Doctoral Dissertation
Doctoral Program in Bioengineering and Medical-Surgical Sciences (36th Cycle)

Influence of root canal treatment and dentinal ageing on the chemical and mechanical properties of radicular dentin and its bonding potential.

Pietro Palopoli
* * * * * *

Supervisors
Prof. Elio Berutti, Supervisor
Prof. Mario Alovisi, Co-Supervisor

Doctoral Examination Committee:
Prof. Mancino Davide, Referee, University of Strasbourg
Prof. Turco Gianluca, Referee, University of Trieste

Politecnico di Torino
April 25, 2024

I hereby declare that the contents and organisation of this dissertation constitute my own original work and does not compromise in any way the rights of third parties, including those relating to the security of personal data.

Pietro Palopoli
Turin, April 25, 2024

Summary

Introduction: Natural teeth, even when structurally compromised, may have a better survival rate compared to implants, as long as a proper treatment is provided. Accordingly, solutions that aim to save even heavily damaged teeth are increasingly being considered. One of the consequences, though, is that clinicians often have to deal with a massively destroyed aged substrate that may have also been modified by previous endodontic or restorative treatments. Ageing produces irreversible modifications to the chemical and mechanical properties of dentin. A gradual reduction in the diameter of the dentinal tubules lumen, due to an accumulation of minerals, and the cross-linking of inter-tubular collagen fibres are the most evident changes with increasing age. Interestingly, root canal treatment appears to accelerate these ageing processes. Endodontically treated teeth with little coronal tissue are often restored with post and core systems. Nonetheless, the aforementioned substrate alterations may significantly affect the bonding ability of adhesive materials. A deep knowledge of the differences between young and aged dentin, as well as between vital and endodontically treated dentin may help to understand the different bonding behaviour of resin cements on these different substrates. This could be fundamental to improve the clinical effectiveness of the adhesive materials. Therefore, the purpose of this study was to assess whether different dentin substrates, and, the ageing of the substrates had a role on root canal adhesion through the analysis of the adhesive strength, the morphological characteristics of the root dentin-luting cement-fibre post interfaces and the mechanical and chemical characteristics of the dentinal substrates. The evaluation was performed on freshly devitalized teeth compared with elements with an aged root canal treatment, and on young patients compared to older ones.

Materials and methods: Thirty-two glass-fibre posts (D.T. Light Post n.1) were cemented into the root canals of human anterior teeth, in patients of the same age group (45 to 55) that, at the moment of extraction, were either vital or with an aged endodontic treatment. After root canal treatment (RCT) or root canal re-treatment (RCRT) fibre posts were luted using a self-adhesive resin cement (iCEM) and a self-etching bonding system plus resin cement (Clearfil Universal Bond Quick +

Clearfil DC Core Plus). The roots were sectioned into 1 mm slices, categorised in coronal and apical, and bond strength was measured using a micro push-out test at baseline (T0) and after one year ageing (T1). Analysis of the failure mode was carried out through stereomicroscope (40X). Fractures were classified as adhesive (between cement and dentin), cohesive (inside the cement or at the post-cement interface) or mixed. In a second section of the study, sixteen fibre posts were luted, as described before, in 8 multi-rooted teeth, to the same two types of radicular substrates. Before insertion of the post, the adhesive system was labelled with fluorescein and the resin cements were labelled with rhodamine. The roots were sectioned and analysed using confocal laser scanning microscopy (CLSM) to determine hybrid layer thickness and the number of resin tags.

Micro-mechanical and chemical characteristics of root canal treated and re-treated dentin were investigated, respectively, by nano-indentation and Raman spectroscopy on the slices that were submitted to the push-out test at T0. To assess the mechanical characteristics, 100 indentations (divided in four 1 mm^2 matrices) were performed on the dentinal tissue around the post. Martens (HM) and Vickers (HV) hardness, elastic modulus (EIT), and plastic deformation (n plast) were assessed. To investigate the chemical characteristics, Raman spectroscopy was performed with the following experimental parameters: 60 s, 12 repetitions, 80X magnification (spot diameter of about 20 μm). The values of Mineral to Matrix ratio, Crystallinity, Phosphate content, Carbonate to Phosphate ratio and the structure, quality and organisation of collagen were extracted by the spectra.

In a third section of the study, thirty-two glass-fibre posts were luted into the root canals of extracted human anterior teeth, equally divided in young (under 20) and old (over 60) specimens, using the same materials and methodology described before. Bond strength and failure mode were assessed as previously described.

