EGAS MONIZ SCHOOL of HEALTH & SCIENCE | INSTITUTO UNIVERSITÁRIO EGAS MONIZ

INSTITUTO UNIVERSITÁRIO EGAS MONIZ

MESTRADO INTEGRADO EM MEDICINA DENTÁRIA

ANÁLISE LABORATORIAL DE DENTINA TRATADA COM PLASMA NÃO TÉRMICO ATMOSFÉRICO UTILIZANDO ESPECTROSCOPIA DE INFRAVERMELHO POR TRANSFORMADA DE FOURIER. (ATR-FTIR)

Trabalho submetido por
Lilou KLEIN
para a obtenção do grau de Mestre em Medicina Dentária

julho de 2026

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Trabalho orientado por

Prof. Doutora Ana Filipa Chasqueira

e coorientado por

Prof. Doutor António Sales Delgado

julho de 2026

AGRADECIMENTOS

Gostaria de expressar o meu profundo agradecimento à minha orientadora de tese, **Prof. Doutora Ana Filipa Chasqueira,** pela sua orientação, disponibilidade constante e pelo apoio científico ao longo de todo o desenvolvimento deste trabalho.

Ao meu coorientador, **Prof. Doutor António Sales Delgado**, agradeço o acompanhamento, os ensinamentos partilhados e a sua contribuição fundamental para a realização deste estudo.

À Egas Moniz School of Health and Science, agradeço a oportunidade de realização deste trabalho e as condições proporcionadas ao longo da minha formação.

Manifesto ainda o meu sincero agradecimento à Professora **Susana Sério**, pela disponibilização do seu laboratório, pelo tempo dedicado, pela partilha de conhecimento e pelas explicações que foram essenciais para a concretização deste trabalho.

À minha parceira de clínica, **Salomé Demellier,** agradeço profundamente a partilha deste percurso, o apoio constante, a entreajuda nos momentos mais exigentes e a força transmitida ao longo de toda esta experiência, que tornou este caminho mais leve e significativo.

Aos meus amigos, **Lucie D., Pierre P., Clotilde T., Flavie T., Carla C., e os outros**, agradeço de coração o apoio, a compreensão e a presença constante. Foram um pilar essencial, trazendo equilíbrio, motivação e alegria ao longo de todo este percurso.

Por fim, dirijo um agradecimento muito especial aos meus pais, **Catherine e Hervé Klein**, pelo seu apoio incondicional, a sua correcção da minha tese toda, pelo amor constante e por estarem sempre presentes em todos os momentos desta caminhada.

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Mais declaro que esta Trabalho de Projeto final Análise laboratorial de dentina tratada por plasma não térmico atmosférico utilizando espectrocopia de infravermelho por transformada de Fourier (ATR-FTIR), não foi apresentada, para efeitos de avaliação, a qualquer outra entidade ou instituição, para além da(s) diretamente envolvida(s) na sua elaboração, e que os conteúdos das versões impressa e eletrónica são inteiramente coincidentes.

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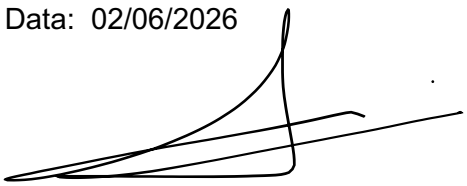

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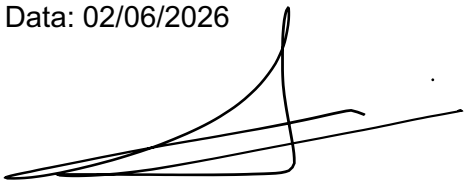

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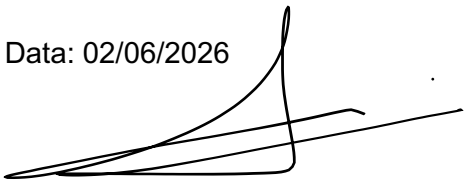

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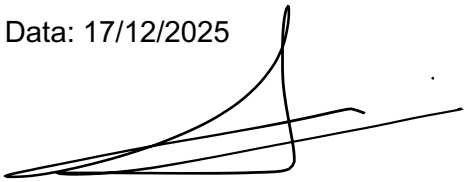

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A seguinte tabela apresenta diferentes opções de partilha de dados, incluindo exemplos de formatos e níveis de acessibilidade. Deverá selecionar a(s) opção(ões) que melhor se adequa(m) ao seu projeto, assegurando o cumprimento das diretrizes éticas e científicas aplicáveis.

Tabela 1: Exemplos de declarações de disponibilidade de dados. Onde aparece [nome do repositório, por exemplo, "pure"] deve ser preenchido

Disponibilidade de dados	**Modelo de declaração de disponibilidade de dados**	**Política de partilha**	**Assinale com "x"**
Dados disponíveis num repositório público que emite conjuntos de dados com DOIs	Os dados que apoiam as conclusões deste estudo estão disponíveis em [nome do repositório, por exemplo, "pure"] em http://doi.org/[doi], número de referência [número de referência].	Partilha geral sem pedido	
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Disponibilidade de dados	**Modelo de declaração de disponibilidade de dados**	**Política de partilha**	**Assinale com "x"**
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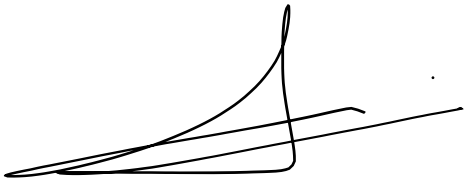
Dados disponíveis no artigo ou nos seus materiais suplementares	Os autores confirmam que os dados que suportam as conclusões deste estudo estão disponíveis no artigo [e/ou] nos seus materiais suplementares.	Básico, partilhar a pedido	
Dados gerados numa instalação central de grande escala, disponíveis mediante pedido	Os dados em bruto foram gerados em [nome da instalação]. Os dados derivados que suportam as conclusões deste estudo estão disponíveis junto do autor correspondente [iniciais], mediante pedido.	Básico, partilhar a pedido	
Embargo dos dados devido a restrições comerciais	Os dados que suportam os resultados estarão disponíveis em [nome do repositório] em [URL / ligação DOI] após um embargo de [6 meses] a partir da data de publicação para permitir a comercialização dos resultados da investigação.	Básico, Partilhar a pedido	
Dados disponíveis a pedido devido a restrições de privacidade/éticas	Os dados que sustentam as conclusões deste estudo estão disponíveis mediante pedido ao autor correspondente, [iniciais]. Os dados não estão disponíveis ao público devido a [restrições, por exemplo, o facto de conterem informações que podem comprometer a	Básico, Partilhar a pedido	

Disponibilidade de dados	Modelo de declaração de disponibilidade de dados	Política de partilha	Assinale com "x"
	privacidade dos participantes na investigação].		
Dados sujeitos a restrições de terceiros	Os dados que suportam as conclusões deste estudo estão disponíveis [junto de] [terceiros]. Aplicam-se restrições à disponibilidade destes dados, que foram utilizados sob licença para este estudo. Os dados estão disponíveis [através dos autores / no URL] com a permissão de [terceiros].	Básico, Partilhar a pedido	
Dados disponíveis a pedido dos autores	Os dados que suportam as conclusões deste estudo estão disponíveis junto do autor correspondente, [L.K.], mediante pedido razoável.	Básico, Partilhar a pedido	**X**
Partilha de dados não aplicável - não foram gerados novos dados	A partilha de dados não é aplicável a este artigo, uma vez que não foram criados ou analisados novos dados neste estudo.	-	
Dados não digitais disponíveis	Os dados não digitais que suportam este estudo são conservados em [adicionar local].	Básicos	
Dados não disponíveis devido a restrições [éticas/legais/comerciais	Devido à natureza da investigação, os dados de apoio [éticos/legais/comerciais] não estão disponíveis.	-	
Dados não disponíveis - consentimento do participante	Os participantes neste estudo não deram consentimento escrito para que os seus dados fossem partilhados	-	

Disponibilidade de dados	Modelo de declaração de disponibilidade de dados	Política de partilha	Assinale com “x”
	publicamente, pelo que, devido à natureza sensível da investigação, os dados de apoio não estão disponíveis.		

O Orientando: Lilou KLEIN Data: 02/06/2026



O Orientador: Prof. Doutora Ana Filipa Chasqueira Data: 11/06/2026

O Coorientador: Prof. Doutor António Sales Delgado Data: 14/06/2026

Resumo

Objetivos: O objetivo deste estudo *in vitro* é avaliar o efeito do plasma atmosférico não térmico (NTAP) na superfície da dentina, em diferentes tempos, utilizando a espectroscopia de infravermelhos por transformada de Fourier em técnica de refletância atenuada total (ATR-FTIR).

Materiais e Métodos: Foram utilizados 7 molares humanos saudáveis, dos quais se seccionou uma fatia de dentina coronal de 3,5 mm. Sobre a superfície da dentina foi aplicado NTAP utilizando uma caneta de plasma de argon a 2mm da dentina durante 10s. Todas as amostras foram analisadas por ATR-FTIR, nos tempos T0, T10min, T1h, T24h, utilizando o acessório de cristal ATR e o modo Spectrum do software Shimadzu LabSolutions IR. Os espectros foram adquiridos na faixa de 4000–400 cm^{-1}, com uma resolução espectral de 2 cm^{-1} e 32 scans por medição, no modo de absorvância. As análises estatísticas foram realizadas em Python 3.11. Os resultados do ATR-FTIR e as rácios derivados foram comparados ao longo do tempo com o teste de Friedman e o W de Kendall como medida do tamanho do efeito. A correção FDR de Benjamini–Hochberg foi aplicada para comparações múltiplas, e a significância foi definida em $\alpha = 0,05$.

Resultados: A análise de medidas repetidas, imediatamente, após 10 min, 1h e 24h, em comparação com o grupo controlo (n=7), não revelou alterações significativas dependentes do tempo nos parâmetros da dentina relacionados com a fase mineral após o tratamento com NTAP. As variações temporais mais pronunciadas foram observadas nas regiões espectrais orgânicas. Contudo, os efeitos devem ser interpretados como tendências temporais.

Conclusão: A análise por ATR-FTIR demonstrou modificações transitórias que afectam predominantemente a fase orgânica da dentina, com os decréscimos mais distintos a ocorrerem passado 60 min e regressão parcial às 24 h. Os resultados sugerem uma resposta dependente do tempo relevante, com alteraçoes transitórias e alterações sustentadas.

Palavras-chave: ATR; dentina; FTIR; plasma não térmico atmosférico

Análise laboratorial de dentina tratada por plasma não térmico atmosférico utilizando espectrocopia de infravermelho por transformada de Fourier. (ATR-FTIR)

Abstract

Objectives: The aim of this *in vitro* study is to evaluate the effect of non-thermal atmospheric plasma (NTAP) on the dentin surface at different time points, using Fourier-transform infrared spectroscopy with the attenuated total reflection (ATR-FTIR) technique.

Materials and Methods: Seven healthy human molars were used, from which a 3.5 mm coronal dentin slice was sectioned. NTAP was applied to the dentin surface using an argon (99% purity, air liquid) plasma pen in continuous mode 4-6 L/min, 2 mm from de dentin during 10 s. All specimens were analysed by ATR-FTIR at baseline (T0), 10 min (T10min), 1 h (T1h), and 24 h (T24h) using an ATR crystal accessory and the Spectrum mode of Shimadzu LabSolutions IR software. Spectra were acquired over the 4000–400 cm^{-1} range, with a spectral resolution of 2 cm^{-1} and 32 scans per measurement, in absorbance mode. Statistical analyses were performed in Python 3.11. ATR-FTIR outcomes and derived ratios were compared over time using the Friedman test, with Kendall's W as the effect-size measure. Benjamini–Hochberg false discovery rate correction was applied for multiple comparisons, and significance was set at $\alpha = 0.05$.

