cause changes in enamel and dentine in the form of roughing, craters, cracking, slicing, carbonification, melting and recrystallisation as described in many previous studies.⁴⁻⁶ These changes depend on the laser type, mode of operation, system for water-cooling and proper operation.³ Additionally, the ability to ablate carious dentine and enamel varies greatly according to different experimental studies.⁴⁻⁶ There is insufficient data that demonstrates the ability of the argon-fluoride and excimer lasers to remove dental caries.⁵ The krypton fluoride excimer laser, which emits in the ultraviolet range, has been shown to remove dentine, but enamel resists ablation.⁵

The high-power and high-frequency Er:YAG laser (LiteTouch) used in the present study has an advanced hydrokinetic system that is claimed to be capable of effective and safe ablation of hard dental tissue. The LiteTouch laser uses unique software that allows for the broadest range of energy and frequency settings. Its unique handpiece prevents loss of energy and, along with precision control over pulse duration, pulse energy and the optimal repetition rate, allows for a wide range of hard tissue procedures. LiteTouch is the first laser in to undesirable thermal effects.³ LiteTouch is the first laser in yet fully explored as a possible opportunity to eliminate acid etching of hard dental tissue and its related adverse effects on the underlying dentine and pulp.

Carisolv is a chemomechanical, minimally invasive method for selective softening of caries in



Figs. 6a & b Dentine surfaces treated with Carisolv gel are clean and highly retentive, with many exposed, open dentinal tubules (x 500; 2,000 magnification).

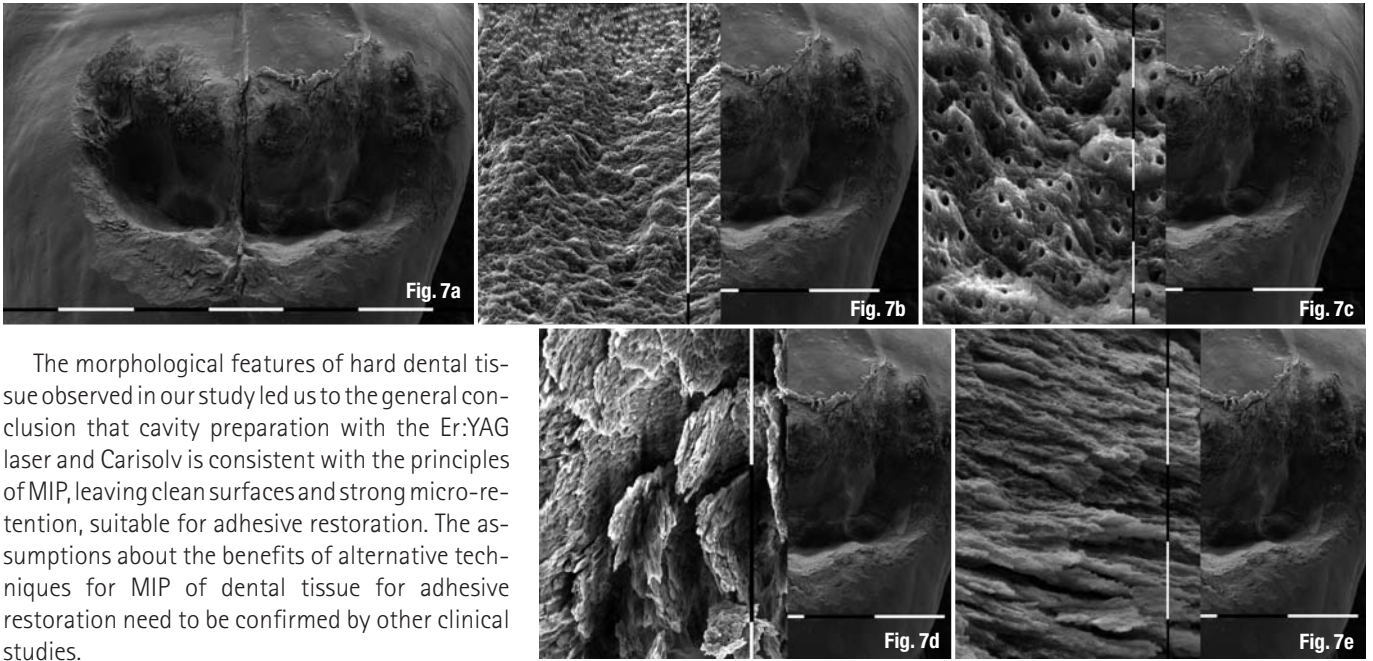
Figs. 6c & d Dentine surfaces treated with Carisolv gel are rough, granular and highly retentive. In some areas, single collagen fibrils are evident (x 3,000 magnification).

