

ABSTRACT

Confocal Laser Scanning Microscopy provides a useful means for the nondestructive examination of ultrastructure characteristics of hard tissues including enamel and dentin (Watson, 1991 *Br. Dent. J.*). In this study, CLSM was used to examine the effects of *in vitro* bleaching on enamel and dentin. Crowns of extracted human third molars were sectioned below the occlusal fissure revealing subsurface dentin and the outer surface ring of enamel. Specimens were polished (1200 followed by alumina on lapidary film) and cut into four equal sections per tooth - allowing each tooth to serve as internal control. DE sections were mounted in acrylate for handling and were bleached: 0 h, 15 h and 30 h in 0.25 grams of commercial Opalescence® bleaching gel (10 % carbamide peroxide) or a laboratory batch of 5.3 % hydrogen peroxide in a carbomer glycerin gel. Blank glycerin served as control. *In vitro* bleaching was confirmed by colorimeter readings of DE and DL with a PR 650 spectrophotometer/colorimeter. Treated teeth were examined with confocal laser scanning microscopy comparing enamel surface and dentin at 5 mm subsurface to polishing under an oil immersion objective. Internal comparison of glycerin (no bleaching) controls (0, 15 and 30 hour exposures) revealed reproducible ultrastructure facilitating treatment comparisons. **Teeth bleached with commercial or laboratory prepared bleaching gels revealed no significant micromorphological changes associated with bleaching process in subsurface enamel, and dentin areas. The direct bleaching of cross sections permitted simple access of all areas to bleaching gel, thereby eliminating the possibility of diffusion limitation of bleach in producing artifacts.**

INTRODUCTION

Vital tooth bleaching continues to gain in popularity in the United States. Today, peroxides are used for tooth whitening in in-office applied trays, dentist directed nightguard bleaching systems and, more recently, in over-the-counter trays. Experience suggests that vital tooth bleaching is indeed a safe procedure. Extensive research and longitudinal clinical observations have shown no important side effects to either soft or hard tissues. Studies on hard

tissues have included examination of bleached surfaces for changes in roughness, hardness, porosity, fracture sensitivity and acid resistance. Few studies or techniques have been available which permit detailed examination of possible ultrastructural effects of bleaching on hard tissues. Confocal laser scanning microscopy permits nondestructive examination of subsurface hard tissue ultrastructural changes. This study applied CLSM to the examination of bleaching effects on enamel and dentin ultrastructure *in vitro*.

MATERIALS AND METHODS

CLSM Measurements

Treated teeth were examined with confocal laser scanning microscopy on a Leica DIAPLAN with illumination provided by a mixed He-Ar laser at 488 nm. Specimens were examined under an oil immersion objective at a focal plane 5 mm below the polished surface.

Tooth Preparation & Bleaching

Methods described in abstract. An additional group of specimens was bleached in 5% HClO₄.

CONCLUSION

-Results for various bleaching regimens appear self consistent - that is peroxide bleaching delivered from commercial gel (Opalescence®) and laboratory prepared H₂O₂ gel appear consistent - as do results for bleaching with perchlorate solution.

-Images from bleached specimens produced major increases in laser reflection - undoubtedly derived from bleaching process on colored (light absorbing) components in dental tissues. The bleaching process is clearly observed in CLSM reflections.

-Peroxide treatment of enamel revealed increased light reflection, but no direct evidence for ultrastructural changes.

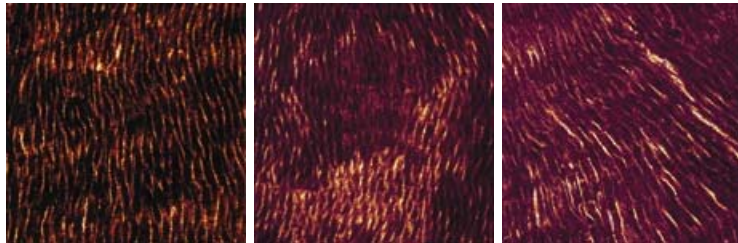
-Peroxide treatment within dentin produced more intensive light reflection changes than within enamel - demonstrative of the increased proportional bleaching process in these tissues.

-Odontoblast processes appeared to bleach extensively as did intertubular dentin - with little changes in tubules or within peritubular dentin.

RESULTS

Confocal Laser Scanning Microscopic Images of Specimens

ENAMEL



HClO₄: 0 Hrs

HClO₄: 15 Hrs

HClO₄: 30 Hrs

ENAMEL

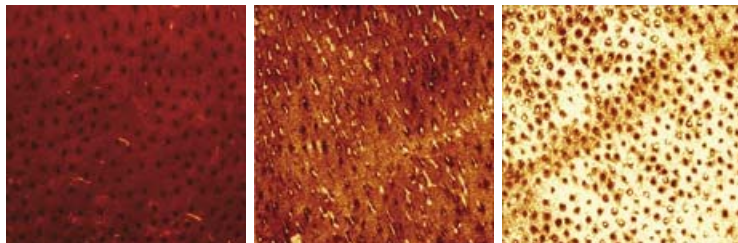


Opalescence: 0 Hrs

Opalescence: 15 Hrs

Opalescence: 30 Hrs

DENTIN

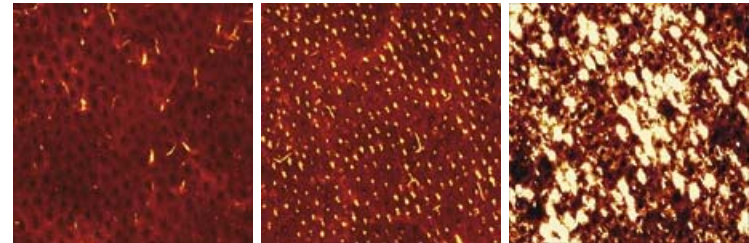


HClO₄: 0 Hrs

HClO₄: 15 Hrs

HClO₄: 30 Hrs

DENTIN



Opalescence: 0 Hrs

Opalescence: 15 Hrs

Opalescence: 30 Hrs

Avg. Dentin Color During Bleaching

Time	Glycerin		H ₂ O ₂		Opalescence		HClO ₄	
	L	b	L	b	L	b	L	b
t=0hrs	77.9	24.9	80.5	24.5	74.4	25.9	75.6	26.6
t=15hrs	71.0	21.2	80.8	15.1	78.2	20.6	79.9	19.5
t=30hrs	70.9	22.3	83.8	13.5	81.2	19.2	88.6	4.63

SUMMARY

In this experiment, the bleaching process delivered high concentrations directly and equally to both enamel and dentin via the cross-sectioned bleaching technique. As a result, bleaching activity on subsurface material was significantly greater than would be seen clinically in longitudinal bleaching through the enamel surface. Nevertheless, the bleaching process produced mainly increases in light reflectivity of structures - without apparently influencing ultrastructure. These preliminary results support the safety and efficacy of the bleaching process and illustrate the power of the CLSM technique.