Results: Repeated-measures analysis across baseline, immediate, 10 min, 1 h, and 24 h time points (n=7) revealed no significant time-dependent changes in dentin parameters associated with the mineral phase following NTAP treatment. The most pronounced temporal variations were observed in the organic spectral regions. However, these findings should be interpreted as temporal trends rather than statistically significant effects.

Conclusion: ATR-FTIR analysis demonstrated transient modifications predominantly affecting the organic phase of dentin, with the most marked decreases occurring at 1 h and a persistent effect observed after 24 h. These findings indicate a biologically relevant time-dependent response characterised by both transient modifications and partial regression of alterations.

Keywords: ATR; dentin; FTIR; non-thermal atmospheric plasma.

Análise laboratorial de dentina tratada por plasma não térmico atmosférico utilizando espectrocopia de infravermelho por transformada de Fourier. (ATR-FTIR)

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Análise laboratorial de dentina tratada por plasma não térmico atmosférico utilizando espectrocopia de infravermelho por transformada de Fourier. (ATR-FTIR)

List of abbreviations

DEJ – Dentino-enamel Junction

ASTM – The American Society for Testing and Materials

NTAP – Non-Thermal Atmospheric Plasma

RONS – Reactive Oxygen and Nitrogen Species

FTIR – Fourier Transform InfraRed

ATR – Attenuated Total Reflectance

CAP Jet – Cold Atmospheric Plasma Jet

Análise laboratorial de dentina tratada por plasma não térmico atmosférico utilizando espectrocopia de infravermelho por transformada de Fourier. (ATR-FTIR)

I. Introduction

Dental adhesive systems have significantly influenced modern restorative dentistry. *G. V. Black's* traditional "*extension for prevention*" principle in 1917, was gradually replaced by minimally invasive dentistry also aided by the advent of Buonocore's discovery of acid etching benefits in enamel, in 1955, enabling adhesive retention, and promoting conservative operative techniques over purely mechanical approaches (1,2).

Indeed modern dental restorations are highly aesthetic and conservative, but the long-term stability restorative interfaces remains a major clinical concern, especially in restorations involving dentin surfaces. Adhesive degradation often results in marginal leakage, which leads to restoration failures, contributing to secondary caries, and bacterial infiltrations (1,3).

These limitations are largely due to incomplete mineral replacement and bonding difficulties inherent to the biological and structural properties of dentin as a substrate. In this context, non-thermal atmospheric plasma has emerged as an alternative approach to prepare the surface of dentin, making it potentially more receptive for the restorative complex (4).

1.1. Dentin as an adhesive substrate: structure and clinical relevance

1.1.1. Dentin microscopic structure and biochemical composition

Dentin is a mineralised tissue that forms the bulk of the tooth (5). It provides mechanical support to the crown and serves as a substrate for the root cementum (6). Biochemically, dentin consists of a highly organised inorganic matrix phase and an organic counterpart. The inorganic component approximately accounts for 70% of the weight (7). It consists of 45% by volume of hydroxyapatite shaped as nanocrystals, smaller and less dense than those found in enamel, providing a balance between rigidity and elasticity (6,8).

The organic fraction represents about 20% of the weight and 30% by volume (7). It is composed of predominantly type I collagen in a three-dimensional fibrillar network with minor amounts of type III and V collagen (9,10). Non-collagenous proteins, such as phosphoproteins, proteoglycans and glycosaminoglycans, modulate the matrix mineralisation to maintain structural integrity (7,9). Water constitutes about 10% of the mass and 25% of the volume of dentin, increasing in concentration from the Dentino-Enamel junction (DEJ) to the pulp (7). Water facilitates ion transport, mechanical damping and transmission of stimuli (9).

1.1.2. Adhesion to dentin

The American Society for Testing and Materials (ASTM) as per specification D 907, provides the following definition for adhesion "t*he state in which two surfaces are held together by interfacial forces which may consist of valence forces, interlocking forces or both*" (11).

A key mechanism subjacent to the dentin adhesion is the formation of the hybrid layer, which constitutes the foundation of the resin dentin bond. The hybrid layer, first described by Nakabayashi in 1982, results from resin monomer infiltration into acid-etched dentin, creating a micromechanical resin-dentin interface (9,12). However, exposed collagen fibrils within this layer are susceptible to hydrolytic and enzymatic degradation by endogenous enzymes activated by acid etching. Moisture and mechanical stress lead to progressive bond strength loss, compromised marginal integrity and increased risk of interface failure over time (13,14).

Another challenge for dentin adhesion is the smear layer. The smear layer is a thin heterogeneous layer (0.5 to 5 μm) composed of organic and inorganic debris deposited on after instrumentation (15). It obstructs the dentinal tubules acting as a semi-permeable barrier (16). The smear layer thickness and density can significantly limit the effectiveness of bonding (16). Significant structural and compositional variations directly impacting bonding effectiveness and clinical outcomes (17–19). Its impact on adhesion depends on the technique used: *etch-and-rinse* adhesives remove it completely, while *self-etching* adhesives partially

incorporate it into the interface by means of a shallower acid demineralisation due to the incorporation of functional acidic monomers (20).

1.1.3. Adhesive systems to NTAP

Despite advances in adhesive systems described by Bart Van Meerbeek (*total-etch*, *self-etch* and *universal*), adhesion to dentin remains limited by sensitivity and the biological and physicochemical constraints regardless of the technique used (3,21).

Contemporary adhesive dentistry is undergoing a shift towards alternative surface pretreatment technologies, among which Non-Thermal Atmospheric Plasma (NTAP) is emerging as a promising approach (22). Some experimental studies reported bond strength values comparable to, or higher than, conventional adhesive protocols, suggesting that NTAP may have the potential to simplify certain bonding procedures. However, further studies are required before it can be considered a reliable substitute for the priming step. The results heavily depend on clinical parameters and on the protocol; authors highlight the lack of standardised protocols (23,24).

1.2. Non-Thermal Atmospheric Plasma in dentistry

1.2.1. Definition and physical principles

Non-thermal atmospheric pressure plasma (NTAP) is a plasma-based technology used in dentistry to alter the surface properties of dental tissues by generating highly reactive species (24,25). It is generated by applying electrical energy to gases at atmospheric pressure and is characterised by partial ionisation composed of electrons, ions and neutral particles of gases (4,26). Unlike thermal plasmas, NTAP is differentiated by elevated electron temperatures, whilst ions and neutral particles remain at lower temperatures, close to ambient conditions (26). Temperatures below 40 °C at the application site sugest that plasma is safe for dental tissues thereby avoiding protein denaturation, inflammatory responses and irreversible pulpal damages (27,28).

One of the most commonly used gases in the production of NTAP is argon (Ar). A colourless, odourless noble gas with a very low chemical reactivity. Its complete electronic configuration results in a high chemical stability, meaning that it almost never spontaneously combines with other elements (29).

Borges (2021) provides a narrative overview of NTAP in dentistry summarising existing experimental and clinical studies. Consequently, the biological effects observed are mainly attributed to the generation of reactive species within the plasma rather than to the specific carrier-gas itself. In this context argon is commonly used as a carrier-gas in cold atmospheric pressure plasma systems applied in dentistry (4,30).

1.2.2. NTAP Mechanisms and its effect on biological tissues in dentistry

The NTAP effects are principally based on the generation of Reactive Oxygen and Nitrogen Species (RONS) (e.g. hydrogen peroxide H_2O_2, ozone O_3, hydroxyl radicals •OH or singlet oxygens) (4,26). The generation of RONS by plasma interacts with organic tissues and microbial cells, resulting in oxidative modifications to cell membranes, DNA, and other molecular structures (28,31). RONS induce physical and chemical changes at a molecular scale, without significant thermal elevation, increase surface free energy and wettability while ensuring effective bacterial decontamination (25,32,33).

The introduction of RONS into type I collagen fibres, the principal component of the organic matrix, forms carbonyl groups (C=O) via surface interactions (10,34). At the molecular level, carbonyl groups act as hydrogen bond acceptors, promoting the formation of additional hydrogen bonds with hydrophilic monomers of adhesives or other collagen chains (4).

In an *in vitro* design study on human dentin, Ayres (2018) demonstrated, using atomic force microscopy and Raman confocal spectroscopy, that NTAP treatment applied for 10 s or 30 s does not provoke significant morphological changes on the surface of dentin compared to those observed after acid etching and did not differ significantly from that of untreated controls (35). Topography preservation and structural integrity of the substrate represent a fundamental

benefit, as they maintain tubular architecture for the resin tags conception, preserve the collagen network from collapses, ensure substrate cohesion and ultimately improve clinical longevity of the restoration (18,36,37).

1.2.3. Effects on wettability, contact angle and surface energy

Properties such as wettability, contact angle and the surface free energy directly influence the quality of the adhesive spreadability and consequent infiltration, and then control the formation of the hybrid layer (38). Wettability can be defined as the capacity of a liquid to spread across a solid surface and can be measured through deriving its contact angle θ (39). A high angle value suggests a hydrophobic surface, while a lower one reflects a hydrophilic surface (40).

Interestingly, treatment with NTAP has been shown to enhance substrate hydrophilicity by reducing the contact angle. In an *in vitro* study, Stasic (2019) compared NTAP effect over phosphoric acid etching. It demonstrated that under plasma jet for 30–60 s at different tip-to-surface distances (2, 4, and 8 mm) that NTAP significantly increases dentin wettability and surface free energy. These effects were dependent on the plasma application parameters, thereby promoting improved adhesive spreading, particularly under a *self-etch* application protocol (25). In fact, the reduction of the contact angle following NTAP treatment is particularly pronounced. The use of argon atmospheric pressure plasma have shown that the immediate bond strength significantly increased along with NTAP treatment time, correlating with enhanced wettability (41).

Recently, previous theses from Egas Moniz School of Health and Science corroborated these findings, that NTAP significantly does indeed reduce the contact angle of dentin surfaces, improving its wettability without altering its surface topography. These studies also demonstrated an increase in bond strength and the formation of a thicker and more intact adhesive interface, suggesting extra favourable adhesion conditions (36,37). It can be inferred that NTAP is a unique surface conditioning method. It can modulate surface energy and wettability without altering the properties of the material or creating excessive roughness.

1.3. FTIR assessment of chemical alterations in NTAP-treated dentin

1.3.1. FTIR spectroscopy principles and its relevance in dental materials analysis

In order to understand the mechanisms by which NTAP interacts with dentin, analytical techniques that can illustrate these chemical changes are required. Among all analytical methods, Fourier Transform Infrared (FTIR) spectroscopy is particularly relevant (42). FTIR spectroscopy provides molecular-level information on both the organic and inorganic dentin components, making it particularly well-suited to the analysis of NTAP-treated dentin (43). FTIR spectroscopy is a physicochemical analysis technique used to identify the chemical composition and molecular bonds (e.g. PO_4^{3-}, C–H, O–H, N–H, C=O), of a material (44). It is based on the absorption of an infrared radiation by a sample (44). Different chemical bonds absorb infrared radiations at specific frequencies, the resulting spectrum represents the molecular fingerprint of the material (42,45). When applied in Attenuated Total Reflectance mode (ATR), FTIR spectroscopy provides a non-destructive, rapid method for surface-sensitive chemical analysis with minimal sample preparation requirements, making it particularly suitable for examining dentin surface (46). In this mode, the infrared beam is directed into an ATR crystal (diamond, ZnSe or germanium), an optical material transparent to infrared light coupled with a high refractive index (**Figure 1**) (47).

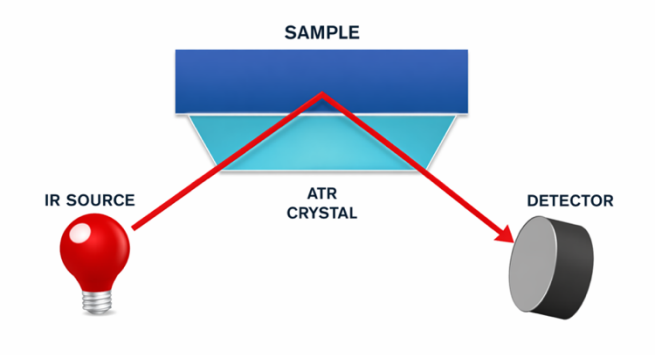

Figure 1. Simplified ATR-FTIR mechanism illustration.

