Non-thermal plasma treatment of dentin surface for bacterial disinfection and improved composite restoration

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Résumé

The objective of this study is to investigate the treatment effects of non-thermal atmospheric gas plasmas on dentin surfaces for oral bacterial disinfection and composite restoration improvement. Oral bacteria of *Streptococcus mutans (S. mutans)* and *Lactobacillus acidophilus (L. acidophilus)* with an initial bacterial population density between 1.0×10^8 and 5.0×10^8 cfu/ml were seeded on various media and their survivability with plasma exposure was examined. The plasma exposure time for a 99.9999% cell reduction was less than 15 seconds for *S. mutans* and within 5 minutes for *L. acidophilus*. To evaluate the dentin/composite interfacial bonding, extracted unerupted human third molars were used by removing the crowns and etching the exposed dentin surfaces with 35% phosphoric acid gel. The teeth thus prepared were sectioned into micro-bars as the specimens for tensile test. Student Newman Keuls (SNK) tests showed that the bonding strength of the composite restoration to peripheral dentin was significantly increased (by 64%) after 30 s plasma treatment of the dentin surfaces. The findings from this study indicated that non-thermal atmospheric plasma technology is promising for dental clinical applications.

Introduction

Polymethacrylate-based dental composites have received widespread clinical acceptance as alternative restorative materials to dental amalgam amid concern regarding the potential health risks associated with mercury released from dental amalgam. As supported by results from multiple clinical and laboratory studies, however, the current dental composite restorations suffer from much reduced longevity mainly due to interfacial failures, which cause microleakage, sensitivity, recurrent caries, and composite restoration failure.^{1,2} The interface failure of composite restoration resulted from adhesive bonding failure of the dental adhesive/composite to the surrounding tooth structure. Adquate dentin/adhesive bonding requires dispersion of the adhesive throughout the dentin surface and micromechanical interlocking of adhesive with collagen fibrils in decalcified dentin.³ In this study, non-thermal atmospheric plasmas was used to prepare and engineer dental surfaces and study the plasma treatment effects on dentin surfaces in terms of bacterial disinfection, dentin surface modification, adhesive wettability improvement, interfacial bonding enhancement. Effective plasma disinfection of oral bacteria will help save healthy dental tissues that are often extensively removed in clinical practice by mechanical drilling to ensure complete removal of caries-causing microorganisms.

Experimental

A recently developed atmospheric cold plasma brush (ACPB)^{4,5} was utilized to clean/disinfect oral bacteria and prepare dentin surfaces for dental adhesive and dental composite application. Two kinds of caries-causing bacteria, *S. mutans* and *L. acidophilus*, were seeded on various media and their survivability with plasma exposure was examined. Extracted unerupted human third molars were used and the occlusal one-third of the crown was sectioned by means of a water-cooled diamond saw. The exposed dentin surfaces were polished with 600 grit SiC sand papers and then etched using 36% phosphoric acid. AdaperTM Single Bond Plus dental adhesive and FiltekTM Z250 composite (3M ESPE Dental Products, USA) were applied and light cured as directed. Dentin/composite bars (8-10×1×1 mm) were cut from the prepared teeth for micro tensile testing and interface characterization.

Results and discussion

Figure 1 shows the cell surviving curve of *S. mutans* with different plasma exposure time on three different supporting media. It can be seen that the argon plasma brush is very effective in killing *S. mutans*

and a very short plasma exposure time of less than 15 s gave a complete kill of the bacteria. It was also found that a longer plasma treatment time of 5 min was required for completely killing L. acidophilus. То investigate bacterial cell damage caused by treatment, a UV-visible the plasma spectrometer was used to monitor the absorbance peak intensities at wavelengths of 260 nm (DNA absorbance) and 280 nm (protein absorbance). For both bacterial samples, a longer plasma exposure did increase the peak intensity of absorbency at wavelengths of 260 nm and 280nm, which suggest the leakage of protein and/or nucleic acids from the bacterial cells due to the cell membrane damages upon plasma exposure. The plasma-induced cell membrane damages were further confirmed by SEM.

FTIR surface analysis showed structural changes in the surface of the demineralized dentin after plasma treatments. A new shoulder peak around 1760 cm⁻¹ associated with carbonyl stretch was found and an amide II shift of ~10 cm⁻¹ was observed (1543 cm⁻¹ before to 1533 cm⁻¹ after), which might indicate the secondary structural changes of dentin collagen after plasma treatment. Figure 2 shows the statistical comparison of ultimate tensile strength data obtained with test specimens prepared from

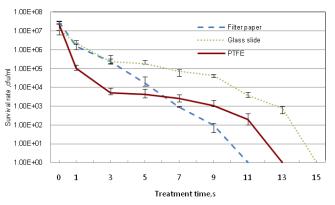


Fig. 1 : Plasma disinfection effect on *S. mutans*. Plasma conditions were 2000 sccm Ar and 10 W DC Power input.

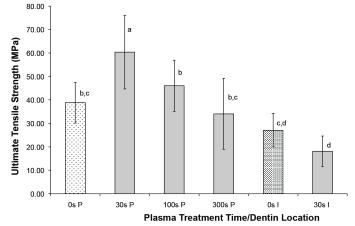


Fig. 2: Statistical comparison of ultimate tensile strength obtained with test specimens prepared from plasma treated dentin and the untreated controls (0s P and 0s I). P: Peripheral dentin, I: Inner dentin; Different letters indicate statistically significant differences ($\alpha = 0.05$).

plasma treated dentin and the untreated controls. Statistically significant differences in tensile strength between all specimens using SNK method were observed. A significant difference was found between the peripheral dentin that was plasma treated for 30 s and all the other treatments. SEM images of the fracture surface generally showed that more composite remained on plasma treated dentin surfaces when compared with the controls, indicating much enhanced dentin/composite interface bonding.

Conclusions

Our experimental results showed that atmospheric plasma treatment was very effective in cleaning/disinfecting caries-causing oral bacteria. Plasma surface preparation of dentin could significantly increase the dentin/adhesive interfacial bonding strength. The findings from this study indicated that non-thermal atmospheric plasmas could be a promising technique in dentistry for many clinical applications such as bacterial disinfection, caries early prevention, and improved composite restoration.

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