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

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

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Abstract

Purpose: In Endodontics, in order to complete a root canal treatment, the use of guttapercha to completely seal the root apex must be coupled with endodontic cements known as sealers to improve the adhesion with dentine, despite their cytotoxicity and solubility. The present study investigates the effect of enhancement of adhesion between these materials and Cold Atmospheric Plasma (CAP)-treated dentine of the apical region of *ex-vivo* teeth as a first step towards the reduction on the use of cytotoxic materials and the use of CAP *in-vivo* at a clinical stage.

Methods: A dielectric barrier discharge (DBD) helium plasma jet was used to treat for 180 sec the shaped root canal dentin. Pushout tests and confocal microscopy analysis have been performed to evaluate the effect of cold plasma treatment in terms of adhesion performances and interaction between filling materials and dentin respectively.

Results: The pushout test results highlight how a CAP treatment of dentin promotes an enhancement of bonding strength between filling materials and dentine in all investigated cases. In particular, the use of guttapercha in the case of a CAP pre-treatment of dentine achieves results comparable with the conventional procedure, avoiding the use of sealers. Moreover, confocal images reveal how the filling materials penetrate deeper in the CAP treated dentin.

Conclusion: CAP treatment of dentin increases the adhesion performances and paves the way for a sealer-free and safer procedure for apical restoration.

Keywords: cold atmospheric plasma, endodontic procedures, apical restoration, adhesion enhancement, guttapercha, biological safety.

1. Introduction

The three dimensional (3D)-obturation of the apical system is the final phase of the Endodontic Triad consisting of shaping, cleaning and filling. Generally, it has been recognized as the most critical phase of endodontic treatment with the final goal of apex sealing and avoiding any residual holes into the filled canal space [1–4]. Manifold materials have been used for this procedure but among all conventional fillers, guttapercha has established itself as the Gold Standard in apical obturation. Specifically, it is characterized by cold and thermal plasticity, high stability after polymerization, inert and biocompatible and low thermal conductivity [5–9]. Although filling materials used in the apical obturation, such as the mentioned guttapercha, present important sealing properties, they are recognized to have lower adhesive features in respect to the restorative materials used in the coronal region of root canal [10–12]. For this reason, a conventional filling material system often consists of guttapercha associated with an endodontic sealer (liquid cement). The sealer serves as a lubricant when inserting the guttapercha point, as a filling material to fill the irregularities of the preparation, and as adhesive component between dentine and guttapercha [10,13,14]. Thus, to enhance the adhesion performances, an endodontic sealer is usually spread in the root canal system before sealing, even though their toxicity and mutagenicity [15–18]. Due to their importance in the 3D-obturation, in recent years, numerous efforts have been made to improve the biocompatibility of cutting-edge sealers such as bioceramic-based sealers. Despite this characteristic, several problems like retreatability and solubility continue to persist [19,20]. In spite of the recent advancement in sealers biocompatibility, there is still a growing need for safer endodontic treatment and characterized by enhanced adhesive performances has prompted the research of innovative materials and therapies [21]. Atmospheric pressure non-equilibrium plasmas, also known as CAPs, thanks to the production of charged particles, reactive species, radiation and electro-magnetic fields at close-to-environmental temperature [22], leading to the application of a CAP treatment directly on living tissues, have shown great potential as innovative and multifunctional technology for dental applications [22–25]. For example, CAP have shown high efficiency in killing planktonic bacteria without damaging human tissues [22,26], and not affect the viability of mesenchymal cells, not inducing morphologic alteration to mucosa and periodontal tissues [27–30]. As a gaseous medium, CAP has the appealing capability to penetrate irregular cavities/fissures, such as dentinal tubules or apical regions and inactivate bacteria, actively fighting bacterial infections [27]. Of particular interest for dental applications is the ability of plasma to induce surface modification on artificial and biological materials. CAP can activate (bio-)surfaces, such as zirconia/titanium, for a higher biocompatibility of dental implants [31] or enamel/dentin for the enhancement of the adhesive restoration [24,32] achieving also a higher spreading capacity of osteoblasts [33].

Moreover, in the research field of CAP-assisted dental restoration, preliminary data has shown that plasma treatment effectively increases the bonding strength at the dentin/composite interface [23]; Ritts and colleagues showed how CAP treatment of dentin before the application of the adhesive system could significantly enhance the adhesion performances [34]. Different studies have shown that, after the chelant/etching phase for the cleaning of dentinal tubules from smear layer, the CAP treatment induced an increase of dentin wettability, and facilitated the adhesive penetration into dentin collagen, promoting a thicker hybrid layer and, consequently, better bonding performance [24,27,32]. By means of scanning electron microscope images, longer and more tortuous resin tags, with lateral projections were observed in the case of plasma pre-treatment of dentin [32,35]. EDX/XPS studies affirmed significant increase in oxygen content on CAP treated dental surfaces, reflecting the introduction of oxygen-containing groups to the substrates which increase dentin wettability [33,36–39].

It is worth mentioning that the long-term stability of plasma-assisted restorations remains an open question, with a limited number of papers dealing with this subject [24,40]. As reported in the study of Hirata and co-workers, after 1 year of aging in water, the improvement in bonding strength provided by CAP treatment of dentin was lost and there was no appreciable difference compared with untreated samples [22].

Supported by literature and our promising results obtained in the frame of coronal restoration assisted by a CAP treatment [41], the present work aims at studying the improvement of adhesion in the apical region of the root canal system through pushout tests, that are generally adopted to evaluate the bonding strength of dentine-adhesive system interface in standardized tooth specimens [42]. The effects of plasma treatment were evaluated in the case of a direct apical filling with guttapercha and in the case of pre-application of an endodontic sealer over the dentin surface. A DBD helium plasma jet, properly designed as lab-prototype to be then exploitable in real endodontic procedures, was used for the experiments, as well as in the previous studies [43] and [41] . By means of a confocal laser microscope analysis, the penetration of restorative materials into dentinal tubules and dentin substrate was qualitatively evaluated.

2. Materials & Methods

2.1. Plasma source

The plasma source employed in the present work, based on DBD plasma jet configuration, was specifically designed (with a relevant contribution by AlmaPlasma s.r.l.) for dental applications and might belong to the same family of the ones for which claims have been defined in patents EP patent 2208404B1 (6 December 2016) and US patent 8482206B2 (9 July 2013) by Pouvesle et al [44,45]. The schematic of the source is shown in Fig. 1 and a detailed description of the plasma source is reported in [41,43]. Helium was used as working gas with a flow rate of 3 slpm. 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%.

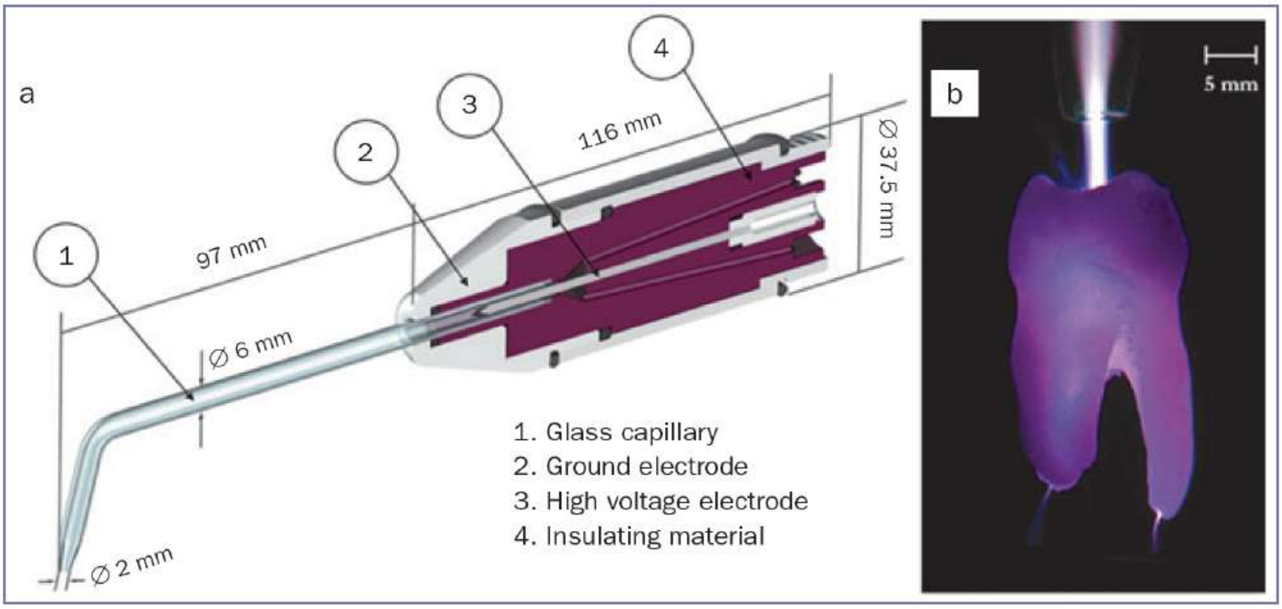


Fig. 1. a) schematic of the DBD-jet plasma source; b) plasma plume, produced by the DBD-jet plasma source and propagating inside a molar model [41].