Notably, the ATR-FTIR variant enables surface-specific investigations that are highly relevant for understanding interfacial phenomena in adhesive dentistry and surface treatment protocols (48). FTIR imaging capabilities permit spatially resolved chemical mapping, facilitating the examination of heterogeneous tissue zones and interface regions with high chemical specificity (44,49).

1.3.2. FTIR spectroscopy application on dentin and dentin–adhesive interfaces

Dentin exhibits distinctive FTIR spectral features which reflect its composite nature. Main spectral bands of the inorganic phase (70% primarily carbonated hydroxyapatite) are phosphate (v_3 and v_4) and carbonate bands defining the basic apatite structure (8). These spectral bands analysis provide information on the chemical composition, structure and biological origin of the material (43). The principal spectral bands characterising the organic phase of dentin are amide bands (43). Examination of these amide bands enables the assessment of collagen integrity, as the triple-helix structure (amide I), confirms proteins presence and evaluates the mineralisation degree (amide II) (48,50). Additionally, band expansion or shifts indicate conformational changes or maturation/degradation rate (amide III) (51).

Consequently, changes in their specific peak intensity ratios following various treatments offer quantitative evidence of chemical modifications occurring at the dentin surface (48,52).

1.3.3. FTIR evidence of NTAP-induced chemical modifications

The ATR-FTIR analytical method is relevant in the present study because it is a highly sensitive chemical surface analysis technique as the NTAP is mainly a superficial treatment (35,44). FTIR thus can enable the rapid analysis the effect of NTAP treatment on the substrate (24,35). While FTIR studies primarily highlight modifications of the organic matrix, the mineral phase can also be considered. Most studies report that clinically relevant NTAP treatments affect mainly the collagen matrix, leaving overall mineral content largely unaltered (35,53).

1.4. Limits in current understanding FTIR evidence of NTAP–dentin interactions

Current understanding of NTAP-dentin interactions remains limited by a lack of biphasic correlation between organic and inorganic spectroscopic data. To date, all FTIR studies on NTAP-dentin have relied on either purified collagen fibrils or completely demineralised dentin, both representing simplified models that do not reflect the complexity of clinical dentin(25,34,54). As a biphasic tissue composed of approximately 70% mineral and 30% organic material, clinical dentin requires a more integrative analytical approach (7). Yet, no studies have applied ATR-FTIR to simultaneously evaluate the effects of NTAP on both organic and mineral phases (43). This dual analysis is usually well established in dentistry, notably in research on caries, ageing and remineralisation, but it has not been extended to the field of NTAP (55).

Although this time-dependent nature raises questions about the NTAP stability and reversibility, its temporal dynamics. The NTAP effect over time remains unclear whether the modifications induced by plasma treatment are permanent, transient, or reversible, which has direct implications for the durability of the final bonding state. The contemporary literature primarily focuses on its immediate effects on dentin chemistry, wettability and bond strength, rely only on immediate time-point measurements or compare before and after plasma exposure (34,35). As a result, the literature clearly demonstrates that plasma effects are time-dependent (56). This phenomenon of plasma ageing is well documented on metals and polymers (57,58). However, there is still a lack of information with spectroscopic data regarding this time-points measurement variation/evolution on treated dentin without any further adhesive application.

Beyond the question of how plasma induced modifications over time, a further limitation concerns their long-term fate. It remains unknown whether the spectroscopic changes induced by NTAP are stable, progressive, or reversible over clinically relevant time periods. It is uncertain whether NTAP transiently activates the surface or induces stable molecular effects, because the dentin surface tends to return to its initial untreated state after NTAP application (23).

Most articles studying post-plasma stability/reversibility use functional measurements such as contact angle, surface energy, adhesion resistance (34,53,54,59,60). They all use ATR-FTIR spectroscopy but not longitudinally. Multi-stage ATR-FTIR analysis establish a causal framework linking plasma-induced functional group modifications to the long-term physicochemical behaviour of dentin.

Our study will help the further research to demonstrate the suitability of dentin treated with NTAP for clinical practice through several temporal ATR-FTIR evaluations. Consequently, this study will provide spectroscopic evidence going along with the previously reported results.

1.5. Sustainable Development Objectives (SDO)

The proposed study has a strong potential for knowledge transfer to society by improving the quality of oral healthcare through the optimisation of dentin adhesion using Non-Thermal Atmospheric Plasma (NTAP). This may reduce retreatment rates, patient discomfort and associated costs (SDO 3). The study also advances scientific innovation and supports the development of cutting-edge biomedical technologies (SDO 9). The findings will be disseminated through professional training, scientific publications, public engagement initiatives and university outreach activities. This will support the integration of novel technologies into everyday clinical practice (SDO 4).

Análise laboratorial de dentina tratada por plasma não térmico atmosférico utilizando espectrocopia de infravermelho por transformada de Fourier. (ATR-FTIR)

II. Objectives

GENERAL OBJECTIVE

The present study aims to evaluate, chemical modifications induced by NTAP treatment on the dentin surface using ATR-FTIR spectroscopy. Thus, multistage FTIR analytical design is used to assess the effects of NTAP on the dentin surface, immediately after treatment and over time.

SPECIFIC OBJECTIVES

- To characterise the ATR-FTIR spectral profile of untreated dentin and dentin treated with non-thermal atmospheric plasma at different post-treatment time points.

- To quantify time-dependent changes in predefined dentin spectral regions, including phosphate, carbonate, amide I, amide II, amide III, CH stretching, and OH/H_2O/NH-associated bands.

- To assess whether NTAP treatment altered derived spectral ratios related to dentin mineral and organic matrix composition, including phosphate/amide I, carbonate/phosphate, and amide I/amide III ratios.

2.1. Research hypothesis

Thus, for this study, the following null and alternative hypotheses were formulated:

- Null hypothesis $H0._1$: NTAP application has no significant effect on the organic and inorganic dentin chemical composition, or on the availability of functional groups, as measured by FTIR spectroscopy.

- Alternative hypothesis $H1_{.1}$: NTAP application modifies the dentin surface chemical composition and the availability of functional groups, as measured by FTIR spectroscopy.

- Null hypothesis $H0_{.2}$: NTAP application effect over time does not influence the organic and inorganic dentin chemical composition as measured by FTIR, or on the availability of functional groups.

- Alternative hypothesis $H1_{.2}$: The chemical modifications induced by NTAP application on dentin surface composition are transient, with a progressive return towards baseline values observed over time, as measured by FTIR spectroscopy.

III. Materials and methods

3.1. Study design: *in vitro* laboratorial study

This study is an *in vitro* laboratorial investigation, carried out in the Biomaterials laboratory at the Egas Moniz University Institute (IUEM). The authorisation for the use of human biological samples was approved, under the internal process n° 1710 by the Ethics Committee (CEEM) of the Instituto Universitario Egas Moniz (IUEM) (appendix).

3.2. Samples and storage

3.2.1. Tooth selection criteria

Seven extracted human posterior teeth were selected from the Egas Moniz University Clinic Tooth Bank for this study. Each specimen was evaluated before and after experimental treatment, thereby serving as its own control, with the tooth defined as the experimental unit (n=7). Inclusion was restricted to premolars and molars without carious lesions, restorations, cracks, or other visible structural defects. All teeth were extracted within six months before the start of the study.

3.2.2. Storage conditions

After extraction, these teeth were disinfected into a 1% chloramine T solution during one week, then transferred into distilled water and stored at 4 °C until specimen preparation.

3.2.3. Specimen preparation

A slice of medium-depth coronal dentin was obtained from each tooth using a hard tissue microtome (Accutom 50 Struers, Ballerup, Denmark) (**Figure 2**) under a speed of 3200 rpm and a feed of 0,500 mm/s (**Figure 3**).

Figure 2. Hard tissue microtome Accutom 50 Struers, Ballerup, Denmark.

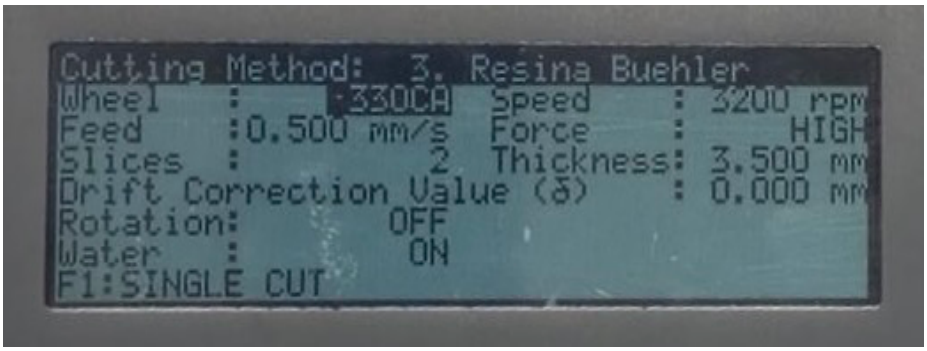

Figure 3. Hard tissue microtome parameters.

The tooth was positioned with sticky wax, occlusal side down along the long axis, on an acrylic base before being placed in the hard tissue microtome. The tooth was cut twice to obtain one slice that is 3.5 mm thick. This resulted in a total of seven dentin disc specimens. The thickness of the discs was not considered a critical parameter, since the studies were carried out exclusively on the dentin surface.

3.3. Experimental groups and NTAP device

3.3.1. Group allocation and randomisation

The specimens were then randomly allocated into two experimental groups (n=7 per group):

- Control group (without NTAP): No additional modification on the dentin surface before analysis.

- Plasma group: An argon NTAP application to the dentin surface using a pen in continuous mode, according to the parameters explained below.

3.3.2. NTAP device and application protocol

Although the general term NTAP is used in this manuscript, the device employed is, more precisely, an argon Cold Atmospheric Plasma Jet (CAP Jet). The argon CAP Jet is a sub-category of NTAP that produces a cold plasma jet focused directly on the substrate.

CAP Jet was applied using an argon (99% purity, Air Liquide®) plasma pen (kINPen® IND Neoplas GmbH) (**Figure 4**) operating in continuous mode 4-6 L/min. The distance between the device and the dentin surface was 2 mm, and the exposure duration was 10 s, both parameters standardised according to an exploratory study occurring in parallel. Plasma application was performed doing gentle sweeping movements to ensure a homogeneous coverage of the dentin surface.

Figure 4. kINPen® Neoplas IND, argon plasma pen devices.

To certify that the NTAP flame was at the correct distance (2 mm) from the dentin disc, a ruler and a plastic stand with sticky wax on it were used for each application (**Figure 5**).

Figure 5. Measurement of the distance between the NTAP flame and the dentin slice.

3.4. ATR-FTIR analysis

3.4.1. Instrumentation and acquisition parameters

ATR-FTIR analyses were performed using a Shimadzu® IRSpirit-X FTIR QATR-S single reflection ATR spectrometer, (**Figure 6**) fitted with a single reflection diamond ATR crystal accessory and the “Spectrum mode” from Shimadzu® LabSolutions IR software. Spectra were acquired over the 4000–400 cm^{-1} range, with a spectral resolution of 2 cm^{-1} and 32 scans per measurement, in absorbance measurement mode and apodization Happ-Genzel.