Results: Results from the micro push-out bond test at T0 showed that:

- The bond strength of fibre posts in aged root canal treated teeth was significantly lower compared to the freshly root canal treated specimens, regardless of the luting cement used and the canal area considered;
- The bond strength registered in the apical area was significantly lower when compared to the values obtained from the coronal region, regardless of the type of substrate and the luting cement selected.

Results from the micro push-out bond test after one year ageing (T1) showed that:

- There was a significant decrease of bond strength after one year ageing, particularly in the coronal half of the post space, regardless of the type of substrate and the cement used;

- After one year ageing the difference in bond strength between RCT and RCRT dentin was not significant anymore;
- The performance of the self-etch and self-adhesive cements were comparable.

Analysis of fractures after the push-out bond strength at T0 and T1 revealed that the majority of failures, both for freshly and aged root canal treated teeth, were adhesive (between dentin and luting cement).

The CLSM analysis found that the hybrid layer (HL) thickness was significantly higher in the freshly root canal treated teeth, regardless of the cement used and the canal area. The number of filled tubules (resin tags) created by resin cements was significantly influenced only by the topography of the post space. More resin tags were visible in the coronal half.

The mechanical analysis performed on the substrates subjected to push-out test at T0 showed that the Young modulus and hardness were significantly higher in the freshly root canal treated tissue. The percentage of plasticization was found, by the statistical test, significantly higher in the aged root canal treated tissue. However the values were so close that this may be considered not scientifically meaningful. For all the mechanical properties considered there was significant difference, between the two tissues analysed, both in the coronal and middle third of the post space, but not in the apical third.

Raman spectroscopy highlighted a worse collagen organisation, structure and quality in the aged root canal treated teeth. Likewise, a significantly higher carbonate/phosphate ratio was evident in the same tissue. This indicates a phosphate substitution with the carbonate in the hydroxyapatite structure, leading to a less pure mineral structure. The intensity of the phosphate peak and the crystallinity found within the two substrates investigated were comparable.

The push-out test in the samples divided by age (under 20 and over 60) showed a higher bond strength in the older group, irrespective of the canal area considered. The self-adhesive cement performed significantly better in the older group compared to the younger one.

Discussion: It can be concluded that root canal treatment, overtime, leads to a significant breakdown of the mechanical and chemical properties. This shift in properties, in the long term, could explain the decreased resistance to fracture of devitalized teeth. Also, this loss of the original characteristics seem to significantly affect the potentiality of radicular dentin to be infiltrated by resin cements. However, in the long term, this does not seem to affect the bond strength when self-etch or self-adhesive adhesive strategies are used. At the same time, these adhesive cements seem to perform better, overall, on older substrates. The strongest difference in performance was registered for the self-adhesive cement. These data

may be coherent with the fact that these materials create a direct chemical link with the hydroxyapatite, and so, a stronger bond may be expected where the mineral component is more represented, as in older dentin. Therefore, in elderly and/or aged root canal treated radicular dentin, simplified luting strategies as single step self-adhesive resin cements seem to be a reliable option.

Contents

Abbreviations

HL hybrid layer
ECM extracellular organic dentinal matrix
PGs Proteoglycans
GAGs Glycosaminoglycans
E&R Etch and rinse
SE self etch
SA self adhesive
UA universal adhesive
10-MDP 10-Methacryloyloxydecyl dihydrogen phosphate
HA hydroxyapatite
DEJ dentin-enamel junction
CDJ cementum-dentin junction
CEJ cemento-enamel junction
PIJ peritubular-intertubular junction
PTD peri-tubular dentin
ITD inter-tubular dentin
deH-DHLNL dehydrodihydroxylysinonorleucine
deH-HLNL dehydrohydroxylysinonorleu-cine
PYD pyridinoline
DPD deoxypyridinoline
NaOCl Sodium hypochlorite
CHX Chlorhexidine
MMPs Metalloproteinases
EDTA ethylenediaminetetracetic acid
CaOH calcium hydroxide
RCT Root Canal Treated
RCR-T Root Canal Re-Treated
R-DI resin-dentin interface
RMS Raman Microspectroscopy
FWHM full width at half maximum
M/M mineral to matrix ratio
C/P carbonate to phosphate ratio
HV Vickers Hardness
HM Martens Hardness
AFM Atomic Force Microscopes
SD Standard deviation