The safety of the plasma source was assessed as recommended by the DIN-specification 91315 and the standard "*DIN EN 60601-1 – Medical electrical equipment*" [46,47]. Even though tooth materials are not soft tissues, in this study was adopted as a reference value the limit imposed for soft tissue (100 μA of leakage current) to be on the conservative side. An investigation of leakage current was carried out following the standard's requirements in the specific cases shown in Fig. 2, when interfacing the source with a human finger at 2, 5 and 12 mm of distance from the source outlet, respectively. The Authors volunteered as samples for these measurements.

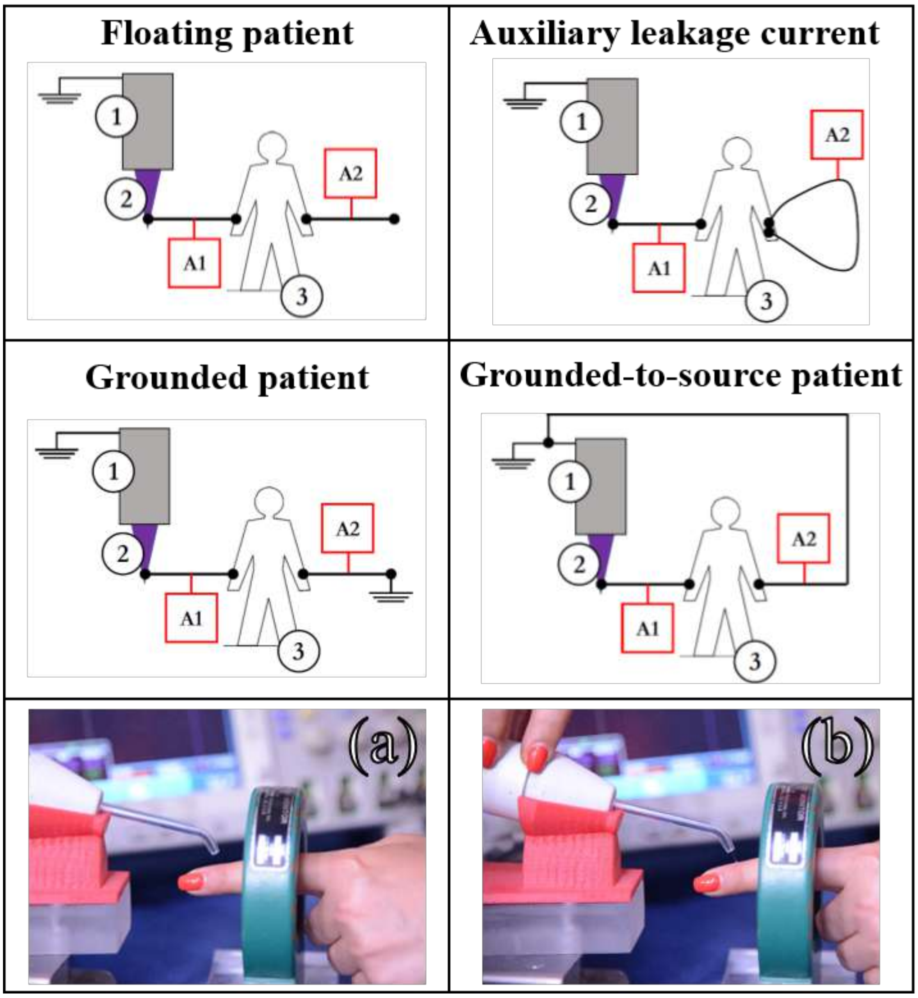


Fig. 2. Schematics of leakage current measurements. 1) plasma source, 2) plasma plume, 3) patient, A1) current probe 1, A2) current probe 2. To show the electrical connection between the plasma plume and the patient, as examples, the photo (a) and photo (b) of the setup for measurement of leakage currents (A1 probe) are given in the cases of floating and grounded-to-source patient, respectively.

As well know, in its expansion in the surrounding air the plasma plume produces ozone, a molecule with recognized important antimicrobial properties that is commonly exploited in the field of dentistry [48–51]. On the other hand, since long exposures at high-concentrated ozone atmosphere could cause hazardous toxic effects to the patient [52], different standards, such as those described by ANNEX XII of Directive 2008/50/EC of the European Parliament and of the European Council of 21 May 2008, impose thresholds for ozone (e.g. O_3 concentration lower than 240 $\mu g/m^3$ for 1 hour of exposure). In light of these considerations, a simplified setup for measurement of the concentration of ozone by means of ozone strips (Ozone Test strip 90736, Macherey-Nagel) placed at the bottom of a chamber with one open boundary (with the almost the same shape and volume of an oral cavity ~ 70 ml, Fig. 3) as a consequence of 1, 3 and 5 min of CAP treatment, defined, placing the end of the capillary 10 mm and 30 mm inside the plastic container.

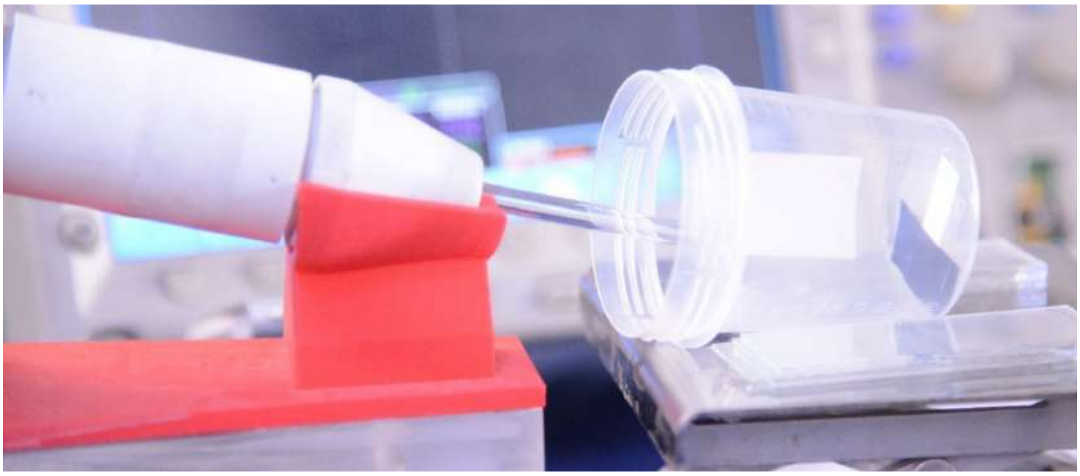


Fig. 3. Photo of ozone concentration measurement inside a chamber with one open boundary by means of ozone strips posed on the bottom of the chamber.

Finally, the UV irradiance produced by the plasma plume was evaluated using the UV power meter Hamamatsu C9536/H9535-222 (measurement range of 0.001–200 mW/cm^2, high spectral response in the range 150–350 nm). As proposed by the DIN-specification 91315, the radiant exposure should not exceed 30 J/m^2 or 3 mJ/cm^2 as a daily dose [46]. The sensor was posed 2 mm far away from the end of capillary of the plasma source.

2.2. Tooth sample preparation and treatment procedures

The procedure adopted for the preparation of teeth sample follows the protocol described in [41] . Forty-four maxillary and mandibular monoradicular human teeth, extracted for periodontal, prosthetic or orthodontic diseases, with straight roots and regular single canals were selected for this study. Teeth were stored in 0,1% thymol solution at 4°C to serve their structural properties and ensure good hydration. The crown was sectioned off at the cemento-enamel junction using a water-cooled diamond blade on a cutting machine (Isomet, Buhler, Lake Bluff, NY, USA) to expose the root canal.

Although guttapercha is used for sealing apical foramen with diameter lower than 1 mm, to standardize the study, the first 8 mm of the root canal were shaped to a regular diameter of 1,6 mm by means of a cylindrical diamond bur (Komet 837/016, Brasseler, Lemgo, D). Teeth were then embedded in epoxy resin cylinders (Enamel Temp Plus, Micerium) using the innovative custom molding procedure proposed in [41] (described in Fig. 4 and Fig. 5) ensuring an accurate position of the specimens for pushout tests. After curing of the epoxy resin (few minutes), the tooth results as perfectly embedded in the mold. The embedded tooth were stored again in thymol solution before further treatment.