Figure 6. Shinadzu® IRSpirit-X FTIR QATR-S single reflection ATR

3.4.2. Spectral processing and evaluation

All spectra were baseline corrected and normalised prior to their analysis. A clean, stable background was mandatory. It is used to correct for atmospheric absorption (ex: CO_2, H_2O), to measure the signal intensity and provide a basis for calculating the final absorbance spectrum (42). As well, the ambient conditions such as temperature and a 50% moisture level were monitored all along experiences duration. This background measurement was collected before every sample analysis, to avoid crystal contamination (**Figure 7).**

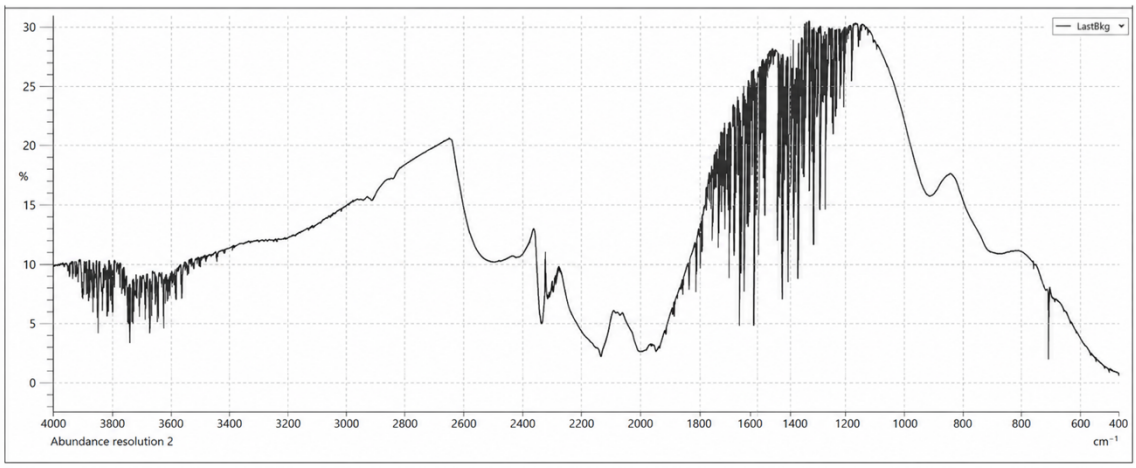

Figure 7. Example of a Background scan (BGK).

The dentin disc is placed on the ATR crystal with the side to be analysed facing downwards, and secured from above (**Figure 8**).

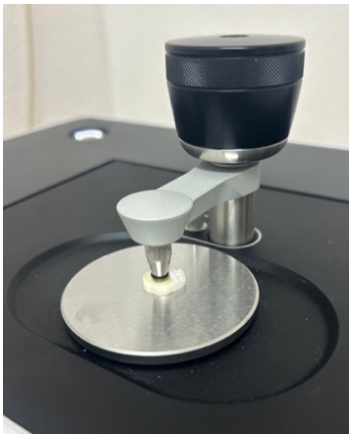

Figure 8. Analysis of a sample.

The characteristic bands of the dentin mineral phase and those of the organic matrix were evaluated. Intensity ratios such as phosphate/amide I and potential band shifts were then identified to assess the chemical changes induced by plasma treatment.

3.5. Time-dependent analysis

3.5.1. Measurement time points

ATR-FTIR spectra were acquired before NTAP application and at predefined post-treatment intervals. The untreated dentin spectrum was used as the baseline control for each specimen. After NTAP application, spectra were collected immediately after treatment, at 10 min, 1 h, and 24 h.

These time points were selected to characterise the short-term temporal evolution of dentin spectral changes after plasma exposure. The immediate measurement represented the first post-treatment condition, while the 10 min and 1 h intervals allowed assessment of early time-dependent changes. The 24 h measurement was used to evaluate whether spectral changes persisted after short-term storage.

When available, an additional 2-week measurement was also performed. Because this time point was not available for all specimens, it was considered exploratory and was not included in the main repeated-measures statistical analysis.

3.6. Statistical Analysis

Statistical analysis was performed using Python 3.11 with NumPy, pandas, SciPy, and Matplotlib. The tooth was considered the experimental unit. When multiple scans were available for the same tooth and time point, values were averaged to obtain one tooth-level value per condition, avoiding pseudo-replication.

Data were summarised as median and interquartile range [IQR]. The complete repeated-measures dataset included Control, Immediate, 10 min, 1 h, and 24 h time points for seven teeth. The 2-week time point included only three teeth and was therefore excluded from inferential repeated-measures testing and analysed descriptively. Predefined ATR-FTIR outcomes and derived ratios were compared across the complete time course using the Friedman test. Kendall's W was calculated as the effect-size estimate. To account for multiple comparisons, Benjamini–Hochberg false discovery rate correction was applied, and both unadjusted p-values and adjusted q-values were reported. The significance level was set at $\alpha = 0.05$.

For the untargeted spectral analysis, paired pointwise comparisons against the control condition were performed across the ATR-FTIR fingerprint region (800–1800 cm^{-1}). The resulting p-values were corrected using the Benjamini–Hochberg procedure. Significant adjacent wavenumbers were summarised as continuous spectral intervals rather than interpreted as isolated points.

IV. Results

4.1. Dataset structure

The primary repeated-measures analysis included the Control, Immediate, 10 min, 1 h, and 24 h time points, each represented by seven teeth. The 2-week time point was available only for teeth 1-3 and was therefore treated as exploratory. For completeness, we analysed them, but they will not be included in the subsequent quantitative analyses.

4.2. Spectral overview

Mean ATR-FTIR spanned the dentin fingerprint profile from 800 to 1800 cm^{-1}. The spectra were broadly similar across groups, indicating that NTAP did not produce a large global distortion of the dentin spectrum. However, time-dependent differences were visible, particularly in the fingerprint region (as seen in **Figure 9.**

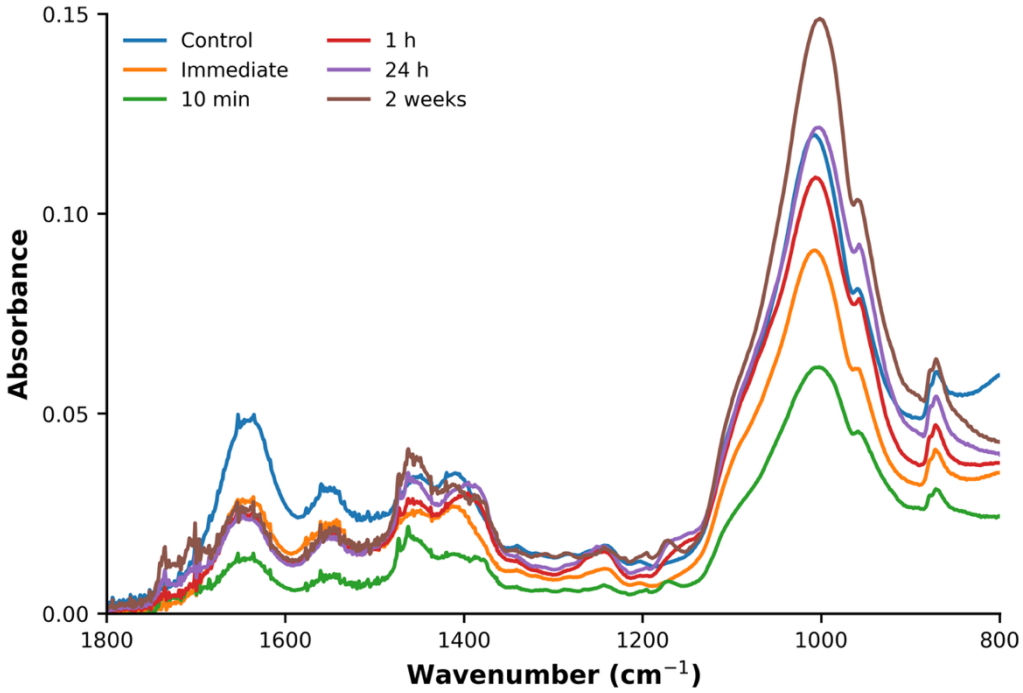

Figure 9. Mean ATR-FTIR spectra of dentin (800–1800 cm^{-1}) for the untreated control group and for dentin after NTAP treatment at the evaluated post-treatment time points.Spectra represent tooth-level mean values. For the repeated-measures time course from control samples to 24 h, n=7 was used (the 2-week time point was exploratory; n=3). Overall, the spectral profiles remained similar, indicating preservation of features. However, increase and decrease of absorbance, particularly in regions associated with phosphate- and amide-related bands were noted.

The difference spectra showed that the NTAP-related response was not uniform across the fingerprint region (**Figure 10**). Immediately after plasma treatment and at 10 min, the difference spectra were predominantly negative, suggesting an overall reduction in absorbance relative to untreated dentin. The most evident negative deviations at these early time points occurred around the phosphate-dominated region near 1009–1010 cm^{-1} and around the amide I region (1639 cm^{-1}).

The 10 min spectrum showed the strongest negative deviation in the phosphate/fingerprint region, with the main trough centred around approximately 1010 cm^{-1}, while a second negative deviation was observed in the amide I region. At 1 h, the spectral pattern changed more clearly. The difference spectrum showed discrete positive deviations in the lower fingerprint region and a broad negative deviation in the higher fingerprint region. Positive changes were observed around approximately 930 cm^{-1}, 982 cm^{-1}, and 1182–1199 cm^{-1}. These regions are located within or close to phosphate- and mineral-associated fingerprint bands. In contrast, the strongest negative deviation at 1 h was observed across the broad 1478–1675 cm^{-1} range, with the largest decrease centred around 1635 cm^{-1}. This region encompasses CH deformation, amide II, and amide I contributions. At 24 h, the difference spectrum suggested partial persistence of the 1 h pattern, although with lower overall magnitude. Positive deviations remained visible particularly around approximately 953 cm^{-1} and 1155 cm^{-1}, while a negative deviation persisted around the amide I region near 1635 cm^{-1}. The 2-week spectrum showed a different profile, with a marked positive deviation around approximately 989 cm^{-1} and a remaining negative deviation near 1636 cm^{-1}.

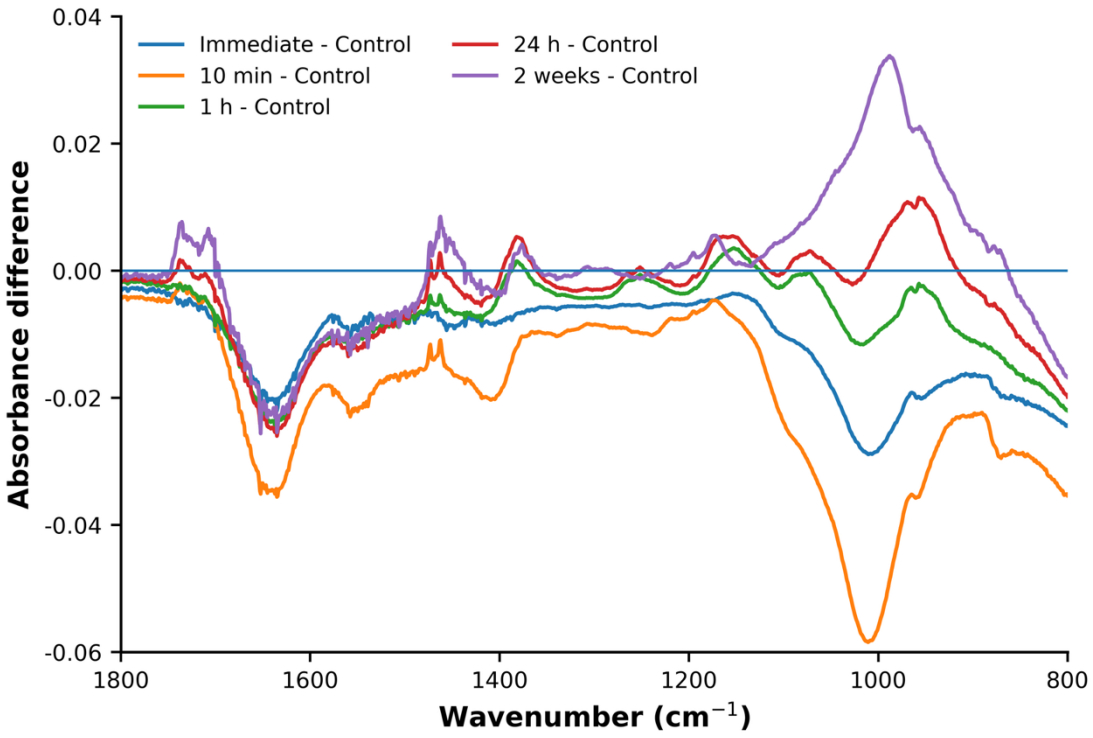

Figure 10. *Difference spectra of NTAP-treated dentin relative to untreated control in the fingerprint region (800–1800 cm⁻¹).* Difference spectra were calculated as treated minus control, such that positive values indicate relatively higher absorbance after NTAP treatment while negative values indicate relatively lower absorbance. Immediate and 10 min time points showed modest spectral deviations from control, whereas the 1 h time point exhibited the most pronounced spectral divergence. The main changes consisted of relative increases in lower-wavenumber fingerprint regions and relative decreases in the higher fingerprint range (within the organic/collagen-associated region). The 24 h spectrum suggested partial persistence of these changes.