Chapter 1

Introduction and specific aims

1.1 The dentinal substrate

1.1.1 Dentinal substrate: chemical characteristics

Dentin is the tissue that lies beneath the enamel and surrounds the pulp chamber and root canals. It is composed of organic and inorganic matter: 45% mineral, 33% organic material, mostly type I collagen, and 22% water by volume. This composition corresponds, on average, to 70% mineral, 20% organic matter, and 10% water by weight (Tjaderhane 2009). This composition can vary in different areas of the tooth, depending on its proximity to the pulp tissue, as well as whether the matrix is affected or infected by caries. These differences can greatly influence the mechanical properties of dentin as well as its bonding potential (Carvalho 2012). The extracellular organic dentinal matrix (ECM) is a complex 3-dimensional network of fibrillar collagen and globular entities that become mineralized by nanoscopic apatite crystallites during the process of dentinogenesis (Veis 2003). Collagen type I constitutes the majority of the collagen structure (90 wt%), that also contains traces of collagen V and III (Tjaderhane 2009). The remaining constituents of the ECM are the non-collagenous proteins, among which the proteoglycans (PGs) are the most represented, followed by dentin sialoproteins, phosphoproteins, bone morphogenic proteins, enzymes and growth factors (Mazzoni 2009). Type I collagen fibrils are perpendicularly connected by non-collagenous proteins (Breschi 1999), as the PGs, which consist of a core protein, glycosaminoglycans (GAGs) and linkage proteins (Goldberg 1993). PGs are involved in the process of dentin mineralization and the maintenance of the structural three-dimensional integrity of collagen fibrillar alignment (Goldberg 1993). Moreover, these proteins can bind and organise water molecules, regulating the affinity of collagen to water and can affect the substitution of water during the formation of the hybrid layer (HL) (Oyarzun 2000).
Collagen chains consist of three domains: a central triple helical region (>95%), a non-helical aminoterminal (N-telopeptide) region and a carboxyterminal (C-telopeptide) region (Yamauchi 2008). These peptide chains spontaneously form

insoluble collagen fibrils by aggregating and stacking in parallel. These collagen fibrils contain a 67 nm gap between the neighbouring collagen molecules, and are further organised in bundles (Veis 2003). During the process of dentin maturation, apatitic mineral crystallites precipitate and first fill the 67 nm gaps between the collagen molecules and then the interfibrillar spaces (Landis 2013), thereby inactivating enzymes that are present in the ECM and were active during the dentinogenesis (Hannas 2007).

Unlike insoluble collagen in other bodily systems, dentinal collagen does not metabolically (Tjaderhane 2009) turn over, meaning that it is not easily degraded, but once it is, it cannot be replaced. This stability is due to the slow formation of covalent inter- and intramolecular cross-links, which occur between the C-terminal of one collagen molecule and the N-terminal of the adjacent collagen molecule (Yamauchi 2008). As dentin collagen does not turn over, the natural cross-links accumulate over time (Miura 2014) and can influence the mechanical properties of collagen fibrils (Miura 2014). As a matter of fact, dentin collagen is the most cross-linked collagen in the body. These cross-links are responsible for the ability of dentin to be acid-etched during bonding procedures without denaturing its collagen (Veis 1964). Therefore, dentin collagen can withstand adhesive procedures that would, for instance, destroy the structure of the dermal collagen (Veis 1964). If the acid etching step is limited to the recommended time (usually 15s), the structural integrity of the ECM is preserved (Oyarzun 2000). Over-etching could, however, induce structural changes in the collagen molecules (Breschi 2003) as well as PGs (Hedbom 1993), introducing a damaged organic part into the HL.

1.1.2 Raman spectroscopy: assessment of chemical properties of dentin.

In the last decades, vibrational spectroscopic techniques, such as Raman Microspectroscopy (RMS), have been employed as emerging methodologies to investigate the structure and macromolecular composition of tissues (Notarstefano 2020, Notarstefano 2021). This is a non-destructive and label-free analytical technique that requires minimal to no preparation (Larkin 2018). Another advantage of this technique is that it can be used on solid and thicker samples and it is not affected by water (Kekkonen 2019). Based on the interaction between the target and electromagnetic radiation, Raman provides information on the chemical bonds and the functional groups of the macromolecules within the analysed specimen (Geraldes 2020). More specifically, RMS is a scattering technique. As such, it estimates the two-photon inelastic light scattering produced when the photons from monochromatic electromagnetic radiation interacting with matter are scattered from the specimen with a lower or higher energy as compared to the incident one (Butler 2016, Larkin 2018).
Furthermore, in recent years, RMS performances have been deeply improved by coupling Raman spectroscopy and optical microscopy allowing this way to acquire morphological and chemical data on the same area of the sample (Akkus 2017).
Therefore, in the dentistry field, RMS can be used to acquire maps of dental tissues, both superficially or at depth, that provide, at the same time, a correlation between the microscopic information acquired from the visual analysis of the specimen and its chemical composition. In this regard, RMS is a new and non-invasive technique to investigate both the inorganic and organic composition of teeth (Lee 2020). In RMS the spectral profile and the position of the peaks can be influenced by the