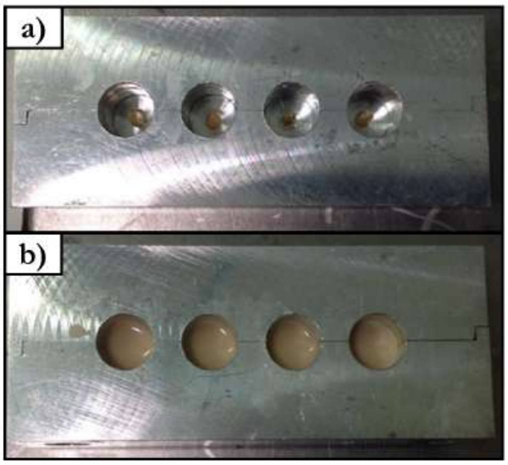


Fig. 4. Molding procedure before (a) and after (b) resin casting.

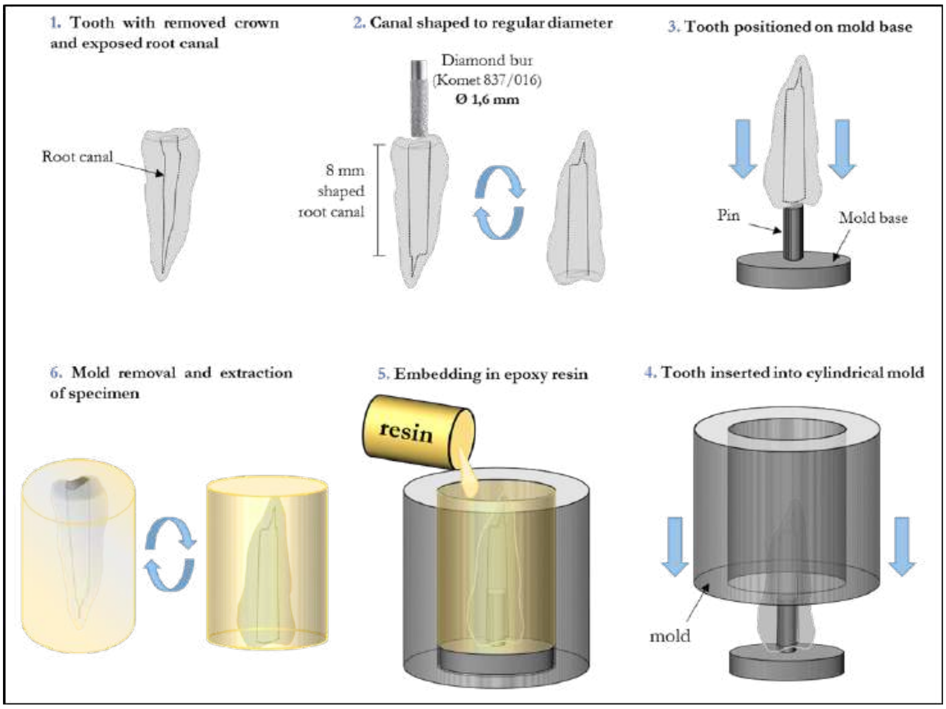


Fig. 5. Molding procedure to ensure the alignment of the root canal along the resin cylinder axis [41].

A solution of EDTA 17% was rinsed for 1 min for the cleaning of each root canal and for the removal of smear layer. Then, teeth were dried with paper points to eliminate any residuals of chelating agent. As reported in Tab. 1, the samples were randomly distributed into four groups and conditioned with different type of dentine pre-treatment: two control groups subjected to standard procedures (C1, C2) and two experimental groups including the pretreatment of dentine by 180 seconds of plasma exposure (P1, P2). CAP treatment was carried out by means of the DBD-jet plasma source (described in paragraph 2.1) at a fixed distance between the source outlet and the root canal opening of 2 mm. More specifically, group C1 included the teeth subjected to no dentine surface conditioning and directly filled with guttapercha (Calamus Dual System – Calamus Flow, Dentsply, Maillefer). Back-packing procedure was used for the application of guttapercha for a better 3D polymerization [53] by means of manual plugger. The group P1 comprised a plasma treatment of the root canal before guttapercha sealing. The group C2 was characterized by the application of an epoxy resin sealer (Top Seal, Dentsply, Maillefer), conventionally used as adhesive material to bond guttapercha with apical dentine. P2 provided a plasma pre-treatment of dentine surface before sealer application.

Group	Filling procedure
C1	Guttapercha
P1	CAP + Guttapercha
C2	Sealer + Guttapercha
P2	CAP + Sealer + Guttapercha

Tab. 1. Sealing protocol applied to each group after root canal shaping.

Photos of single steps of the procedure were collected in Fig. 6. Finally, the specimens were stored for 1 week at 37°C in saturated environment.

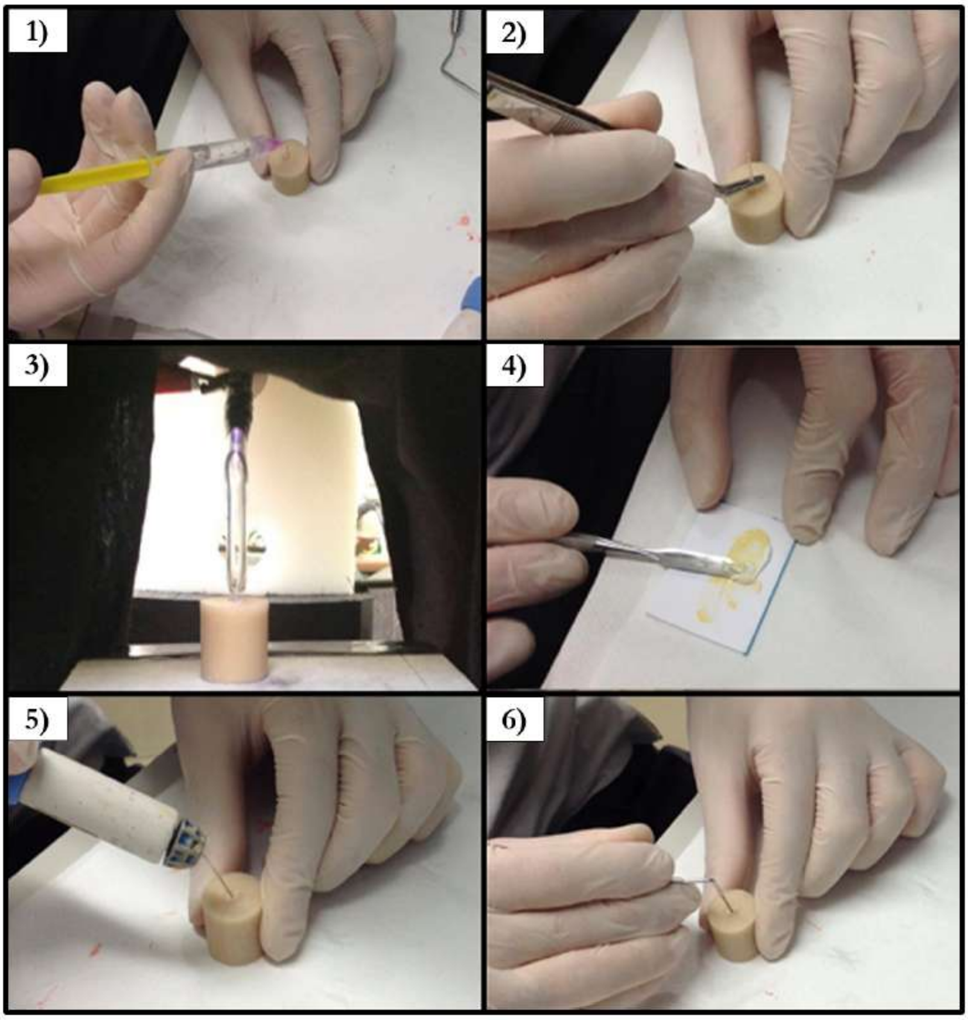


Fig. 6. Different steps of experimental procedures: 1) EDTA rinsing; 2) drying with paper points; 3) CAP treatment; 4) sealer preparation; 5) guttapercha obturation with Calamus Flow; 6) manual compaction/compression of guttapercha.