The OH/H_2O/NH region was further inspected to visualize the spectral behaviour underlying the predefined 3200–3600 cm^{-1} endpoint. Mean spectra showed lower absorbance in the NTAP-treated groups compared with untreated control dentin, particularly from 10 min onward (**Figure 11**). Difference spectra confirmed a predominantly negative shift across the 3200–3600 cm^{-1} interval, with the largest reductions observed at 10 min, 1 h, and 24 h. The 2-week profile was interpreted descriptively because only three teeth were available at this time point.

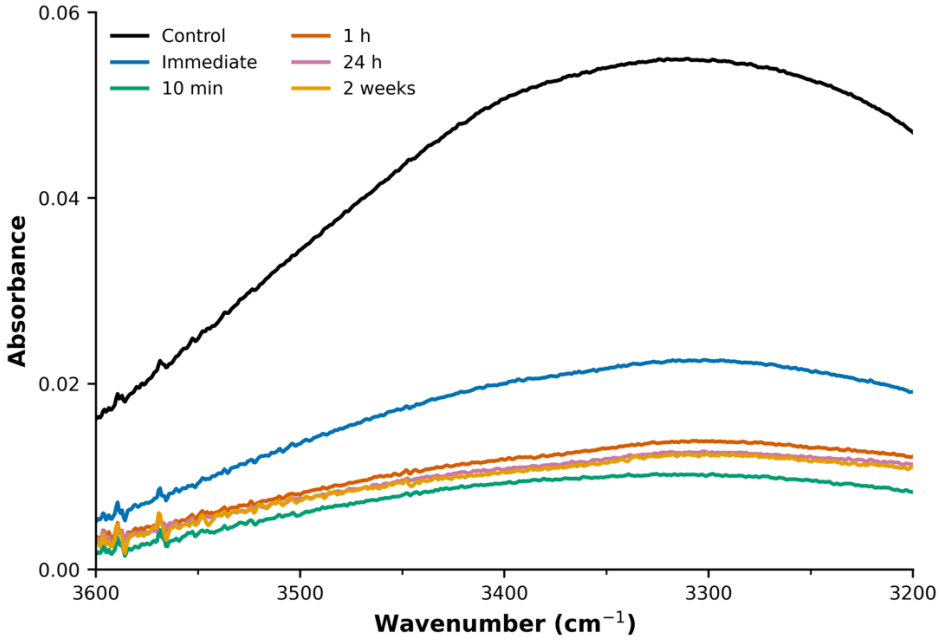

Figure 11. ATR-FTIR spectral changes in the OH/H_2O/NH region after NTAP treatment. Mean spectra and difference spectra of dentin in the 3200–3600 cm^{-1} region. Mean spectra represent tooth-level mean absorbance values for untreated control dentin and NTAP-treated dentin at each evaluated time point. Difference spectra were calculated as NTAP-treated dentin minus untreated control dentin; negative values indicate lower absorbance relative to control. The 2-week time point is shown descriptively because only three teeth were available.

4.3. Time-dependent changes in predefined ATR-FTIR outcomes

Most mineral-related parameters did not show evidence of a consistent time-dependent change after NTAP treatment (see **Table 1**, with Friedman analyses results). Phosphate area in the 900–1200 cm^{-1} region remained broadly comparable across time points, with no significant Friedman effect ($\chi^2(4)=2.51$, $p=0.642$, q=0.785, Kendall's W=0.09). Similarly, carbonate v_2 area (850–890 cm^{-1}) and the carbonate/phosphate ratio showed no relevant time-dependent effect ($p=0.869$ and $p=0.938$, respectively). These results indicate that NTAP did not produce a clear alteration in conventional mineral-associated FTIR markers under the tested conditions.

In contrast, the strongest time-dependent responses were observed in spectral regions associated with organic components, hydration, and collagen-related features. The CH stretching region (2800–3000 cm^{-1}) increased progressively after NTAP, from a median of 0.120 [0.259] in the control group to

1.11 [0.525] at 1 h and 1.23 [2.38] at 24 h. This endpoint showed the largest Friedman effect among the predefined outcomes ($\chi^2(4) = 12.34$, $p=0.015$, q=0.125, Kendall's W = 0.44). The broad OH/H_2O/NH region (3200–3600 cm^{-1}) showed an opposite trend, decreasing from 3.67 [7.07] in the control group to 0.501 [1.33] at 1 h and 0.323 [0.968] at 24 h ($\chi^2(4) = 10.40$, p=0.034, q=0.125, Kendall's W=0.37). Amide I peak position also showed a time-dependent effect before multiplicity correction ($\chi^2(4)=10.68$, $p=0.030$, q=0.125, Kendall's W=0.38), with the most evident shift observed at 10 min.

Although CH stretching, OH/H_2O/NH area, and Amide I peak position showed unadjusted p-values below 0.05, none of the predefined endpoints remained statistically significant after Benjamini–Hochberg correction. Therefore, these findings should be interpreted as time-dependent spectral trends rather than definitive confirmatory effects. Taken together, the predefined endpoint analysis suggests that NTAP did not substantially modify bulk mineral-related dentin markers but may have induced subtle changes in the organic/hydration-related spectral environment of the dentin surface.

Table 1. *Time-dependent changes in predefined ATR-FTIR outcomes after NTAP treatment.* Values are presented as median [interquartile range] from tooth-level mean spectra. Friedman test was performed across the complete repeated-measures time course from control up to 24 h. Significance values are unadjusted Friedman p-values. Q-values correspond to Benjamini–Hochberg correction across the predefined endpoints. Kendall's W is reported as the effect-size estimate for the Friedman test, with larger values indicating stronger time-dependent effects.

Endpoint	Control median [IQR]	Immediate median [IQR]	10 min median [IQR]	1 h median [IQR]	24 h median [IQR]	Friedman $\chi^2(4)$	*p*	BH q-value	Kendall's W
Phosphate area 900-1200 cm^{-1}	6.61 [7.75]	4.26 [7.26]	4.50 [5.63]	6.54 [11.86]	3.97 [14.84]	2.51	0.642	0.785	0.09
Carbonate v_2 area 850-890 cm^{-1}	0.110 [0.174]	0.104 [0.119]	0.095 [0.131]	0.124 [0.184]	0.072 [0.267]	1.26	0.869	0.938	0.04
Amide I area 1600-1700 cm^{-1}	1.25 [1.62]	0.701 [0.964]	0.339 [0.537]	0.604 [0.835]	0.432 [0.954]	6.97	0.137	0.252	0.25
Amide II area 1500-1580 cm^{-1}	0.272 [0.160]	0.137 [0.132]	0.089 [0.126]	0.136 [0.084]	0.064 [0.226]	3.77	0.438	0.688	0.13
Amide III area 1200-1300 cm^{-1}	0.099 [0.058]	0.122 [0.125]	0.046 [0.120]	0.037 [0.309]	0.040 [0.319]	2.74	0.602	0.785	0.10
CH stretching area 2800-3000 cm^{-1}	0.120 [0.259]	0.269 [0.622]	0.687 [2.12]	1.11 [0.525]	1.23 [2.38]	12.34	**0.015**	0.125	0.44
OH/H_2O/NH area 3200-3600 cm^{-1}	3.67 [7.07]	1.13 [3.04]	0.446 [0.657]	0.501 [1.33]	0.323 [0.968]	10.40	**0.034**	0.125	0.37
Amide I peak position 1630-1670 cm^{-1}	1653.1 [1.08]	1653.1 [1.43]	1635.9 [16.49]	1653.1 [3.94]	1653.1 [1.43]	10.68	**0.030**	0.125	0.38

The statistically supported spectral intervals observed at 1 h are shown in **Figure 12.** In the preprocessed 1 h minus Control difference spectrum, three positive regions were identified in the lower fingerprint range: 896.1–934.1 cm^{-1}, 971.3–992.9 cm^{-1}, and 1182.1–1199.3 cm^{-1}. The highest positive difference occurred within the 971.3–992.9 cm^{-1} interval, with the maximum difference centred at approximately 982.1 cm^{-1}. A broader negative region was observed between 1478.2 and 1674.6 cm^{-1}, with the largest negative difference centred around 1637.3 cm^{-1}. This negative interval extended across the CH deformation, amide II, and amide I region.

Compared with the descriptive difference spectra, the corrected analysis confirmed that the most consistent NTAP-associated spectral response occurred at 1 h. The positive intervals were restricted to selected lower-wavenumber

fingerprint regions, whereas the negative response was broader and concentrated in the organic/collagen-associated range. No corrected spectral intervals were identified at the Immediate, 10 min, or 24 h time points.

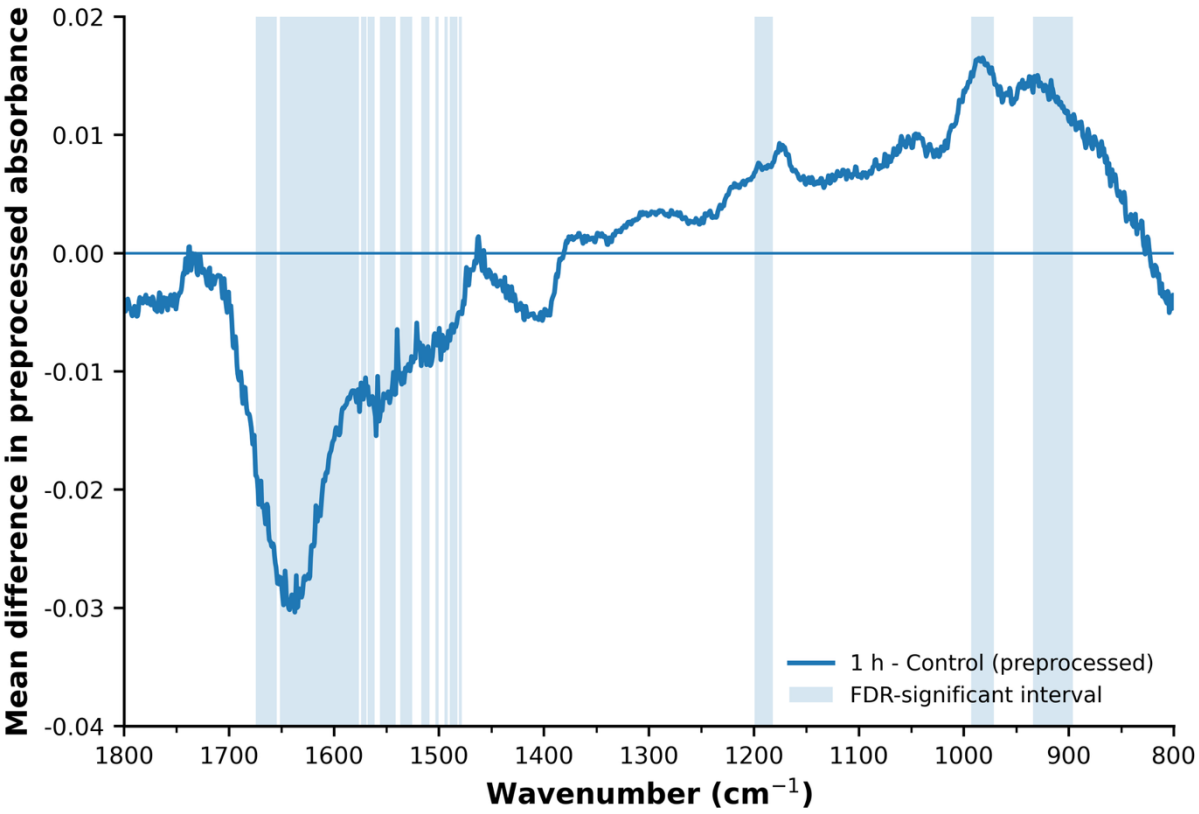

Figure 12. *Corrected spectral intervals at 1 h after NTAP treatment.* Preprocessed mean difference spectrum for 1 h NTAP-treated dentin relative to untreated control in the ATR-FTIR fingerprint region. Positive values indicate higher preprocessed absorbance at 1 h, whereas negative values indicate lower preprocessed absorbance. Shaded areas indicate corrected significant intervals. Positive intervals were detected at 896.1–934.1 cm^{-1}, 971.3–992.9 cm^{-1}, and 1182.1–1199.3 cm^{-1}. A broad negative interval was detected at 1478.2–1674.6 cm^{-1}.