composition and structure of the sample (Penel 1998). Therefore, a precise analysis of the spectral bands related to the chemical composition of the different dental components is of paramount importance.
For instance, the Raman spectrum of dentin (Fig. 1) is mainly characterised by bands at 1660 cm^{-1}, 1450 cm^{-1} and 1242 cm^{-1} attributable to the organic matrix (collagen) and bands at 1070 cm^{-1} and 960 cm^{-1} belonging to the mineral component of the dentinal tissue (Orsini 2021). The bands at 1070 cm^{-1} and 960 cm^{-1} represent, respectively, the carbonate (CO_3^{2-}) and the phosphate (PO_4^{3-}) groups of hydroxyapatite (HA).

Fig. 1

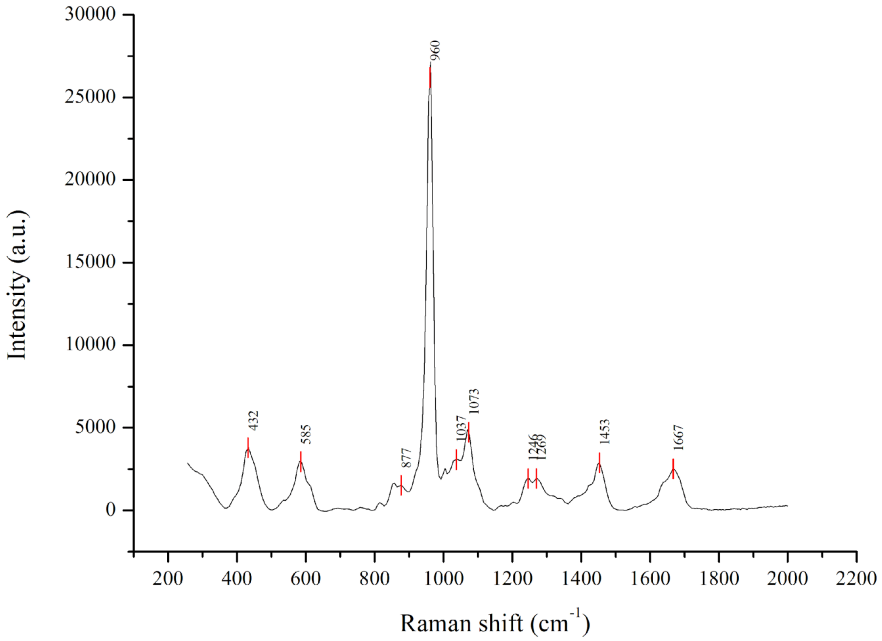

Fig. 1 Representative Raman spectra of dentinal tissue.

However, it is not very common to study absolute band intensities in Raman spectroscopy because they are influenced by the efficiency of the Raman spectroscope and other optical effects such as grain size, refractive index, and surface roughness of the specimen (Wopenka 2008). Therefore, many authors rather report the relative peak intensities or peak areas of select pairs of bands from the Raman spectrum. For example, the ratio between the primary phosphate band (approximately 959 cm^{-1}) and the amide I band (1616–1720 cm^{-1}) corresponds to the mineral-to-matrix ratio, which represents the amount of mineralization (Mc Creadie 2006). Other parameters consider the full width at half maximum of a single band (FWHM), such as the crystallinity that is inversely proportional to FWHM of the band at 960 cm^{-1}.

The Raman-derived markers for the characterization of hard dental tissue are summarised in Table 1.

Table 1

MINERAL / MATRIX	(A_{960}/A_{1660})	Amount of the mineral component respect to the organic one
CRYSTALLINITY	($1/FWHM_{960}$)	Degree of order within the HA mineral crystals
CARBONATE/ PHOSPHATE	(I_{1070}/I_{960})	Extent of carbonate incorporation in the hydroxyapatite lattice
PHOSPHATES	(I_{960})	Mineral compound of HA
CH_2 GROUPS	(I_{1450})	Structural organization and relative amount of collagen
AMIDE I	(I_{1655})	
AMIDE III	(I_{1246})	
AMIDE I / AMIDE III	(I_{1655}/I_{1246})	Collagen organization
AMIDE III / CH_2 AMIDE I/ CH_2	(I_{1246}/I_{1450}) (I_{1655}/I_{1450})	Differences in collagen structure and quality

Table 1 Raman-derived markers for the characterization of hard dental tissues (I: intensity of the band; A: area of the band; and FWHM: full width at half maximum) (Orsini 2021).