2.3. Pushout test

According to the procedure reported in [42] for teeth not embedded in resin, 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 as schematically described in Fig. 7. The coronal and apical sections were respectively cut 4 and 1 mm above the apical terminal. Through the molding system procedure, the accurate positioning of the specimens in the pushout testing machine was guaranteed. Images of sample slices preparation and collection are reported in Fig. 8 and Fig. 9.

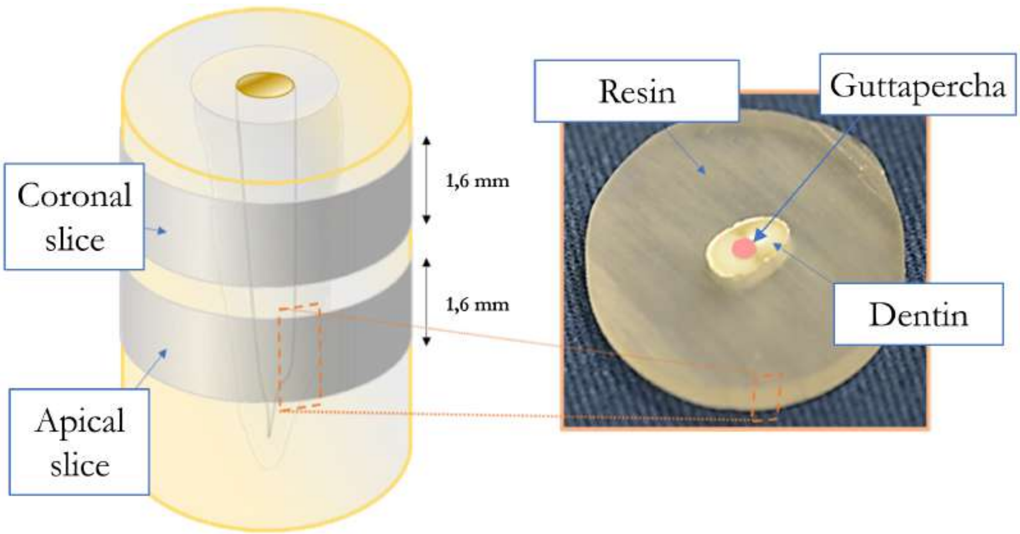


Fig. 7. Tooth coronal and apical section specimens for pushout test.

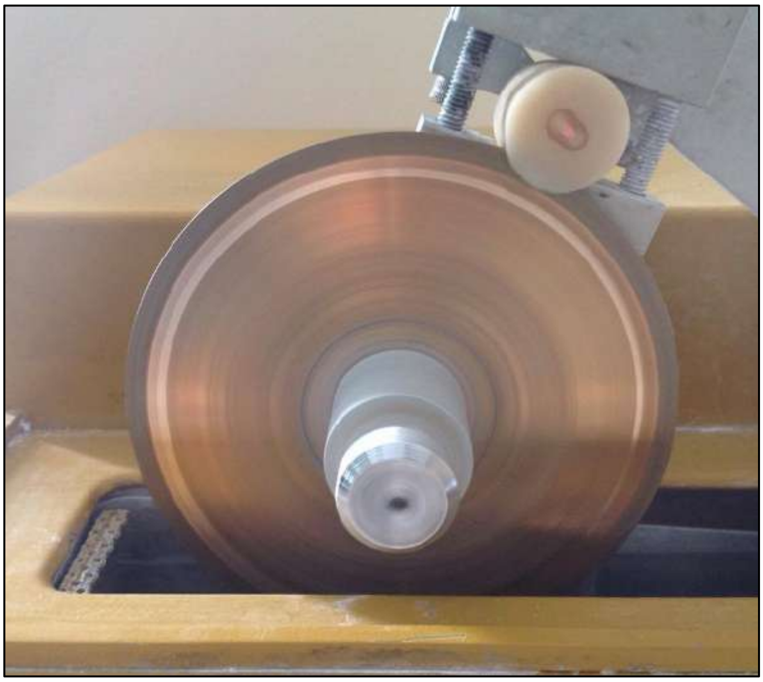


Fig. 8. Isomet diamond saw for the samples section cutting.

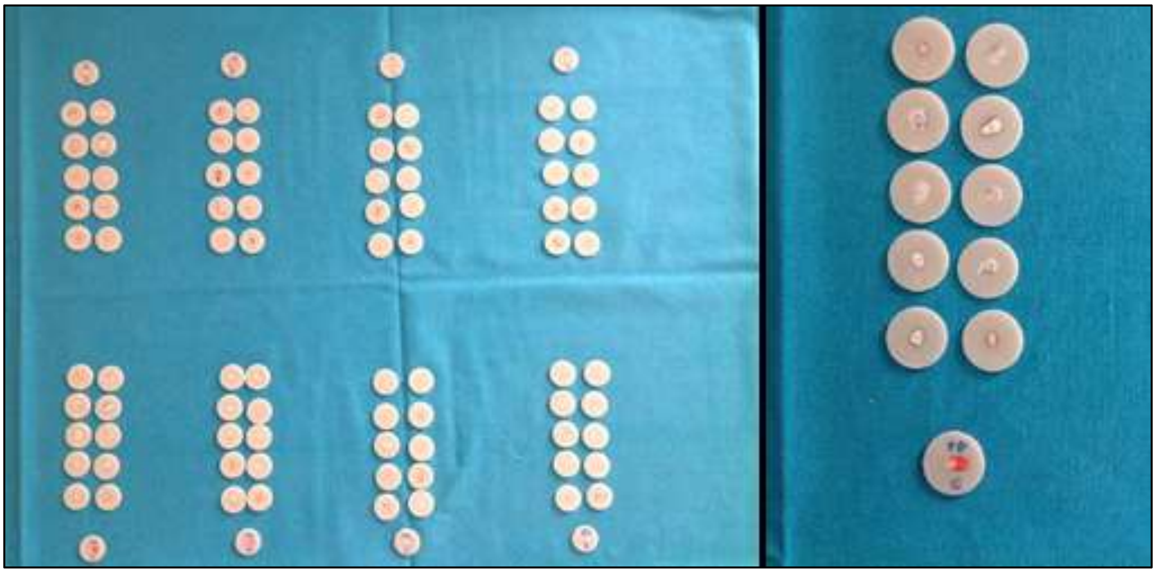


Fig. 9. Specimens prepared for pushout test.

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 $p<0,05$.

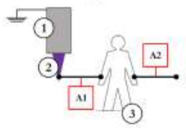
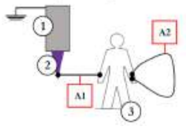
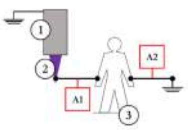
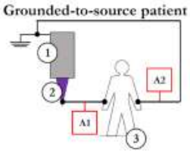
2.4. CLSM analysis

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 described in paragraph 2.2. Since sealer was applied in liquid state, a mixture of colourant-sealer was prepared and then used for C2 and P2 groups. On the other hand, being guttapercha plugger in plastic-solid phase, for the case C1 and P1, 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 C2 and P2, while in the C1 and P1 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 keep open at 100 µm for all acquisitions (512x512 px).

3. Results

3.1. Measurements of leakage currents, ozone production and UV irradiance

In Tab. 2, the recorded values of maximum current are reported for the investigated cases and distances, as described in paragraph 2.1. As clearly shown by the results, the highest values of leakage current are observed in the case of floating patient for which in two conditions the measured current overcomes the threshold value of 100 µA. the safest condition was individuated when the patient is grounded-to-source: all the recorded values of I_{rms} are under the threshold limit. The distance between the source outlet and the soft tissue (the human finger in the present study) relevantly affects the leakage current in the case of not-grounded patient.

	Gap [mm]	**A1_I_{rms} [µA]**	**A2_I_{rms} [µA]**		**Gap [mm]**	**A1_I_{rms} [µA]**	**A2_I_{rms} [µA]**
Floating patient 	2	140	30	Auxiliary leakage current 	2	90	110
	5	120	<10		5	20	40
	12	100	30		12	<10	230
Grounded patient 	2	87	70	Grounded-to-source patient 	2	80	50
	5	38	10		5	100	100
	12	130	40		12	80	50

Tab. 2. Recorded values of Irms for three different distances between human finger and the source outlet: 2, 5 and 12 mm.

In Tab. 3 the values of O_3 concentration are reported. Considering 5 min of CAP treatment as the maximum exposure time envisaged for the entire clinical procedure, the O_3 density does not exceed the threshold of 240 µg/m^3; even so, a suction hose might be used in the future during plasma treatment to be sure to operate the plasma in fully safe conditions for the patient.

