Cold atmospheric pressure plasma (CAP) treatment to assist the restoration of the apical region of a root canal in endodontic procedures

E. Simoncelli[1], M. Manzini[2], A. Stancampiano[1*], M. Gherardi[1,4], V. Colombo[1,4], R. Tonini[2]

[1,3] Alma Mater Studiorum-Università di Bologna, Bologna, Italy
[1]Department of Industrial Engineering (DIN),
[2]School of Dentistry, Università degli Studi di Brescia, Brescia, Italy
[3]Industrial Research Center for Advanced Mechanics and Materials (C.I.R.I.-M.A.M.),
**presently at GREMI, UMR7344 CNRS/Université d'Orléans, Orléans, France*

Abstract: In the field of dental restoration, guttapercha is generally used to completely seal the root apex, coupled with an endodontic sealer, despite its cytotoxicity. The present study aims at investigating the enhancement of adhesion between these materials and the dentine of the apical region of ex-vivo teeth when treated by a DBD Helium plasma jet; pushout tests and confocal microscopy analysis have been performed to evaluate the effect of cold plasma treatment.

Keywords: cold atmospheric plasma, dental restoration, tooth root canal, guttapercha, adhesion enhancement, endodontic sealer, apical region, bond strength, push-out, confocal analysis.

1. Introduction

The restoration of the apical region of root canal aims at avoiding a new bacterial colonization in the tooth apex, that may result in a hazardous abscess. Filling materials, such as guttapercha (GUT), are generally used to completely seal the root apex with the purpose of entombing the residual bacteria remaining on the root canal walls or within dentinal tubules (dentinal substrate) and preventing any contacts with the peri-apical tissues and nerves [1,2]. In the conventional procedures, to achieve a well-adherent apical monoblock, endodontic cements known as sealers (SEA) are applied before guttapercha filling, despite their cytotoxicity [3]. CAP treatment can be considered as an attractive solution to improve the performances of conventional procedures involved in apical restoration thanks to CAP ability to modify the surface chemistry of dentine [4]. In order to evaluate the enhancement of bonding strength between sealing material and CAP-treated dentine, pushout tests were performed on extracted teeth, with single root-canal, and a confocal laser scanning microscopy analysis was run to evaluate the interaction of dental materials with the dentine substrate.

2. Materials and Methods

Forty extracted teeth with standardized shape of the only root-canal were used for the experiments. 180s CAP treatment was performed with a plasma jet, generating a He DBD plasma propagating through a dielectric capillary, that fits for endodontic procedures. The plasma source was driven by a micropulsed generator producing high-voltage sinusoidal pulses operating at peak voltage V=15 kV, frequency f = 22 kHz, with duty cycle DC = 7.5% in all investigated cases.

The restorative procedure was performed either with direct filling of guttapercha or with preliminary application of a sealer, comparing the results of untreated or CAP-treated dentine. Indeed, the investigated cases are: G1 (GUT), G2 (CAP+GUT), G3 (SEA+GUT), G4 (CAP+SEA+GUT). G3 was considered the reference case, as it represents the standard procedure for the apical restoration.

A common guttapercha points, composed by an organic component (guttapercha; wax and/or resin) and an inorganic component (zinc oxide, metal sulfates), in proportion 1:1, are used in all the experiments; while the Topseal (Dentsply Maillefer), an epoxy resin sealer also marketed as AH Plus, that exploits an epoxide amine chemistry in its interaction with the (bio-)substrates, is used as sealer.

Photos of single steps of the procedure were collected in Fig. 1.

The adhesive performances of restorative procedure were evaluated through push-out tests. After water storage, the samples were sectioned transversally to the long axis of the tooth by means of a diamond saw (Isomet) irrigated with water. Tooth sections 1,6 mm-thick, were obtained from coronal and apical portions of the root canal. The coronal and apical sections were respectively cut 4 and 1 mm above the apical terminal. Through a molding system procedure, the accurate positioning of the specimens in the pushout testing machine was guaranteed. Tests were performed using a universal testing machine (Instron model 5848, load cell HBM U2A 200 kg, Micro Tester MTS electronic Test Star IIs). Specimens were axially loaded on the luting material section (Ø 1,6 mm) with a cylindrical metallic plunger (Ø 1,4 mm) at a cross-head speed of 1 mm/min. When dislodgement occurred, the maximum failure load was recorded and converted into MPa considering the real dimensions of each specimen. Statistical analysis was performed applying one-variable analysis of variance (ANOVA) as post-hoc comparison at a significance level set at $p<0,05$.