Specifically the Mineral to Matrix Ratio (M/M) (A960/A1660) indicates the ratio between phosphate and collagen (amide I). It is related to the amount of the mineral component with respect to the organic one. It can be calculated as the ratio between the area or the intensity of the band at 960 cm^{-1} , which is the most intensive band of HA (hydroxyapatite), attributed to phosphates, and that of the amide I band centred at 1660 cm^{-1}. Mineral-to-matrix ratio is a strong predictor of the dentinal mechanical properties (Morris 2010).

Crystallinity ($1/FWHM_{960}$) can be defined as the degree of order within the HA mineral crystals. Crystallinity increases when crystals are larger and/or more perfect, and when there is less substitution of Phosphate (960 cm^{-1}) with B-type carbonate (CO_3^{2-}, 1070 cm^{-1}), since ion substitution may introduce structural distortions. As explained before, this parameter is directly proportional to the inverse of the full width at half maximum (FWHM) of the band centred at 960- cm^{-1}, meaning that a narrow band width indicates less structural variations and high mineral crystallinity and vice versa (Karan 2009). Practically, the lower the carbonate content in apatite is, the narrower the phosphate band (Xu 2009).

Carbonate to phosphate ratio (C/P), also known as gradient in mineral content, is calculated as the ratio between the intensities of the bands centred at 1070 cm^{-1} and 960 cm^{-1} attributed, respectively, to the carbonate and phosphate groups in HA. This ratio indicates the extent of carbonate incorporation in the HA lattice. Therefore, a prominent carbonate band around 1070 cm^{-1} in the Raman spectrum is indicative of the degree of carbonate substitution in the lattice structure of the apatite (Salehi 2013). Furthermore, the curve-fitting of the carbonate band shows

whether the carbonate has replaced hydroxide (A-type) or phosphate (B-type) in the apatite structure. However, it must be pointed out that the carbonate band can be partially overlapped by another phosphate band around 1076 cm^{-1} which could eventually reduce measurement accuracy, especially for apatite with minimal carbonate content (Morris 2011).

As stated before, RMS can give important information about the organic matrix of the dentinal tissue. For instance, the ratio between amide I and amide III is indicative of collagen organisation. It is calculated as the ratio between the area of the bands at 1655 cm^{-1} and 1246 cm^{-1}. A higher value of this ratio indicates better organisation (Toledano 2014), better recovery (Xu 2012) and improved structure and quality of the collagen matrix (Salehi 2013).

Similar information about collagen organisation can be also given by the ratio of the intensity of the bands at 1246 cm^{-1} and 1450 cm^{-1} (Xu 2012, Salehi 2013, Toledano 2014). These peaks represent, respectively, amide III and CH_2. More specifically, the intensity of amide III band is a marker of the orientation of collagen supramolecular structure (Salehi 2013). A partial loss of order in fibrils orientation, due to ageing or caries, influences the fluorescence signal, thus providing an understanding of the spatial arrangement of collagen structure within tissues (Salehi 2013). A higher ratio is also representative of higher collagen content (Salehi 2013).

Collagen quality is also indicated by the ratio of the intensity of the bands at 1655 cm^{-1} and 1450 cm^{-1}, which represent, respectively, amide I and CH_2. A decrease of this value stands for altered collagen quality (Salehi 2013). Thus, an increase of the peak at CH_2 suggests an alteration of the collagen that can be due to ageing (Ager 2005), dehydration (Ager 2006) or radiological damage (Barth 2010).

Considering the single bands, the intensity of the one at 1655 cm^{-1} represents amide I. The characteristic vibrational modes of amide I is mostly an in-plane carbonyl stretch (stretches between C and O) (Ager 2006). A lower value of this peak suggests damage or removal of collagen fibrils due to a degradation process (Xu 2012). This peak is independent of tissue organisation (Paschalis 2017).
The intensity of the band at 1246 cm^{-1} is related to amide III. This peak, that involves C-N stretching and N-H bending (Ager 2006), contrary to amide I, seems to be influenced by the amount of inorganic and organic content of the sample, as well as by collagen fibres and mineral crystallites orientation (Paschalis 2017). A decrease of this peak may indicate a structural disorganisation in the secondary structure of the protein unit that composes the collagen fibril (Campi 2018).