D [mm]	**Time** [min]	**Ozone concentration** [µg/m^3]
10	1	< 90
30		90 – 150
10	3	90 - 150
30		150 – 210
10	5	150 – 210

Tab. 3. Measured concentration of ozone for 1, 3 and 5 minutes of CAP treatment. D is the distance from the edge of plastic container and the end of capillary.

As far as the UV irradiance is concerned, the UV radiation produced by the DBD Helium plasma jet is always under the instrument sensitivity ($<1\mu W/cm^2$) and, in turn, under the imposed limit.

3.2. Pushout tests

Tab. 4 shows the mean bonding strength obtained by pushout tests for coronal and apical sections. Results clearly show a statistically significant enhancement, around three times (~ +200% with a $p<<0,05$) compared to control, 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. Relevant increase of adhesion performances, around +50% ($p<<0,05$) is observed for P2 group both in coronal and apical sections, where sealer was used as in conventional treatment. The comparison of P1 and C2 groups, highlights how a plasma treatment of the dentine could replace the application of endodontic sealer because the values of achieved bonding strength are similar to each other.

	Average bonding strength [MPa]	**Increase [+%]**
C1 – Coronal	1,05 ± 0,36	**+ 238,1%**
P1 (plasma) – Coronal	3,56 ± 1,04	
C1 – Apical	1,25 ± 0,40	**+ 194,1%**
P1 (plasma) – Apical	3,69 ± 1,21	
C2 – Coronal	3,10 ± 0,61	**+ 58,6%**
P2 (plasma) – Coronal	4,92 ± 0,85	
C2 – Apical	3,49 ± 0,78	**+ 47,6%**
P2 (plasma) – Apical	5,15 ± 0,71	

Tab. 4. 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.

3.3. CLSM analysis

In Fig. 11 the confocal acquisitions are reported for the case C1, P1, C2 and P2. Comparing the control groups C1 and C2, 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 (P1 and P2), characterized by a CAP pre-treatment of dentine, the penetration of guttapercha (P1) and of endodontic sealer (P2) is extremely amplified for the whole length of the root canal.

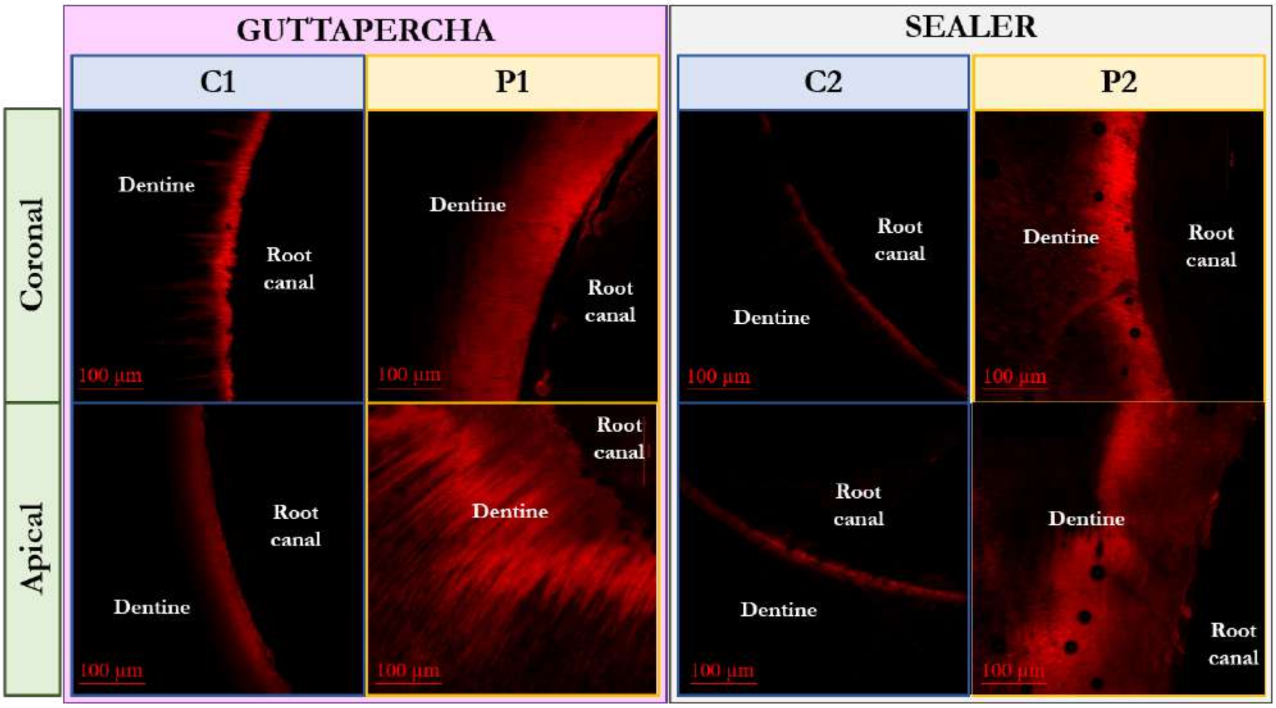


Fig. 10. Collection of CLSM images divided for each investigated group (C1, C2, P1 and P2) and for different regions of root canal: coronal and apical.

4. Discussion of results

As far as the endodontic materials used in root canal restoration, it is well know that the adhesion properties depend on the surface energy of the involved materials (dentine or guttapercha), the surface tension of the adhesive (sealer), the adhesive's quality to be spread over the surfaces [54]. Moreover, an effective bonding, based both on chemical and micromechanical mechanisms [54], requires a dentinal surface that is free of debris and pulp residues from an imperfect mechanical and chemical treatment of the canal [12]. The control groups (C1 and C2) report pushout values in accordance with literature, highlighting the low bonding strength of guttapercha with the dentine substrate [13,14], while the application of an endodontic sealer before guttapercha filling leads to values of mean adhesion strength around 3 MPa, as also found by Lee et al. [10].

Pushout results clearly show a relevant enhancement of the mean bonding strength between the plasma treated dentine and the filling materials used in these experiments, guttapercha or endodontic sealer as well. P2, that represents 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 dentine. Generally, a substrate exposed to a CAP produced in air (or generally O_2/N_2 mixture gases) is functionalized and grafted by the insertion of carbonyl and amine functional groups, resulting in an increased amount of O and N content in the surface [34,37,55,56], leading to an increase of surface wettability. The epoxy resin-based sealer used in this work should be able to react with any exposed amino groups in collagen to form covalent bonds with dentine collagen when the epoxide ring opens [10]. Moreover, as Topseal operates in fluid state characterized by a polar component (amine paste), it could be spread better in a plasma-activated surface with higher hydrophilicity resulting in deeper sealer infiltration into the demineralized dentine and, thus, in a thicker hybrid layer [12].

CLSM acquisitions are focused on the infiltration of filling material essentially in dentinal tubules and support the hypothesis of an enhanced penetration of sealer into dentine substrate along the whole length of root canal. It is worth mentioning that the formation of resin tags in lateral tubules contributes only for a percentage <15% to the total adhesion strength [12,57,58], while hybridization, with the resulted hybrid layer, is considered the primary process involved in resin-based restoration [12].

Surprisingly, the adhesion performances of group P1 show an unexpected increase (+238%, $p<<0,05$), revealing how guttapercha was able to “self-bond” directly with the dentine substrate without any sealer application. The guttapercha used in this work does not present any chemical characteristic that can promote an adhesive restoration; and, in light of this consideration, the pushout results appear even more interesting. As demonstrated in [41], CAP treatment induces an improvement of the wettability of dentine surface, that may support the spreading of guttapercha in viscous state during the filling procedure. Compared to the resin tags formed by sealers or adhesive systems, which are characterized by high rigidity, guttapercha tags could probably play a major role of anchor of the sealed block to the dentine for their higher flexibility. As a matter of fact, CLSM images show the increased penetration of guttapercha in lateral tubules both in coronal and apical region, and pushout results of P1 group highlight that the achieved adhesion performances are comparable to ones obtained with the application of endodontic sealer before guttapercha filling. 180 sec of CAP treatment increase the adhesion by an enhanced mechanical anchorage of guttapercha into dentine substrate, as well as endodontic sealer can favour the formation of hybrid layer promoting, chemically and micromechanically, the adhesion of a monoblock to dentine. However, the highest value of adhesion of sealed monoblock and dentine is achieved performing the conventional procedure (sealer + guttapercha) with a CAP pre-treatment of the cleaned root canal (P2).