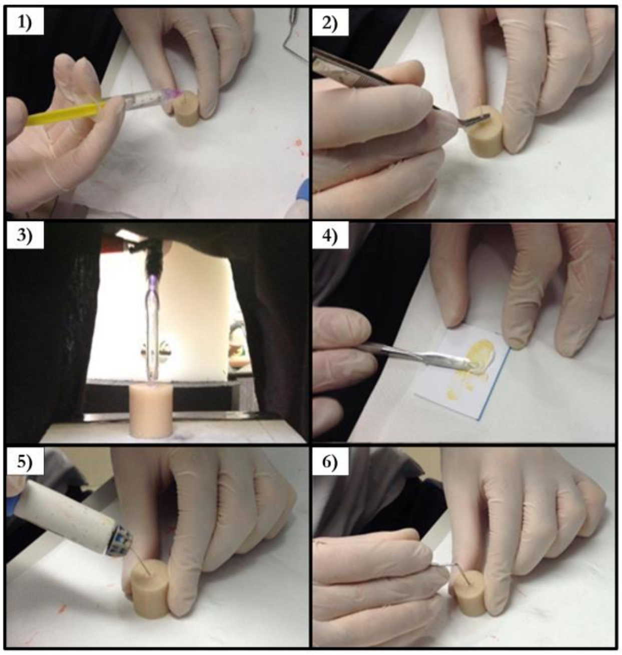

Fig.1. Different steps of experimental procedures: 1) EDTA rinsing; 2) drying with paper points; 3) CAP treatment; 4) sealer preparation; 5) guttapercha obturation with Calamus Flow; 6) manual compaction/compression of guttapercha.

Moreover, a confocal (CLSM) analysis was carried out to examine the depth of penetration of guttapercha and sealer in the dentinal substrate. Confocal analysis was carried out using a colourant with eosin 0,1%, a red-fluorescent molecule (Eosin Yellowish 1B 425, Chroma-Gesellschaft, red emission around 532 nm). Teeth specimens were prepared as in the pushout tests. Since sealer was applied in liquid state, a mixture of colourant-sealer was prepared and then used for G3 and G4 groups. On the other hand, being guttapercha plugger in plastic-solid phase, for the case G1 and G2, the colourant was spread in the root canal before guttapercha filling. Thus, in the confocal images, the red fluorescence represents the sealer penetration in the cases G3 and G4, while in the G1 and G2 groups the red signal shows the colourant itself pushed into dentinal tubules by the guttapercha penetration. The confocal analysis was performed by means of confocal microscope (510 META, Zeiss) with a 40x lens and an additional zoom of 3x as magnification factor. Pinhole was kept open at 100 µm for all acquisitions (512x512 px).

3. Results and Discussion

Table 1 clearly shows a relevant increase of the mean bonding strength between the plasma treated dentine and the filling materials used in these experiments for coronal and apical sections, guttapercha or endodontic sealer as well.

Results underline a statistically significant increase, around three times (~ +200%, $p<<0,05$) compared to control G1, of the bonding strength along the whole axial length of the root canal when plasma is applied on dentine before the direct application of guttapercha, revealing how guttapercha was able to "self-bond" directly with the dentine substrate without any sealer.

G4, representing the conventional apical sealing procedure supported by a plasma pre-treatment of dentine surface, shows an improvement around +50% underlining an enhanced interaction of endodontic sealer with the dentinal substrate. The similarity in the values of bonding strength between G2 and G3 groups highlights how a plasma treatment of the dentine could replace the application of endodontic sealer in terms of adhesion performances.

Table 1. Mean bonding strength ± standard deviation of sealing system (guttapercha or sealer+guttapercha) to dentine evaluated through pushout test. Last column reports the average % improvement in bonding strength due to plasma treatment of dentin with respect to untreated control.

	Average bonding strength [MPa]	Increase [+%]
G1 – Coronal	1,05 ± 0,36	**+ 238,1%**
G2 – Coronal	3,56 ± 1,04	
G1 – Apical	1,25 ± 0,40	**+ 194,1%**
G2 – Apical	3,69 ± 1,21	
G3 – Coronal	3,10 ± 0,61	**+ 58,6%**
G4 – Coronal	4,92 ± 0,85	
G3 – Apical	3,49 ± 0,78	**+ 47,6%**
G4 – Apical	5,15 ± 0,71	

In Fig. 2, the confocal acquisitions are reported for the case G1, G2, G3 and G4. Comparing the control groups G1 and G3, a higher penetration is observed in the case in which guttapercha was directly posed in contact with the dentine substrate, with no relevant difference between coronal and apical regions. For both cases (G2 and G4), characterized by a CAP pre-treatment of dentine, the penetration of guttapercha (G2) and of endodontic sealer (G4) is extremely increased for the whole length of the root canal.