dentine and its subsequent removal with hand excavators.¹⁶ The system consists of gel containing three amino acids (glutamine, lysine and leucine) and a transparent liquid (0.5% NaOCl), which are mixed immediately before application. The chlorinated amino acids obtained selectively tear the damaged collagen fibres in carious dentine without damaging the underlying demineralised but not denaturated collagen. The macerated, infected dentine is removed manually using excavators. Carisolv gel is colourless and its amino acid concentration is twice as small, while the sodium hypochlorite concentration is increased twofold. The mechanism of action of Carisolv gel is based primarily on the proteolytic effect of NaOCl, which dissolves the denaturated collagen in the carious lesion.¹⁶ It is thought that the three amino acids enhance the effect of NaOCl on the collagen and reduce the involvement of healthy dental tissue. Carisolv chemical effects on the underlying pulp have been assessed as safe, and the alkaline pH (~11) of the gel neutralises acids and has a bactericidal effect on cariogenic flora.^{1,16} The presence of NaOCl in Carisolv is problematic, however, because of the danger of NaOCl inhibiting the bonding agent's polymerisation. Another clinical problem is the inability of Carisolv to affect the enamel and that requires combination with rotary instruments to excavate caries.¹⁶ Additionally, the results reported by studies on Carisolv's capacity to remove the smear layer are conflicting. According to some studies, Carisolv almost completely removes the smear layer, leaving visible and patent dentinal tubules.^{15,17} According to another study, however, Carisolv is unable to eliminate the smear layer and no patent dentinal tubules result.¹⁸ The latter study was conducted on a non-carious dentine surface and the researchers observed an irregular smear layer over enamel and dentine, and all dentinal

tubule orifices filled with debris. A third group of researchers found results that lay in between the findings of the other two: Carisolv does not eliminate the smear layer entirely. They observed partially patent dentinal tubules and residue of a contaminant smear layer covering the dentinal surface.²

The dentine surfaces treated with Carisolv and observed by SEM in the present study were clean, free of a smear layer, with some remnants of denaturated collagen fibres. Conventional rotating burs formed a smear layer on the dental surface, while Carisolv increased the surface roughness, leaving a relatively clean area. The dentine topography following Carisolv application was granular and rough compared with preparation with rotating instruments and exhibited roughness similar to that observed after laser preparation. The marked structural changes in the dental tissue and the surface roughness observed in our study may play a crucial role in composite material adhesion, possibly without requiring the use of etching agents. However, data in the literature on structural changes following Carisolv preparation varies considerably and we can conclude that this system for the chemomechanical removal of dental caries is likely sensitive to the application technique, mineralisation and other dentine characteristics.^{2,19}

The results of some contemporary studies have demonstrated that despite the differences between individual studies, in general the amount of smear layer after treatment with the Er:YAG laser and Carisolv in all cases is less than that after preparation with conventional rotating instruments, and surface changes are characterised by markedly rugged topography.^{2,3,12,15}



The morphological features of hard dental tissue observed in our study led us to the general conclusion that cavity preparation with the Er:YAG laser and Carisolv is consistent with the principles of MIP, leaving clean surfaces and strong micro-retention, suitable for adhesive restoration. The assumptions about the benefits of alternative techniques for MIP of dental tissue for adhesive restoration need to be confirmed by other clinical studies.

Conclusion

SEM analysis of hard dental tissue treated with steel and diamond burs showed surfaces covered with a thick layer of debris, which could compromise adhesion of filling materials. Dental tubule orifices were obturated with debris, with the exception of the areas under water turbulence, where the debris was partially removed.

Carisolv gel does not affect the enamel or healthy dentine. The surface topography of the dentine remaining after complete caries removal with Carisolv was rougher than that after conventional preparation with rotating burs. No typical smear layer was observed, but thin patches of contaminants, much less prominent than after drilling, were visible.

All laser-treated samples showed no evidence of thermal damage or signs of carbonification or melting. The SEM examination revealed characteristic micro-irregularities of the laser-prepared dentine surface without any smear layer and with

open dentinal tubules. Intertubular dentine was ablated more than peri-tubular dentine and that made the dentinal tubules appear to be better exposed. The Er:YAG laser ablated enamel effectively, leaving well-exposed enamel prisms without debris. The surfaces were very retentive.

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Fig. 7a A cavity prepared with the Er:YAG laser shows unclear cavity outlines and craters shading into one another (x 20 magnification). There are no precise outlined cavity elements.

Figs. 7b & c Laser-treated dentine surfaces are clean and free from debris, and all dentinal tubules are open. The surfaces are also irregular and rough, and therefore highly retentive. At greater magnification, the more effective removal of intertubular dentine is seen and this makes dentinal tubule orifices appear convex (x 500; 2000 magnification).

Figs. 7d & e Enamel surfaces treated with the Er:YAG laser revealed characteristic architectures of bundles of enamel prisms with different orientation. The surface is highly retentive and free from contaminants and a smear layer (x 2,000; 500 magnification).

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Dr Georgi Tomov

Department of Operative Dentistry and Endodontics
Faculty of Dental Medicine
Medical University of Plovdiv
Bulgaria