In addition to direct spectral-region measurements, selected ATR-FTIR ratios were calculated to provide normalized indicators of dentin mineral and organic-matrix composition. Descriptive and inferential ratio values are presented in **Table 2**. The phosphate/amide I ratio showed the largest time-dependent variation among the ratio outcomes, but the Friedman test did not reach statistical significance after correction ($\chi^2(4)= 8.69$, $p=0.069$, q=0.191, Kendall's W=0.31).

The carbonate/phosphate ratio showed no evidence of a time-dependent change across the evaluated time points ($\chi^2(4)=0.80$, $p=0.938$, q=0.938,

Kendall's W=0.03). The amide I/amide III ratio also did not show a statistically significant time-dependent effect ($\chi^2(4)=7.77$, $p=0.100$, q=0.221, Kendall's W=0.28). No derived ratio remained statistically significant after Benjamini–Hochberg correction. The 2-week time point was excluded from the Friedman analysis because only teeth 1–3 were available and was therefore treated descriptively.

Table 2. Derived ATR-FTIR ratios calculated from tooth-level mean spectra. Values are presented as median [interquartile range]. The Friedman test was performed across control, Immediate, 10 min, 1 h, and 24 h groups (n=7 teeth). The 2-week time point was excluded from the Friedman analysis because only teeth 1–3 were available and was interpreted descriptively. χ^2 indicates the Friedman test statistic; df=4 for all comparisons. p-values are unadjusted Friedman p-values and q-values correspond to Benjamini–Hochberg correction across the derived ratios.

Endpoint	Control median [IQR]	Immediate [IQR]	10 min [IQR]	1 h [IQR]	24 h [IQR]	Friedman χ^2	*p*	BH q-value	Kendall's W
Mineral-to-matrix ratio phosphate/Amide I	4.91 [2.46]	6.49 [1.75]	7.87 [8.56]	10.83 [3.77]	10.17 [4.87]	8.69	0.07	0.191	0.31
Carbonate/phosphate ratio 850-890/900-1200	0.017 [0.006]	0.017 [0.005]	0.020 [0.02]	0.021 [0.006]	0.018 [0.01]	0.8	0.94	0.94	0.03
Amide I/Amide III ratio	13.73 [7.95]	11.82 [2.92]	4.42 [8.41]	8.67 [8.42]	3.23 [7.36]	7.77	0.1	0.22	0.28

V. Discussion

5.1. Significance of the study and justification

The present study aims to assess chemical modifications induced by NTAP on intact dentin surfaces without any prior adhesive preparations using ATR-FTIR spectroscopy. Transformations were monitored through a multi-stage analytical design at four predefined time points. In order to characterise the temporal evolution, stability, and biphasic nature of NTAP-induced spectroscopic changes on dentin.

Despite the growing interest in NTAP as a surface conditioning tool in dentistry, the current level of understanding of its actual effects, immediately and over time, on the surface of clinically-prepared dentin, remains incomplete. Existing FTIR-based investigations have predominantly relied on simplified tissue models, which did not reflect the comprehensive compositional and structural complexity of intact dentin (53). Furthermore, the contemporary literature is largely restricted to immediate post-treatment measurements or simple before-and-after comparisons, leaving unresolved whether plasma-induced chemical changes are transient, progressive, or stable over time (30). Accordingly, the experimental time points were selected to capture spectroscopic responses following NTAP application within a mechanistic framework, rather than representing strictly clinical time intervals.

In this study, ATR-FTIR spectroscopy was chosen as the primary characterisation technique due to its well-established, non-invasive, simultaneous examination of both the inorganic and the organic phases of intact dentin. The technique provides surface-sensitive measurements within an approximate penetration depth of 0.5–2 μm, which is directly relevant to the surface-localised nature of NTAP-induced modifications (61).

5.2. Control group

5.2.1. Baseline and spectral characteristics

The band assignments and relative intensities observed across the seven control specimens were consistent with those reported in the references to sound dentin in the scientific literature; confirming the presence of the characteristic organic (Amide I, II, III; C–H) and inorganic (v_3 PO_4^{3-}, v_1 PO_4^{3-}, v_4 PO_4^{3-}, CO_3^{2-}) spectral signatures (51,52,62,63).

The control group revealed stable spectral profiles across the observation period, establishing reliable baseline values against which treatment-induced modifications could be compared. Phosphate area (900–1200 cm^{-1}) gave a median of 6.61 [7.75], carbonate v_2 area (850–890 cm^{-1}) measured 0.110 [0.174] and the OH/H_2O/NH region (3200–3600 cm^{-1}) showed elevated absorbance with 3.67 [7.07]. But the interquartile ranges observed in control measurements particularly for phosphate and OH/H_2O/NH area, reflected expected biological variability inherent to dentin samples. This heterogeneity, whilst often complicating statistical power, validated the comparison approach where each tooth served as its own control, well neutralising inter-specimen differences.

Therefore the control group baseline established that untreated mineralised dentin exhibited stable mineral composition with pronounced hydration-related absorbance (52). These consistent baseline values provided a decent reference frame to assess if spectral shifts in the experimental group represented true treatment effects or spontaneous variations.

5.3. Experimental group

Regarding treatment with NTAP, results of the present study demonstrated that applying it for 10 s induced a dynamic and superficial physicochemical modification in dentin. Due to the short time exposure, the thermal potential of the plasma is expected to be practically negligible, eliminating

any micro-heating effect and ensuring that the observed alterations result exclusively from exposure to RONS.

5.3.1. Effects of NTAP on dentin chemical composition

Based on the spectroscopic findings, the first null hypothesis was partially rejected since NTAP application had significant effect on the organic chemical composition, although less pronounced in the mineral phase. Notably, changes in conventional mineral-related markers (phosphate and carbonate areas) were not statistically significant, but substantial modifications in organic and hydration-related spectral regions were observed. Specifically, CH stretching (2800–3000 cm^{-1}) increased progressively from of 0.120 in the control group to 1.11 at 1 hour and 1.23 at 24 hours and the OH/H_2O/NH region (3200–3600 cm^{-1}) decreased significantly from 3.67 in the control group to 0.323 at 24 h, demonstrating that NTAP did effectively alter the chemical environment at the dentin surface.

The absence of significant changes in phosphate and carbonate areas aligns with studies indicating that the primary mechanism of NTAP, generated RONS, on dental tissues may be through superficial molecular conformational alteration of surface hydration, collagen structure, and hydrogen-bonding networks rather than actual mineral modification (30,35,54). The selective alteration of the organic phase rather than the mineral phase reflects the high reactivity of RONS (e.g. Hydroxyl radical (OH^-), delta oxygen singlet ($^1O_2$) and superoxide anion (O_2^-)) with surface-bound water and polar functional groups combined with their limited penetration depth (64). This may be also why the carbonate v_2 area and the carbonate/phosphate ratio presented no relevant changes. The stability of mineral-related parameters, under these study conditions, suggests that NTAP treatment does not induce demineralisation/remineralisation phenomena detectable by FTIR, which seems to be a clinically favourable result in the preservation of dentin integrity.

The data demonstrated a progressive increase in CH stretching following NTAP treatment. This linear rise represented the spectral alteration with the largest effect size among the predefined parameters. This increase can be interpreted as indicative of the organic phase surface modification, potentially

involving structural reorganistation of collagen (63). That may also be due to the removal of water within the matrix and altered accessibility of functional groups on the dentin surface (30,31).

Although the Amide area parameters showed no significant alterations, the peak position exhibits a remarkable displacement of ~17 cm^{-1} at 10 minutes (from 1653.1 to 1635.9 cm^{-1}), partially reversing at later timeframe. This transitory pattern suggests a dynamic and time-dependent response surpassing simple denaturation. Then, the transitory Amide I shift was consistent with a model of reversible protein denaturation and conformational rearrangement (62). This supports the interpretation that NTAP induced possible substantial conformational stress in the collagenous matrix, and then a partial structural relaxation. Also, the stability of the Amide I/Amide III ratio confirms that this phenomenon is purely conformational, with no chemical cleavage of the protein chains occurring. For clinical applications, it implied that the optimal timing between NTAP application and adhesive procedures might be a potential window between 10 minutes to 1 hour, the maximum conformational changes.

5.3.2. Hydration-related changes

The impact of the gas flow and the reactive species removed residual structural moisture strongly bound to the tissue, causing the collapse of the OH/H_2O/NH band (3200–3600 cm^{-1}). The most pronounced decrease occurred at 10 minutes post-treatment, suggesting a transient effect of NTAP exposure. The absorbance dropped to approximately 0.020, compared to 0.050 in the control group. This represents a circa 60% reduction and most likely reflected the peak impact of reactive species removal of bound water and alterations in hydrogen-bonding networks rather than a true decrease in surface hydrophilicity.

The NTAP generated RONS exposure effect increases in the OH/H_2O/NH region for up to 10 minutes after treatment increases, due to their lifetime, reactivity, and selectivity; thereafter, a gradual recovery is observed, with the return of hydration. It indicated that NTAP-induced dehydration was reversible, reflecting a temporary perturbation of surface hydration rather than irreversible tissue degradation.

This finding may be associated with the known capacity of NTAP to interact with water molecules and water-derived reactive species and potentially alter hydrogen bonding networks in biological tissues (31). In agreement with previous studies reporting increased dentin wettability after NTAP treatment, these findings suggested that plasma exposure promoted the formation or exposure of reactive species, thereby increasing surface free energy (36,37).

5.3.3. Temporal evolution

The temporal profile observed after NTAP treatment was not characterized by a simple monotonic recovery towards baseline. This study shows consistent time-dependent patterns, with the most pronounced spectral divergence at 1 hour post-treatment. Nevertheless, previous measurements suggested the emergence of a distinct spectral profile rather than a complete return to baseline, indicating a more complex temporal dynamic than simple transient recovery. These findings support a dynamic and time-dependent response of dentin to NTAP treatment with a clear peak effect around 1 hour post-exposure and evidence of partial persistence thereafter.

Immediate post-treatment measurements revealed only limited spectral deviations from the control group. At 10 minutes, the most pronounced shift in amide I peak position was observed, suggesting an early response of the organic matrix. The greatest overall spectral divergence occurred at 1 hour post-treatment, representing the peak response identified in the present study. Although several spectral features showed partial recovery by 24 hours, deviations from baseline remained detectable. As the samples were maintained strictly in a non-humid environment, this restoration of the spectral profile reflects the spontaneous dissipation of accumulated surface electrostatic stress and the intrinsic elastic relaxation of collagen. Free from the plasma's energy stimulus, the dry organic matrix dissipates surface charges and expands back to its original equilibrium three-dimensional conformation over 24 hours. However, the 2-week measurements, given the limited sample size (n=3), should be considered only as exploratory.