Moreover, the results of G2 group gain interest and importance in the context of biological safety. As anticipated above, the possibility to cause an apical extrusion during the endodontic treatment may result into hazardous risk for patient related to the toxicity and mutagenicity of conventional resin-based sealers [3]. Although Topseal is recognized to be one of the most biocompatible sealers on the market, it was demonstrated that its cytotoxicity can be related to the contained small amount of formaldehyde and to the release of the amine and epoxy resin components from this material [5]. Generally, the biological risk of using an endodontic sealer is critically dependent on both the cytotoxicity of the material and the practitioner's ability to seal the apex of the root canal [3]. CAP can reduce the cytotoxic risk favouring an apical 3D obturation performed with the use of only guttapercha characterized by adhesion and sealing performances comparable to the ones achieved in conventional procedures (G3).

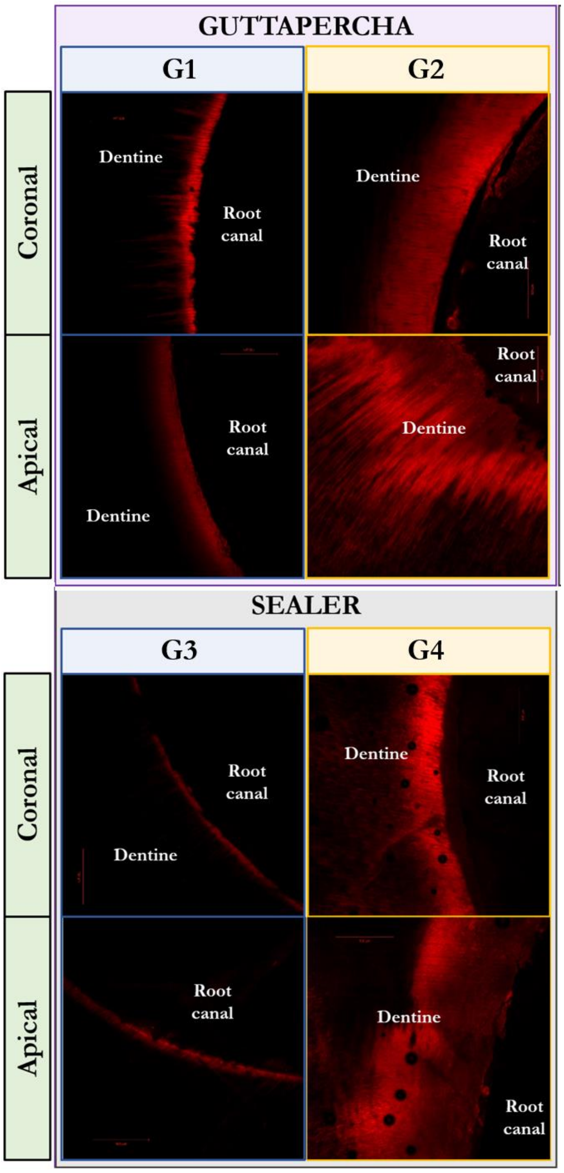

Fig. 2. Collection of CLSM images divided for each investigated group and for different regions of root canal.

4. Conclusions

This work, based on the study of the CAP effects in the field of dental composite restoration, was focused on the CAP-induced enhancement of adhesive performances of apical sealing. The acquired data, through pushout tests on ex-vivo teeth, show how the simple addition of a 180s plasma treatment to the conventional procedures can lead to a statistically relevant improvement of the mean bonding strength along the entire root canal length. Moreover, considering the results obtained in a previous study [6], positive effects of DBD-jet plasma treatment were observed for both sets of materials used in the coronal-medial and apical restoration of the root canal (self-etch adhesive systems and sealer+guttapercha, respectively). In particular, the study highlighted the possibility to avoid the use of cytotoxic endodontic sealers in the apical sealing in terms of adhesion performances between the guttapercha and the plasma treated dentine. Confocal images confirmed the higher spreading and penetration of both guttapercha and sealer into dentinal tubules.

Even if future clinical in vivo studies are still required, the potential for a future success of a CAP-assisted endodontic procedure appears day by day more certain.

5. References

[1] R. S. Schwartz, Journal of Endodontics **32**, 1125 (2006).

[2] K.-W. Lee, M. Williams, J. J. Camps, and D. H. Pashley, Journal of Endodontics **28**, 684 (2002).

[3] S. Bouillaguet, J. C. Wataha, P. E. Lockwood, C. Galgano, A. Golay, and I. Krejci, European Journal of Oral Sciences **112**, 182 (2004).

[4] M. Gherardi, R. Tonini, and V. Colombo, Trends in Biotechnology **20**, 1 (2017).

[5] P. T. da Silva, F. G. Pappen, E. M. Souza, J. E. Dias, I. Bonetti Filho, I. Z. Carlos, and R. T. Leonardo, Brazilian Dental Journal **19**, 228 (2008).

[6] E. Simoncelli, D. Barbieri, R. Laurita, A. Liguori, A. Stancampiano, L. Viola, R. Tonini, M. Gherardi, and V. Colombo, Clinical Plasma Medicine **3**, 77 (2015).