Therefore, the amide bands, mostly amides I and III, can be considered good indicators of protein conformation because of the role of the amide moiety in the organisation of the collagen matrix (Bandekar 1992).

Finally, the intensity of the band at 1450 cm^{-1} is assigned to the CH_2 wag bands of collagen I (Ager 2006), and it is mainly associated with the organic matrix of dentin (Xu 2009). This peak is related to the angular deformation of NH and bonding stretching CN, usually typical of proteins, lipids, and carbohydrates (Campi 2018).

1.1.3 Dentinal substrate: structural characteristics

Dentinal structure is mainly characterised by the presence of dentin tubules. They are a mesh of microscopic tubules that run radially from the pulp toward the DEJ and CDJ. Each tubule is surrounded by a highly mineralized cuff of apatite crystals known as peri-tubular dentin (Ten Cate 2008). The inter-tubular dentin occupies the region between the tubules and is made of a collagen fibrils mesh oriented essentially perpendicular to the tubules and bound by apatite crystallites (Kinney 2003). The diameter of collagen fibers ranges from 50 to 100 nm, while the apatite crystals are approximately 5 nm thick and their morphology depends on distance from the pulp (needle-like near the pulp and plate-like at the DEJ) (Kinney 2001). Due to this wide range of dimensional scales, dentin is often described as a complex hierarchical structure (Kinney 2003).
Many tubules characteristics are important to the structural behaviour of dentin, its permeability, and in achieving strong adhesive bonds. Of primary importance are the tubule density, tubule diameter, and spatial variations in these measurements. At the crown level, the average density ranges from approximately 15,000 to 65,000 tubules per mm^2 (Garberoglio 1976). The tubule diameter decreases from approximately 2.5 mm (near the pulp) to less than 1 mm at the DEJ (Arola 2012) (Fig 1). The mean tubules density ranges from approximately 8,000 to 58,000 tubules/mm^2 and the density of the tubules is significanly higher at the crown level than the root region where, however, the highest number of tubule branches can be detected (Mjor 1996).

Fig. 2

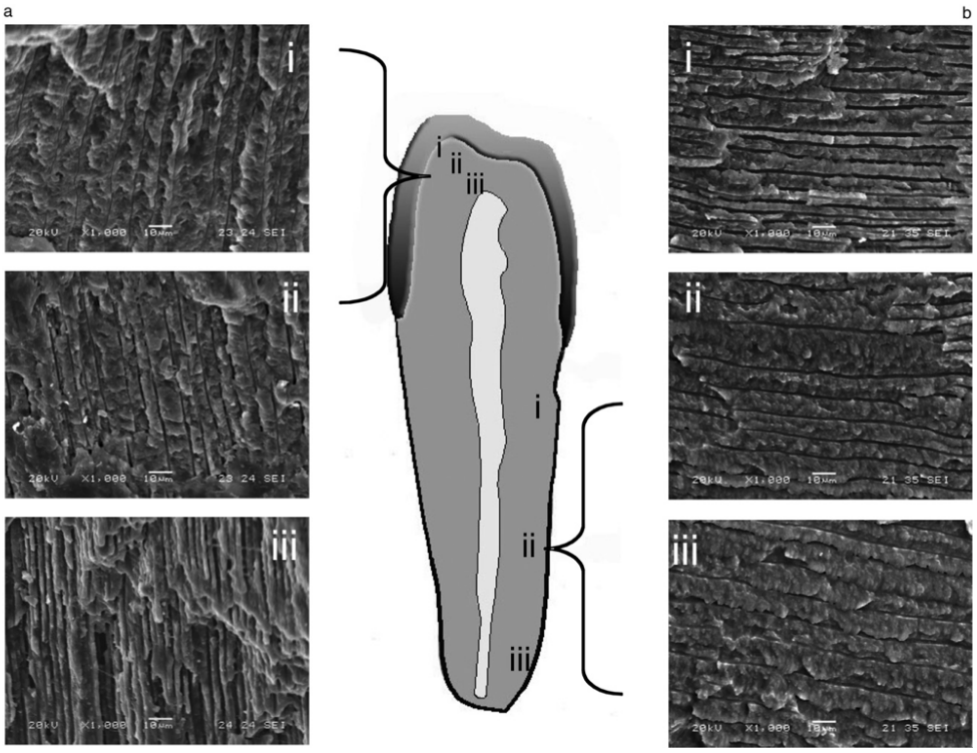

Fig. 2. These pictures show differences in the dentinal microstructure in a 25 year old premolar. Differences in tubule dimensions and density can be appreciated moving from the pulp to the outer region and from the crown to the root. (a) Crown region. (b) Root region. (Arola 2012).