It is worth considering that the primary function of the guttapercha is to seal the apical region avoiding gaps, which can provide pathways for bacteria and toxins to the periapex and apical connective tissues [59]. Even if CLSM acquisitions cannot confirm the absence of gaps in the whole coronal-apical perimeter, confocal images of group P1 and P2 clearly show a higher infiltration and spreading of both materials (sealer and guttapercha) into dentine substrate because of plasma treatment, that may result in an enhancement of apical sealing. Moreover, the results of P1 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 [15–18,60]. 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 [18]. Thus, 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 (sealer + guttapercha).

Although the electrical characterization, the measurements of ozone concentration and UV irradiance have shown how the plasma source can operate in safety conditions, to assess the potentialities of a CAP treatment in the restoration of root canals, future studies are required to investigate the long-term stability of adherent monoblock to dentine surface and to evaluate the sealing performances by means of dye filtration tests or total-canal scanning imaging.

5. 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 [41], 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.

The experimental results, combined with ones obtained in the field of root canal disinfection [43], demonstrated the efficacy of CAP treatment, performed with a DBD-jet plasma source operating in the same conditions, in the phases of both disinfection and restoration of the root canal. 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.

A. Appendices

Further efforts were undertaken by AlmaPlasma s.r.l. to improve the ergonomics of the device shown in Fig. 1. More specifically, to comply with the dimensions of devices commonly used in the dental field, the geometry of each component of the first prototype of DBD-jet plasma source (Fig. A.1a) was optimized and adapted to an external housing design having a diameter of 21.5 mm (Fig A.1b). In addition, the need to minimise the weight of the device resulted in changes of the external housing material: instead of aluminium, it was realized in polymethylmethacrylate (PMMA), a transparent material of low density that also allows the operator to monitor the discharge zone during operation of the plasma source (Fig. A.1c).

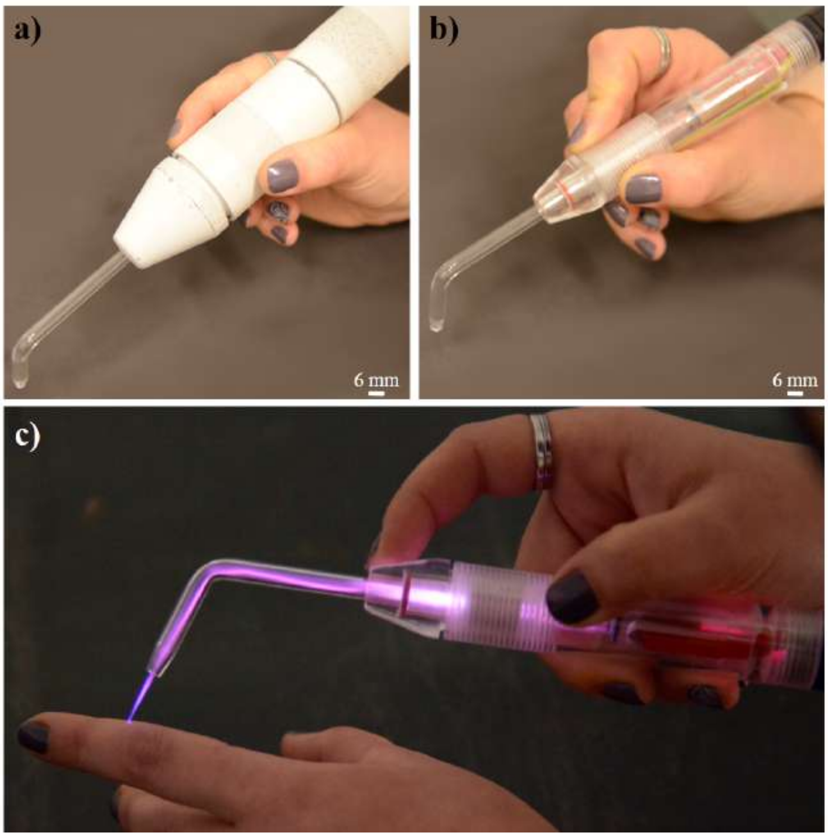


Fig. A.1. a) first prototype of the DBD-jet plasma source designed by AlmaPlasma s.r.l.; b) second prototype of the DBD-jet plasma source designed by AlmaPlasma s.r.l.; c) plasma plume, produced by the second prototype of DBD-jet plasma source.

Fig. A.2. shows the section view of both prototypes; even if the number of components was unchanged, electrical and gas connections were improved to take up as little space as possible. More in detail, the axial inlet of helium gas through the upper part of high voltage electrode has allowed a considerable reduction of the plasma source diameter (from 31.7 mm to 21.5 mm). The design changes envolved only the geometry of the support elements necessary to encase the electrodes and the dielectric capillary and to fix gas and electrical connections. Therefore, the relative position between high voltage and ground electrode has remained unchanged. As a consequence of this, no significative differences of plasma propagation have been identified; tests will be performed in the future to confirm the efficacy and the safety levels of this new prototype of DBD-jet plasma source.

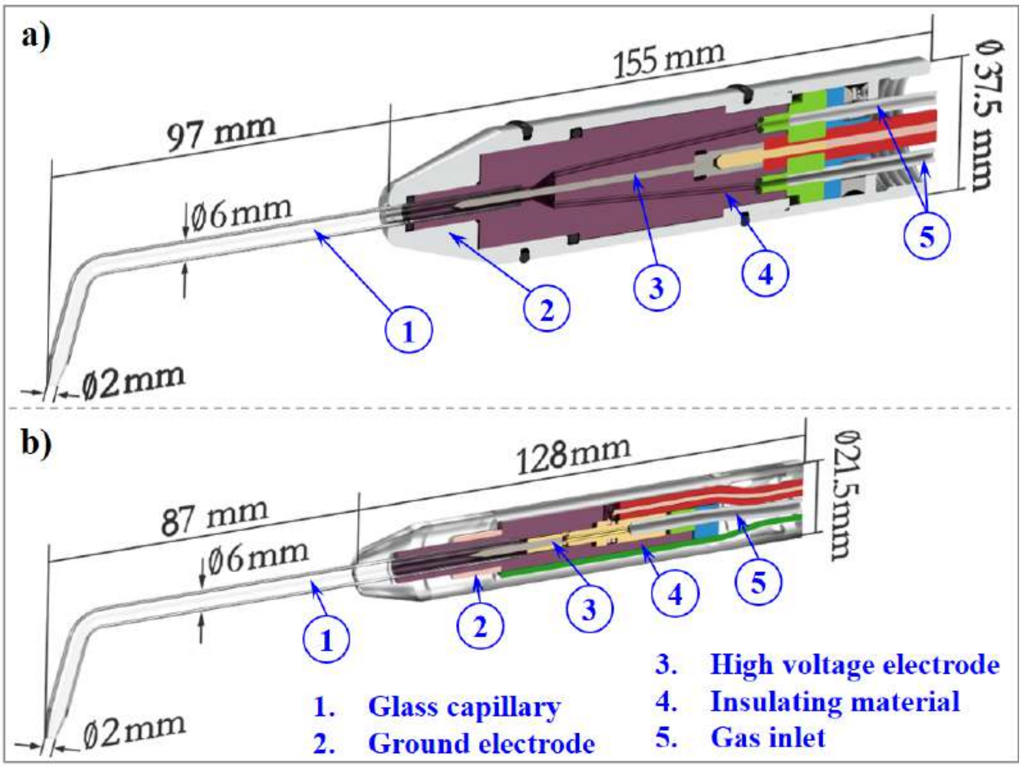


Fig. A.2. Section view of the a) first prototype and the b) second prototype of the DBD-jet plasma source, by AlmaPlasma s.r.l.

References

[1] G.H. Li, L.N. Niu, L.C. Selem, A.A. Eid, B.E. Bergeron, J.H. Chen, D.H. Pashley, F.R. Tay, Quality of obturation achieved by an endodontic core-carrier system with crosslinked gutta-percha carrier in single-rooted canals, J. Dent. 42 (2014) 1124–1134. https://doi.org/10.1016/j.jdent.2014.04.008.

[2] U. Sjögren, B. Hägglund, G. Sundqvist, K. Wing, Factors affecting the long-term results of endodontic treatment, J. Endod. 16 (1990) 498–504. https://doi.org/10.1016/S0099-2399(07)80180-4.

[3] Y. Zhong, J. Chasen, R. Yamanaka, R. Garcia, E.K. Kaye, J.S. Kaufman, J. Cai, T. Wilcosky, M. Trope, D.J. Caplan, Extension and Density of Root Fillings and Postoperative Apical Radiolucencies in the Veterans Affairs Dental Longitudinal Study, J. Endod. 34 (2008) 798–803. https://doi.org/10.1016/j.joen.2008.03.022.