5.4. Limitations

Some limitations of the present study must be acknowledged, which may have affected the scope and generalisability of the findings.

Firstly, the potential variability associated with the dentin substrate itself presents a challenge. High inter-variability affected by the degree of dentin mineralisation, tooth age may all influence the physicochemical properties of dentin and its response to plasma treatment. Although efforts were made to standardise specimen preparation, these biological variations could have affected the observed outcomes.

Secondly, it is important to highlight that the experimental parameters of the NTAP application such as the argon flow rate, applied voltage, exposure time as well as the distance of the plasma jet from the dentin surface can significantly affect the outcomes and are, therefore, essential for reproducibility. These parameters critically determine the nature and density of reactive species generated and, consequently, the extent of the chemical modifications induced on the dentin surface (34). Since NTAP studies are still incipient, these parameters are subject to further optimisations and are not considered final yet.

Finally, this study was a laboratory study using ex vivo tooth models that were stored under controlled conditions. Logically, the oral environment introduces additional biological variables, such as the presence of saliva, biofilm proteins and/or enzymatic activity that can significantly affect the stability of NTAP-induced chemical changes. Consequently, caution is mandatory when extrapolating the observed controlled temporal dynamics to in vivo clinical context.

5.5. Future perspectives

The present findings support the need for future studies that move beyond isolated spectroscopic characterisation and that can clearly establish a causal effect between NTAP-induced dentin surface changes and long-term adhesive performance. Although NTAP appears to induce time-dependent modifications in

the dentin spectral profile, particularly in organic- and hydration-associated regions, it remains unclear whether these changes translate into clinically relevant improvements in resin–dentin bonding. Future investigations should therefore combine surface chemistry, wettability, adhesive infiltration, and bond longevity within the same experimental design.

A first priority, as mentioned above, should be the standardisation of NTAP application parameters. The current literature remains heterogeneous regarding plasma source, gas composition, gas flow, exposure time, distance from the substrate, dentin hydration state, and timing between plasma treatment and adhesive application (23,25,30). These variables are paramount, as they may determine what may be the dominant effect. Factors such as surface activation, dehydration, oxidation, potential cleaning of the smear layer, or collagen modification are yet to be confirmed.

The temporal stability of NTAP-induced dentin changes also requires further investigation. The present results suggest that plasma-related surface changes may evolve after treatment rather than being limited to the immediate post-exposure period. Future studies should therefore include time-resolved evaluations at clinically relevant intervals, even after producing hybrid layers, such as immediate bonding, 10-30 min, 1 h, 24 h, 48–72 h. This would help determine whether NTAP produces a transient activation window, a delayed surface response, or a reversible recovery of the dentin substrate. This information is essential to define the ideal timing for adhesive placement after plasma treatment.

Future work should also combine ATR-FTIR with more surface-sensitive and spatially resolved techniques. ATR-FTIR provides useful information on dentin spectral changes but has limited depth specificity and cannot fully identify the outermost chemical changes induced by plasma. Other analytical techniques should therefore be used to quantify changes in surface elemental composition and oxygenated carbon species, such as Raman mapping, SEM, AFM, contact-angle analysis, and surface free-energy measurements may help relate chemical modification to morphology, wetting behaviour, and substrate accessibility (35,36,54). From an adhesive perspective, future studies should evaluate whether NTAP effects depend on the bonding strategy. Different categories of

adhesives may respond as differently to plasma-conditioned. Studies should therefore compare adhesive classes and clinically relevant protocols, including NTAP before adhesive application, NTAP after acid-etching, and NTAP combined with adhesive systems containing functional monomers.

Finally, the translational relevance of NTAP should be tested under more clinically representative conditions. Ex vivo models incorporating pulpal pressure, controlled dentin moisture, salivary contamination or acquired pellicle, thermocycling, mechanical loading, and long-term water storage would provide a more realistic assessment of whether NTAP-induced surface activation remains beneficial over time. Only after these parameters are clarified should in vivo studies be considered.

VI. Conclusion

This study evaluated the chemical modifications induced by argon NTAP on intact, mineralised, dentin surfaces using ATR-FTIR spectroscopy, through a multi-stage longitudinal design encompassing four time stages (T0, T10min, T1h, T24h) and simultaneously monitoring both the organic and inorganic phases in a control and then plasma-treated group. ATR-FTIR analysis demonstrated transient modifications predominantly affecting organic spectral features, with the most distinct decreases occurring at 1 hour and a persistent divergence at 24 hours. It demonstrates a relevant time-dependent response with transient modifications and sustained alterations. Notably, mineral-related parameters remained unchanged, indicating that NTAP operates through selective remodelling of the organic matrix rather than global demineralisation. These results suggest that a defined post-treatment window exists, which has direct implications for the clinical integration of NTAP as a dentin conditioning strategy.

VII. References

1. Cardoso M, de Almeida Neves A, Mine A, Coutinho E, Van Landuyt K, De Munck J, et al. Current aspects on bonding effectiveness and stability in adhesive dentistry. Aust Dent J. 2011 Jun 13;56(s1):31–44. doi:10.1111/j.1834-7819.2011.01294.x
2. Buonocore MG. A Simple Method of Increasing the Adhesion of Acrylic Filling Materials to Enamel Surfaces. J Dent Res. 1955 Dec 1;34(6):849–53. doi:10.1177/00220345550340060801
3. Van Meerbeek B, De Munck J, Yoshida Y, Inoue S, Vargas M, Vijay P, et al. Buonocore memorial lecture. Adhesion to enamel and dentin: current status and future challenges. Oper Dent. 2003;28(3):215–35. PubMed PMID: 12760693.
4. Borges AC, Kostov KG, Pessoa RS, de Abreu GMA, Lima G de MG, Figueira LW, et al. Applications of Cold Atmospheric Pressure Plasma in Dentistry. Applied Sciences. 2021 Feb 24;11(5):1975. doi:10.3390/app11051975
5. Shahmoradi M, Bertassoni LE, Elfallah HM, Swain M. Fundamental Structure and Properties of Enamel, Dentin and Cementum. In. 2014. p. 511–47. doi:10.1007/978-3-642-53980-0_17
6. Arola DD, Gao S, Zhang H, Masri R. The Tooth: Its Structure and Properties. Dent Clin North Am. 2017 Oct 1;61(4):651–68. doi:10.1016/J.CDEN.2017.05.001 PubMed PMID: 28886762.
7. Goldberg M, Kulkarni AB, Young M, Boskey A. Dentin: structure, composition and mineralization. Front Biosci (Elite Ed). 2011 Jan 1;3(2):711–35. doi:10.2741/e281 PubMed PMID: 21196346.
8. Pushpalatha C, Gayathri VS, Sowmya S V, Augustine D, Alamoudi A, Zidane B, et al. Nanohydroxyapatite in dentistry: A comprehensive review. Saudi Dent J. 2023 Sep;35(6):741–52. doi:10.1016/j.sdentj.2023.05.018 PubMed PMID: 37817794.
9. Breschi L, Maravic T, Cunha SR, Comba A, Cadenaro M, Tjäderhane L, et al. Dentin bonding systems: From dentin collagen structure to bond preservation and clinical applications. Dental Materials. 2018 Jan;34(1):78–96. doi:10.1016/j.dental.2017.11.005

10. Moradian-Oldak J, George A. Biomineralization of Enamel and Dentin Mediated by Matrix Proteins. J Dent Res. 2021 Sep;100(10):1020–9. doi:10.1177/00220345211018405 PubMed PMID: 34151644.
11. ASTM International. Standard Terminology of Adhesives. ASTM International. D907 15(2023) ; 2015.
12. Nakabayashi N, Kojima K, Masuhara E. The promotion of adhesion by the infiltration of monomers into tooth substrates. J Biomed Mater Res. 1982 May 13;16(3):265–73. doi:10.1002/jbm.820160307
13. Tjäderhane L, Nascimento FD, Breschi L, Mazzoni A, Tersariol ILS, Geraldeli S, et al. Optimizing dentin bond durability: control of collagen degradation by matrix metalloproteinases and cysteine cathepsins. Dent Mater. 2013 Jan;29(1):116–35. doi:10.1016/j.dental.2012.08.004 PubMed PMID: 22901826.
14. H. S. Delgado A, Belmar Da Costa M, Polido MC, Mano Azul A, Sauro S. Collagen-depletion strategies in dentin as alternatives to the hybrid layer concept and their effect on bond strength: a systematic review. Sci Rep. 2022 Jul 29;12(1):13028. doi:10.1038/s41598-022-17371-0
15. Violich DR, Chandler NP. The smear layer in endodontics – a review. Int Endod J. 2010 Jan 7;43(1):2–15. doi:10.1111/j.1365-2591.2009.01627.x
16. Saikaew P, Sattabanasuk V, Harnirattisai C, Chowdhury AFMA, Carvalho R, Sano H. Role of the smear layer in adhesive dentistry and the clinical applications to improve bonding performance. Japanese Dental Science Review. 2022 Nov;58:59–66. doi:10.1016/j.jdsr.2021.12.001
17. Hosseini M, Raji Z, Kazemian M. Microshear Bond Strength of Composite to Superficial Dentin by Use of Universal Adhesives with Different pH Values in Self-Etch and Etch & Rinse Modes. Dent Res J (Isfahan). 2023 Jan;20(1). doi:10.4103/1735-3327.367904
18. Pegado REF, do Amaral FLB, Flório FM, Basting RT. Effect of different bonding strategies on adhesion to deep and superficial permanent dentin. Eur J Dent. 2010 Apr;4(2):110–7. PubMed PMID: 20396440.
19. Montanari M, Fiorillo L, Cervino G, Sambataro S, Herford AS, Cicciù M. The Effect of Different Condition of Pulpal Pressure on Microtensile Bond Strength of Several Dentin Bonding Agents on Deep and Superficial Dentin. Materials. 2021 Oct 19;14(20):6200. doi:10.3390/ma14206200

20. Perdigão J. Current perspectives on dental adhesion: (1) Dentin adhesion – not there yet. Japanese Dental Science Review. 2020 Nov;56(1):190–207. doi:10.1016/j.jdsr.2020.08.004
21. Saitamon P, Pravalpruekskul S, Vongphan N, Harnirattisai C, Sattabanasuk V. Universal Adhesive Application Modes Differentially Affect the Fracture Resistance of Resin Composite Restored Teeth. Oper Dent. 2024 Mar 1;49(2):200–9. doi:10.2341/23-092-L PubMed PMID: 38196084.
22. Morfill GE, Zimmermann JL. Plasma Health Care - Old Problems, New Solutions. Contributions to Plasma Physics. 2012 Aug 16;52(7):655–63. doi:10.1002/ctpp.201210062
23. Awad MM, Alhalabi F, Alshehri A, Aljeaidi Z, Alrahlah A, Özcan M, et al. Effect of non-thermal atmospheric plasma on micro-tensile bond strength at adhesive/dentin interface: A systematic review. Materials. 2021 Feb 2;14(4):1–13. doi:10.3390/MA14041026
24. Stasic JN, Pficer JK, Milicic B, Puač N, Miletic V. Effects of non-thermal atmospheric plasma on dentin wetting and adhesive bonding efficiency: Systematic review and meta-analysis. J Dent. 2021 Sep;112:103765. doi:10.1016/j.jdent.2021.103765
25. Stasic JN, Selaković N, Puač N, Miletić M, Malović G, Petrović ZLj, et al. Effects of non-thermal atmospheric plasma treatment on dentin wetting and surface free energy for application of universal adhesives. Clin Oral Investig. 2019 Mar 18;23(3):1383–96. doi:10.1007/s00784-018-2563-2
26. Arora V. Cold Atmospheric Plasma (CAP) In Dentistry. Dentistry. 2013;04(01). doi:10.4172/2161-1122.1000189
27. Lau XE, Liu X, Chua H, Wang WJ, Dias M, Choi JJE. Heat generated during dental treatments affecting intrapulpal temperature: a review. Clin Oral Investig. 2023 Apr 6;27(5):2277–97. doi:10.1007/s00784-023-04951-1
28. Braný D, Dvorská D, Halašová E, Škovierová H. Cold Atmospheric Plasma: A Powerful Tool for Modern Medicine. Int J Mol Sci. 2020 Apr 22;21(8):2932. doi:10.3390/ijms21082932
29. Encyclopaedia Britannica. The Editors of Encyclopaedia Britannica. 2025. Argon.