1.1.3.1 Dentinal substrate: structural composition of radicular dentin.

Several authors have studied the composition and structure of radicular dentin and found differences from coronal dentin and along the root. For instance, Komabayashi et al. reported a density of 14,000 to 32,000 tubules/mm^2 1–2 mm below the cemento-enamel junction (CEJ) of human canines, that was much lower than equivalent measures in the crown: the density effectively doubled from the outer wall (near the periodontal ligament) to the region adjacent to the pulp (Komabayashi 2008).

Another study (Camargo 2007) sorted the specimens into cervical, middle, and apical root thirds and found that the tubules density decreased moving from the cervical area toward the apex. Moreover, the peri-tubular cuff was found less thick in root dentin compared to the crown. The lower number of tubules and the decreased thickness of the peritubular cuff in the root correspond to a higher inter-tubular dentin/volume of tissue ratio and a larger amount of matrix (Marchetti 1992). Furthermore, in root dentin collagen fibrils have larger diameter (Marchetti 1992) and a different primary orientation than those found in the tooth crown (Yasui 2004). As inorganic (mineral) and organic (collagen) matter are considered responsible, respectively, for the hardness and toughness of dentin (Marshall 1997), the structural spatial variations are of paramount importance to the mechanical behaviour of this tissue.

1.1.4 Mechanical behaviour of dentin

Dentin is the most abundant mineralized tissue in the human tooth. Therefore, knowledge of its mechanical properties is essential to predict the effects of microstructural alterations due to caries, sclerosis, and ageing on tooth strength. In clinical dentistry, knowledge of dentin properties is important to understand the effects of the wide variety of restorative dental procedures, such as the choice of bonding methods.

The main mechanical properties of teeth include elasticity, hardness, visco-elasticity and fracture behaviour. However, fracture behaviour is not a focus of this study.

Elasticity is a term used to describe the characteristic in which a material changes under external force and recovers when the force is removed. The elastic property indices of natural teeth primarily include the elastic modulus (ratio of normal stress to normal strain), shear modulus (ratio of shear stress to shear strain) and Poisson's ratio (ratio of transverse contraction strain to longitudinal extension strain in the direction of the stretching force). However, the elastic modulus is the property that has been studied most extensively (Zhang 2014). The elastic modulus, also known as Young's modulus, is a value used to measure the rigidity of a material and is defined as the ratio of stress and strain under an elastic state. For human teeth, Young's modulus indicates the ability of enamel and dentin to resist elastic deformation (Zhang 2014). According to Kinney (Kinney 2003), the Young's Modulus of young dentin ranges from 20 GPa to 25 GPa.

Hardness is a measure of the hardness or softness of a material and also represents the ability of a solid material to resist elastic deformation, plastic deformation and destruction (Zhang 2014). Measurements of the hardness of natural teeth have shifted from macroscopic to microscopic with the advancement of new methods, enabling the accurate measurement of hardness at different sites (Habelitz 2001).
Hardness is commonly used to characterise the ability to resist compression deformation and fracture of a local area of a material. The hardness of teeth can be divided into static hardness and dynamic hardness (Zhang 2014). The static indentation hardness test is the most commonly used method for characterisation (Zhang 2014). The frequently used indices for static indentation hardness include the Vickers hardness, Knoop hardness and nano-hardness.
The Vickers hardness can be obtained by calculating the bare testing force unit area in indentation. The indenter is a diamond square pyramid formed by opposite angles of 136°. The measured value is the quotient of the load to surface area of the indentation (Zhang 2014).
The microhardness is the hardness of materials measured using a load of 10 mN to 10 N (Zhang 2014). The nano-hardness is the hardness of materials measured with a load of less than 700 mN, with indentation on the scale of microns or even nanometres (Zhang 2002).

Due to the complex structure of the dentinal tissue, the factors influencing the dentinal mechanical properties include the location, density and direction of the dentinal tubules, the direction of the collagen fibres, and the average density of the mineral phase (Cohen 2008).