[4] B.M. Gillen, S.W. Looney, L.S. Gu, B.A. Loushine, R.N. Weller, R.J. Loushine, D.H. Pashley, F.R. Tay, Impact of the quality of coronal restoration versus the quality of root canal fillings on success of root canal treatment: A systematic review and meta-analysis, J. Endod. 37 (2011) 895–902. https://doi.org/10.1016/j.joen.2011.04.002.

[5] H. Schilder, A. Goodman, W. Aldrich, The thermomechanical properties of gutta-percha. Part V. Volume changes in bulk gutta-percha as a function of temperature and its relationship to molecular phase transformation, Oral Surg. Oral Med. Oral Pathol. 59 (1985) 285–296. https://doi.org/10.1016/0030-4220(85)90169-0.

[6] A. Goodman, H. Schilder, W. Aldrich, The thermomechanical properties of gutta-percha. Part IV. A thermal profile of the warm gutta-percha packing procedure, Oral Surg. Oral Med. Oral Pathol. 51 (1981) 544–551. https://doi.org/10.1016/0030-4220(81)90017-7.

[7] H. Schilder, A. Goodman, W. Aldrich, The thermomechanical properties of gutta-percha: III. Determination of phase transition temperatures for gutta-percha, Oral Surg. Oral Med. Oral Pathol. 38 (1974) 109–114. https://doi.org/10.1016/0030-4220(74)90321-1.

[8] A. Goodman, H. Schilder, W. Aldrich, The thermomechanical properties of gutta-percha: II. The history and molecular chemistry of gutta-percha, Oral Surg. Oral Med. Oral Pathol. 37 (1974) 954–961. https://doi.org/10.1016/0030-4220(74)90448-4.

[9] H. Schilder, A. Goodman, W. Aldrich, The thermomechanical properties of gutta-percha: I. The compressibility of gutta-percha, Oral Surg. Oral Med. Oral Pathol. 37 (1974) 946–953. https://doi.org/10.1016/0030-4220(74)90447-2.

[10] K.-W. Lee, M. Williams, J.J. Camps, D.H. Pashley, Adhesion of Endodontic Sealers to Dentin and Gutta-Percha, J. Endod. 28 (2002) 684–688. https://doi.org/10.1097/00004770-200210000-00002.

[11] D. McComb, D.C. Smith, Comparison of physical properties of polycarboxylate-based and conventional root canal sealers, J. Endod. 2 (1976) 228–235. https://doi.org/10.1016/S0099-2399(76)80162-8.

[12] R.S. Schwartz, Adhesive Dentistry and Endodontics. Part 2: Bonding in the Root Canal System-The Promise and the Problems: A Review, J. Endod. 32 (2006) 1125–1134. https://doi.org/10.1016/j.joen.2006.08.003.

[13] L. Pommel, I. About, D. Pashley, J. Camps, Apical Leakage of Four Endodontic Sealers, J. Endod. 29 (2003) 208–210. https://doi.org/10.1097/00004770-200303000-00011.

[14] G. ichiro Hata, S. Kawazoe, T. Toda, F.S. Weine, Sealing ability of Thermafil with and without sealer, J. Endod. 18 (1992) 322–326. https://doi.org/10.1016/s0099-2399(06)80481-4.

[15] S. Bouillaguet, J.C. Wataha, P.E. Lockwood, C. Galgano, A. Golay, I. Krejci, Cytotoxicity and sealing properties of four classes of endodontic sealers evaluated by succinic dehydrogenase activity and confocal laser scanning microscopy, Eur. J. Oral Sci. 112 (2004) 182–187. https://doi.org/10.1111/j.1600-0722.2004.00115.x.

[16] J. Bratel, M. Jontell, U. Dahlgren, G. Bergenholtz, Effects of root canal sealers on immunocompetent cells in vitro and in vivo, Int. Endod. J. 31 (1998) 178–188. https://doi.org/10.1046/j.1365-2591.1998.00148.x.

[17] A.U. Eldeniz, K. Mustafa, D. Ørstavik, J.E. Dahl, Cytotoxicity of new resin-, calcium hydrooxide- and silicone-based root canal sealers on fibroblasts derived from human gingiva and L929 cell lines, Int. Endod. J. 40 (2007) 329–337. https://doi.org/10.1111/j.1365-2591.2007.01211.x.

[18] P.T. da Silva, F.G. Pappen, E.M. Souza, J.E. Dias, I. Bonetti Filho, I.Z. Carlos, R.T. Leonardo, Cytotoxicity evaluation of four endodontic sealers, Braz. Dent. J. 19 (2008) 228–231. https://doi.org/10.1590/S0103-64402008000300010.

[19] A. Al-Haddad, Z.A.C.A. Aziz, Bioceramic-Based Root Canal Sealers: A Review, Int. J. Biomater. 2016 (2016) 1–10. https://doi.org/10.1155/2016/9753210.

[20] M. Colombo, C. Poggio, A. Dagna, M.V. Meravini, P. Riva, F. Trovati, G. Pietrocola, Biological and physico-chemical properties of new root canal sealers, J. Clin. Exp. Dent. 10 (2018) e120–e126. https://doi.org/10.4317/jced.54548.

[21] P. Spencer, Q. Ye, J. Park, E.M. Topp, A. Misra, O. Marangos, Y. Wang, B.S. Bohaty, V. Singh, F. Sene, J. Eslick, K. Camarda, J.L. Katz, Adhesive/Dentin Interface: The Weak Link in the Composite Restoration, Ann. Biomed. Eng. 38 (2010) 1989–2003. https://doi.org/10.1007/s10439-010-9969-6.

[22] G.C. Kim, H.W. Lee, J.H. Byun, J. Chung, Y.C. Jeon, J.K. Lee, Dental applications of low-temperature nonthermal plasmas, Plasma Process. Polym. 10 (2013) 199–206. https://doi.org/10.1002/ppap.201200065.

[23] V. Arora, V. Nikhil, N.K. Suri, P. Arora, Cold Atmospheric Plasma (CAP) In Dentistry, Dentistry. 4 (2014) 1–5. https://doi.org/10.4172/2161-1122.1000189.

[24] M. Gherardi, R. Tonini, V. Colombo, Plasma in Dentistry: Brief History and Current Status, Trends Biotechnol. 36 (2018) 583–585. https://doi.org/10.1016/j.tibtech.2017.06.009.

[25] S. Cha, Y.-S. Park, Plasma in dentistry, Clin. Plasma Med. 2 (2014) 4–10. https://doi.org/10.1016/j.cpme.2014.04.002.

[26] G. Fridman, M. Peddinghaus, M. Balasubramanian, H. Ayan, A. Fridman, A. Gutsol, A. Brooks, Blood Coagulation and Living Tissue Sterilization by Floating-Electrode Dielectric Barrier Discharge in Air, Plasma Chem. Plasma Process. 26 (2006) 425–442. https://doi.org/10.1007/s11090-006-9024-4.

[27] Y. Liu, Q. Liu, Q.S. Yu, Y. Wang, Nonthermal atmospheric plasmas in dental restoration, J. Dent. Res. 95 (2016) 496–505. https://doi.org/10.1177/0022034516629425.

[28] D. Liu, Z. Xiong, T. Du, X. Zhou, Y. Cao, X. Lu, Bacterial-killing effect of atmospheric pressure non-equilibrium plasma jet and oral mucosa response, J. Huazhong Univ. Sci.

Technol. [Medical Sci. 31 (2011) 852–856. https://doi.org/10.1007/s11596-011-0690-y.

[29] I. Molnar, J. Papp, A. Simon, S.D. Anghel, Deactivation of Streptococcus mutans Biofilms on a Tooth Surface Using He Dielectric Barrier Discharge at Atmospheric Pressure, Plasma Sci. Technol. 15 (2013) 535–541. https://doi.org/10.1088/1009-0630/15/6/09.

[30] O. Lunov, O. Churpita, V. Zablotskii, I.G. Deyneka, I.K. Meshkovskii, A. Jäger, E. Syková, Š. Kubinová, A. Dejneka, Non-thermal plasma mills bacteria: Scanning electron microscopy observations, Appl. Phys. Lett. 106 (2015) 053703–053707. https://doi.org/10.1063/1.4907624.

[31] K. Duske, I. Koban, E. Kindel, K. Schröder, B. Nebe, B. Holtfreter, L. Jablonowski, K.D. Weltmann, T. Kocher, Atmospheric plasma enhances wettability and cell spreading on dental implant metals, J. Clin. Periodontol. 39 (2012) 400–407. https://doi.org/10.1111/j.1600-051X.2012.01853.x.