30. Liu Y, Liu Q, Yu QS, Wang Y. Nonthermal Atmospheric Plasmas in Dental Restoration. J Dent Res. 2016 May;95(5):496–505. doi:10.1177/0022034516629425 PubMed PMID: 26848068.
31. Jha N, Ryu JJ, Choi EH, Kaushik NK. Generation and Role of Reactive Oxygen and Nitrogen Species Induced by Plasma, Lasers, Chemical Agents, and Other Systems in Dentistry. Oxid Med Cell Longev. 2017 Jan 24;2017(1). doi:10.1155/2017/7542540
32. Nima G, Harth-Chu E, Hiers RD, Pecorari VGA, Dyer DW, Khajotia SS, et al. Antibacterial efficacy of non-thermal atmospheric plasma against Streptococcus mutans biofilm grown on the surfaces of restorative resin composites. Sci Rep. 2021 Dec 10;11(1):23800. doi:10.1038/s41598-021-03192-0
33. Laroussi M. Sterilization of contaminated matter with an atmospheric pressure plasma. IEEE Transactions on Plasma Science. 1996 Jun;24(3):1188–91. doi:10.1109/27.533129
34. Ritts AC, Li H, Yu Q, Xu C, Yao X, Hong L, et al. Dentin surface treatment using a non-thermal argon plasma brush for interfacial bonding improvement in composite restoration. Eur J Oral Sci. 2010 Oct 16;118(5):510–6. doi:10.1111/j.1600-0722.2010.00761.x
35. Ayres AP, Bonvent JJ, Mogilevych B, Soares LES, Martin AA, Ambrosano GM, et al. Effect of non-thermal atmospheric plasma on the dentin-surface topography and composition and on the bond strength of a universal adhesive. Eur J Oral Sci. 2018 Feb 11;126(1):53–65. doi:10.1111/eos.12388
36. Ramos MSJ. Aplicação do plasma não térmico atmosférico na dentisteria restauradora – um desafio promissor [estudo laboratorial]. [Caparica (Portugal)]: Egas Moniz School of Health and Science; 2023.
37. Eliard. M. Propriedades superficiais da dentina tratada por NTAP: avaliação do ângulo de contacto e análise topográfica [Supervisor: Chasqueira AF.]. [Caparica (Portugal)]: Egas Moniz School of Health and Science; 2025.
38. Bourgi R, Kharouf N, Cuevas-Suárez CE, Lukomska-Szymanska M, Haikel Y, Hardan L. A Literature Review of Adhesive Systems in Dentistry: Key

Components and Their Clinical Applications. Applied Sciences. 2024 Sep 10;14(18):8111. doi:10.3390/app14188111

39. Young T. III. An essay on the cohesion of fluids. Philos Trans R Soc Lond. 1805 Dec 31;95:65–87. doi:10.1098/rstl.1805.0005
40. Rüttermann S, Trellenkamp T, Bergmann N, Raab WHM, Ritter H, Janda R. A new approach to influence contact angle and surface free energy of resin-based dental restorative materials. Acta Biomater. 2011 Mar;7(3):1160–5. doi:10.1016/j.actbio.2010.10.002
41. Wang DY, Wang P, Xie N, Yan XZ, Xu W, Wang LM, et al. study on non-thermal argon plasma in improving the bonding efficacy between dentin and self-etch adhesive systems. Dent Mater J. 2022 Jul 25;41(4):2021–215. doi:10.4012/dmj.2021-215
42. Gong Y, Chen X, Wu W. Application of fourier transform infrared (FTIR) spectroscopy in sample preparation: Material characterization and mechanism investigation. Advances in Sample Preparation. 2024 Aug;11:100122. doi:10.1016/j.sampre.2024.100122
43. Al-Akhras MAH, Alebrahim MA, Ba Rajjash AS, Al jarrah K, Hammouri HM, Mousa MS, et al. Ancient and modern bone diagnosis: Towards a better understanding of chemical and structural feature alterations. Spectrochim Acta A Mol Biomol Spectrosc. 2025 Feb;326:125259. doi:10.1016/j.saa.2024.125259
44. Liu GL, Kazarian SG. Recent advances and applications to cultural heritage using ATR-FTIR spectroscopy and ATR-FTIR spectroscopic imaging. Analyst. 2022;147(9):1777–97. doi:10.1039/D2AN00005A
45. Pasieczna-Patkowska S, Cichy M, Flieger J. Application of Fourier Transform Infrared (FTIR) Spectroscopy in Characterization of Green Synthesized Nanoparticles. Molecules. 2025 Feb 4;30(3):684. doi:10.3390/molecules30030684
46. Delgado AH, Young AM. Modelling ATR-FTIR Spectra of Dental Bonding Systems to Investigate Composition and Polymerisation Kinetics. Materials. 2021 Feb 5;14(4):760. doi:10.3390/ma14040760
47. Xu L, Ma F, Zhou J, Du C. Attenuated Total Reflectance Crystal of Silicon for Rapid Nitrate Sensing Combining Mid-Infrared Spectroscopy. ACS Omega. 2024 Dec 3;9(48):47613–20. doi:10.1021/acsomega.4c06701

48. Gandolfi MG, Taddei P, Zamparini F, Ottolenghi L, Polimeni A, Prati C. Dentine surface modification and remineralization induced by bioactive toothpastes. Int J Dent Hyg. 2024 Aug 10;22(3):554–74. doi:10.1111/idh.12710
49. Al Jedani S, Whitley CA, Ellis BG, Triantafyllou A, Smith CI, Gunning PJ, et al. Image fusion of IR and optical microscopy for mapping of biomolecules in tissue. Analyst. 2021 Sep 27;146(19):5848–54. doi:10.1039/d1an01161h PubMed PMID: 34498612.
50. Murphy B, D'Antonio J, Manning M, Al-Azzam W. Use of the Amide II Infrared Band of Proteins for Secondary Structure Determination and Comparability of Higher Order Structure. Curr Pharm Biotechnol. 2014 Nov 7;15(9):880–9. doi:10.2174/1389201015666141012181609
51. Anastasiadis K. Correlation of amide I and III components in the human dentine infrared spectrum. Dent Mater J. 2025 Nov 25;44(6):2025–73. doi:10.4012/dmj.2025-073
52. Ramírez-Bommer C, Gulabivala K, Ng Y -L., Young A. Estimated depth of apatite and collagen degradation in human dentine by sequential exposure to sodium hypochlorite and <scp>EDTA</scp> : a quantitative <scp>FTIR</scp> study. Int Endod J. 2018 Apr;51(4):469–78. doi:10.1111/iej.12864
53. Chen M, Zhang Y, Dusevich V, Liu Y, Yu Q, Wang Y. Non-thermal atmospheric plasma brush induces HEMA grafting onto dentin collagen. Dent Mater. 2014 Dec 1;30(12):1369. doi:10.1016/J.DENTAL.2014.10.004 PubMed PMID: 25458523.
54. Dong X, Chen M, Wang Y, Yu Q. A Mechanistic study of Plasma Treatment Effects on Demineralized Dentin Surfaces for Improved Adhesive/Dentin Interface Bonding. Clin Plasma Med. 2014 Jul 1;2(1):11–6. doi:10.1016/j.cpme.2014.04.001 PubMed PMID: 25267936.
55. Liu Y, Yao X, Liu YW, Wang Y. A Fourier transform infrared spectroscopy analysis of carious dentin from transparent zone to normal zone. Caries Res. 2014;48(4):320–9. doi:10.1159/000356868 PubMed PMID: 24556607.
56. Hirata R, Sampaio C, Machado LS, Coelho PG, Thompson VP, Duarte S, et al. Short- and Long-term Evaluation of Dentin-Resin Interfaces Formed

by Etch-and-Rinse Adhesives on Plasma-treated Dentin. J Adhes Dent. 2016;18(3):215–22. doi:10.3290/j.jad.a36134 PubMed PMID: 27200431.

57. Mozetič M. Aging of Plasma-Activated Polyethylene and Hydrophobic Recovery of Polyethylene Polymers. Polymers (Basel). 2023 Dec 11;15(24):4668. doi:10.3390/polym15244668
58. Zhong G, Xu S, Yu C, Atashbahar M, Liu Q. Effect of Non-Thermal Atmospheric Plasma Treatment on Bonding Performance of Customized Ceramic Post-and-Cores. Journal of Esthetic and Restorative Dentistry. 2025 Sep 17. doi:10.1111/jerd.70031
59. Zhang Z xing, Huang C, Zheng T li, Wang S, Cheng X rong. Effects of residual water on microtensile bond strength of one-bottle dentin adhesive systems with different solvent bases. Chin Med J (Engl). 2005 Oct 5;118(19):1623–8. PubMed PMID: 16232347.
60. Chen M, Zhang Y, Sky Driver M, Caruso AN, Yu Q, Wang Y. Surface modification of several dental substrates by non-thermal, atmospheric plasma brush. Dental Materials. 2013 Aug;29(8):871–80. doi:10.1016/j.dental.2013.05.002
61. Milosevic M. Internal Reflection and ATR Spectroscopy. Appl Spectrosc Rev. 2004 Dec 31;39(3):365–84. doi:10.1081/ASR-200030195
62. Stani C, Vaccari L, Mitri E, Birarda G. FTIR investigation of the secondary structure of type I collagen: New insight into the amide III band. Spectrochim Acta A Mol Biomol Spectrosc. 2020 Mar;229:118006. doi:10.1016/j.saa.2019.118006
63. Prado M do, Roizenblit RN, Pacheco LV, Barbosa CA de M, Lima CO de, Simão RA. Effect of Argon Plasma on Root Dentin after Use of 6% NaOCl. Braz Dent J. 2016 Feb;27(1):41–5. doi:10.1590/0103-6440201600486
64. Milhan NVM, Chiappim W, Sampaio A da G, Vegian MR da C, Pessoa RS, Koga-Ito CY. Applications of Plasma-Activated Water in Dentistry: A Review. Int J Mol Sci. 2022 Apr 8;23(8). doi:10.3390/ijms23084131 PubMed PMID: 35456947.

Análise laboratorial de dentina tratada por plasma não térmico atmosférico utilizando espectrocopia de infravermelho por transformada de Fourier. (ATR-FTIR)

Appendix

CEEM
COMISSÃO DE ÉTICA EGAS MONIZ

Processo Interno: 1710

PT-402/25

Ex.ma Senhora

Lilou Margaux Jeanne Marie Klein

Monte de Caparica, 17 de dezembro de 2025.

Ex.ma Senhora,

Em resposta ao Pedido de Parecer que submeteu à apreciação da Comissão de Ética da Egas Moniz, com o tema denominado: "ANÁLISE LABORATORIAL DE DENTINA TRATADA COM PLASMA NÃO TÉRMICO ATMOSFÉRICO UTILIZANDO ESPECTROSCOPIA DE INFRAVERMELHO POR TRANSFORMADA DE FOURIER. (ATR-FTIR)", foi aprovado.

A Presidente da Comissão de Ética da Egas Moniz

Profa Doutora Ana Filipa Vicente