[32] Y. Zhang, Q. Yu, Y. Wang, Non-thermal atmospheric plasmas in dental restoration: Improved resin adhesive penetration, J. Dent. 42 (2014) 1033–1042. https://doi.org/10.1016/j.jdent.2014.05.005.

[33] I. Koban, K. Duske, L. Jablonowski, K. Schröder, B. Nebe, R. Sietmann, K.D. Weltmann, N.O. Hübner, A. Kramer, T. Kocher, Atmospheric Plasma Enhances Wettability and Osteoblast Spreading on Dentin In Vitro: Proof-of-principle, Plasma Process. Polym. 8 (2011) 975–982. https://doi.org/10.1002/ppap.201100030.

[34] A.C. Ritts, H. Li, Q. Yu, C. Xu, X. Yao, L. Hong, Y. Wang, Dentin surface treatment using a non-thermal argon plasma brush for interfacial bonding improvement in composite restoration, Eur. J. Oral Sci. 118 (2010) 510–516. https://doi.org/10.1111/j.1600-0722.2010.00761.x.

[35] G.J. Han, J.H. Kim, S.N. Chung, B.H. Chun, C.K. Kim, D.G. Seo, H.H. Son, B.H. Cho, Effects of non-thermal atmospheric pressure pulsed plasma on the adhesion and durability of resin composite to dentin, Eur. J. Oral Sci. 122 (2014) 417–423. https://doi.org/10.1111/eos.12153.

[36] N.R.F.A. Silva, P.G. Coelho, G.B. Valverde, K. Becker, R. Ihrke, A. Quade, V.P. Thompson, Surface characterization of Ti and Y-TZP following non-thermal plasma exposure, J. Biomed. Mater. Res. Part B Appl. Biomater. 99 B (2011) 199–206. https://doi.org/10.1002/jbm.b.31887.

[37] A. Lehmann, A. Rueppell, A. Schindler, I.M. Zylla, H.J. Seifert, F. Nothdurft, M. Hannig, S. Rupf, Modification of Enamel and Dentin Surfaces by Non-Thermal Atmospheric Plasma, Plasma Process. Polym. 10 (2013) 262–270. https://doi.org/10.1002/ppap.201200088.

[38] G.B. Valverde, P.G. Coelho, M.N. Janal, F.C. Lorenzoni, R.M. Carvalho, V.P. Thompson, K.D. Weltemann, N.R.F.A. Silva, Surface characterisation and bonding of Y-TZP following non-thermal plasma treatment, J. Dent. 41 (2013) 51–59. https://doi.org/10.1016/j.jdent.2012.10.002.

[39] W. Chen, J. Huang, N. Du, X.-D. Liu, X.-Q. Wang, G.-H. Lv, G.-P. Zhang, L.-H. Guo, S.-Z. Yang, Treatment of enterococcus faecalis bacteria by a helium atmospheric cold plasma brush with oxygen addition, J. Appl. Phys. 112 (2012) 13304–133048. https://doi.org/10.1063/1.4732135.

[40] R. Hirata, A. Paula, A. Ayres, S. Machado, P.G. Coelho, V.P. Thompson, Long-term Adhesion Study of Self-etching Systems to Plasma-treated Dentin, J. Adhes. Dent. 17 (2015)

227–233. https://doi.org/10.3290/j.jad.a34138.

[41] A. Stancampiano, D. Forgione, E. Simoncelli, R. Laurita, R. Tonini, M. Gherardi, V. Colombo, The Effect of Cold Atmospheric Plasma (CAP) Treatment at the Adhesive-Root Dentin Interface, J. Adhes. Dent. 21 (2019) 229–237. https://doi.org/10.3290/j.jad.a42521.

[42] L. Boschian Pest, G. Cavalli, P. Bertani, M. Gagliani, Adhesive post-endodontic restorations with fiber posts: push-out tests and SEM observations, Dent. Mater. 18 (2002) 596–602. https://doi.org/10.1016/s0109-5641(02)00003-9.

[43] E. Simoncelli, D. Barbieri, R. Laurita, A. Liguori, A. Stancampiano, L. Viola, R. Tonini, M. Gherardi, V. Colombo, Preliminary investigation of the antibacterial efficacy of a handheld Plasma Gun source for endodontic procedures, Clin. Plasma Med. 3 (2015) 77–86. https://doi.org/10.1016/j.cpme.2015.11.001.

[44] C. Cachoncinlle, R. Viladrosa, A. Khacef, E. Robert, J.M. Pouvesle, S. Dozias, Transient plasma ball fenerator system at long distance, WO2009/050240A1, 2009.

[45] J.-M. Pouvesle, C. Cachoncinlle, R. Viladrosa, A. Khacef, E. Robert, S. Dozias, Transient plasma ball generation system at long distance, US8482206B2, 2013.

[46] M.S. Mann, R. Tiede, K. Gavenis, G. Daeschlein, R. Bussiahn, K.D. Weltmann, S. Emmert, T. von Woedtke, R. Ahmed, Introduction to DIN-specification 91315 based on the characterization of the plasma jet kINPen® MED, Clin. Plasma Med. 4 (2016) 35–45. https://doi.org/10.1016/j.cpme.2016.06.001.

[47] K.-D. Weltmann, T. von Woedtke, Basic requirements for plasma sources in medicine, Eur. Phys. J. - Appl. Phys. 55 (2011) 13807. https://doi.org/10.1051/epjap/2011100452.

[48] A. Azarpazhooh, H. Limeback, The application of ozone in dentistry: A systematic review of literature, J. Dent. 36 (2008) 104–116. https://doi.org/10.1016/j.jdent.2007.11.008.

[49] M. Sharma, J.B. Hudson, Ozone gas is an effective and practical antibacterial agent, Am. J. Infect. Control. 36 (2008) 559–563. https://doi.org/10.1016/j.ajic.2007.10.021.

[50] U. Kogelschatz, B. Eliasson, M. Hirth, Ozone generation from oxygen and air: Discharge physics and reaction mechanisms, Ozone Sci. Eng. 10 (1988) 367–377. https://doi.org/10.1080/01919518808552391.

[51] Z.B. Guzel-Seydim, A.K. Greene, A.C. Seydim, Use of ozone in the food industry, Leb. Technol. 37 (2004) 453–460. https://doi.org/10.1016/j.lwt.2003.10.014.

[52] M.G. Mustafa, Biochemical basis of ozone toxicity, Free Radic. Biol. Med. 9 (1990) 245–265. https://doi.org/10.1016/0891-5849(90)90035-H.

[53] C.J. Ruddle, Filling root canal systems: the Calamus 3D obturation technique, Adv. Endod. 29 (2010) 78–81.

[54] I.M. Saleh, I.E. Ruyter, M. Haapasalo, D. Ørstavik, The effects of dentine pretreatment on the adhesion of root-canal sealers, Int. Endod. J. 35 (2002) 859–866. https://doi.org/10.1046/j.1365-2591.2002.00585.x.

[55] J.M. Grace, L.J. Gerenser, Plasma Treatment of Polymers, J. Dispers. Sci. Technol. 24 (2003) 305–341. https://doi.org/10.1081/DIS-120021793.

[56] J.-H. Kim, M.-A. Lee, G.-J. Han, B.-H. Cho, Plasma in dentistry: a review of basic concepts and applications in dentistry, Acta Odontol. Scand. 72 (2014) 1–12. https://doi.org/10.3109/00016357.2013.795660.

[57] J. Tagami, L. Tao, D.H. Pashley, Correlation among dentin depth, permeability, and bond strength of adhesive resins, Dent. Mater. 6 (1990) 45–50. https://doi.org/10.1016/0109-5641(90)90044-F.

[58] P.N. Pereira, M. Okuda, H. Sano, T. Yoshikawa, M.F. Burrow, J. Tagami, Effect of intrinsic wetness and regional difference on dentin bond strength, Dent. Mater. 15 (1999) 46—53. https://doi.org/10.1016/S0109-5641(99)00013-5.

[59] W. Geurtsen, G. Leyhausen, Biological aspects of root canal filling materials - histocompatibility,cytotoxicity, and mutagenicity., Clin. Oral Investig. 1 (1997) 5–11. https://doi.org/10.1007/s007840050002.

[60] M. Bernáth, J. Szabó, Tissue reaction initiated by different sealers, Int. Endod. J. 36 (2003) 256–261. https://doi.org/10.1046/j.1365-2591.2003.00662.x.