Considering the macrostructure of dentinal tissue, Wang et al. found that the micro-hardness of dentin adjacent to the DEJ is low, increases rapidly to a peak and decreases slowly towards the pulp cavity. Therefore, the middle portion of the dentin has a higher hardness and elastic modulus than the outer portions (Wang 1997).
Cohen et al. assessed the microstructural mechanical properties of dentin with AFM, with which they measured the hardness and elastic modulus of the tubule lumen edge, peritubular dentin, peritubular-intertubular junction (PIJ) and intertubular dentin. The results showed that also the microstructural mechanical properties vary according to topography and mineral content. The hardness decreases gradually from the dentinal tubule cavity wall to the inter-tubular dentin and corresponds to the decrease in mineral content (Cohen 2008). Furthermore, highly mineralized peritubular dentin has a Young's modulus of 40–42 GPa, whereas weakly mineralized intertubular dentin has a Young's modulus of 17 GPa (Ziskind 2011).

However, the mechanical properties of dentin are not only exclusively related to its internal structure and composition. They are also influenced by the external environment: a study showed that the elastic modulus decreases by 35% and the hardness decreases by 30% in a hydrated environment (Kinney 2005). Kinney et al. confirmed that dentin is anisotropic in a moist environment, while it becomes isotropic in a dry environment (Guidoni 2006). These studies indicate that the mechanical properties of dentin are greatly influenced by the environment.

Many studies have studyed the properties of coronal dentin. On the contrary, only a few have assessed the mechanical properties of the root region. As well as the

crown, root dentin has an anisotropy behaviour and topographical variations in strength. When loads are applied parallel to the long axis of the tooth, the strength obtained is the highest. Furthermore, in accordance with the reduced number of tubules from the cervical to the apical root third, a higher strength in the apical region is expected. Assessments of the mechanical behaviour of coronal and radicular dentin seem to point toward greater strength and higher fracture toughness in root dentin, as long as the direction of loading is parallel to the long axis of the tooth.

1.1.5 Nanoindentation: assessment of mechanical properties of dentin.

Each dental calcified tissue has a unique microstructure that significantly influences the variations in the mechanical properties. Specifically, the relative proportions between mineral content, organic material, and water, are determinant for tissues' mechanical behaviour. Physiological processes, such as ageing, and many pathological conditions affecting dental hard tissues may lead to changes in mineral levels, crystalline structures, and mechanical properties. In recent years, the most widely used methodology to measure the mechanical behaviour of dental calcified tissues has been the nano-indentation technique, that allows the assessment of the mechanical properties of enamel, dentin and cementum on a nano-scale.

The use of nanoindentation in the dental field began in the early 90s (Van Meerbeek 1993); since then, a considerable number of studies has been published using this technology. The nanoindentation allows the measurement of the mechanical properties, such as hardness and elastic modulus, at the surface of a given sample. This technique is much simpler compared with more conventional mechanical tests such as compressive, tensile, bending, shear strength, and punch shear tests, especially on small complex composite materials such as enamel, dentin, and cementum (Waters 1980). Other advantages of the indentation system are that measurements are relatively nondestructive, and the specimen preparation is less time consuming as the test can be done on a bulk-polished specimen (Waters 1980, Mencik 1994). However, this technique still has some disadvantages. The most important drawback is that a nanoindenter can not position two separate indentations closer than a few micrometres. To overcome this issue Atomic Force Microscopes (AFM) have been developed and equipped in order to allow, at the same time, the measurement of hardness and Young modulus while imaging the sample at nearly atomic resolution. This technological advancement is very important for the assessment of the specific properties of inhomogeneous structures such as the dentinal tissue. For instance, AFM allows the selective measurement of peritubular and intertubular dentin, and this has brought a massive contribution to our current understanding of the mechanical behaviour of dental hard tissues correlated with their microstructural compositions.

Generally, nanoindentation systems work as load and displacement sensing systems where information is taken from the penetration of indenter on loading as well as from the elastic recovery of the specimen on unloading. Indentation into specimens can proceed continuously or in incremental steps with gradual increases of force loads until a preset maximum force is reached. After this point, seemingly, the unloading phase can be continuous or in a multiple load/partial-unload cycle where the indenter progresses in several incremental steps and partially unloads at each

step. In this process, an additional hold period at maximum load can be incorporated to allow the creep relaxation before unloading, enabling a more reliable measure of the elastic modulus from the unloading slope. This particular feature makes it possible to test soft hydrated tissue with highly elastic recovery behaviour such as carious dentin (Angker 2004, Angker 2005). Indentations can be done using a pyramid pointed indenter or a spherical one. A diamond-pointed tipped indenter with an equilateral triangular base (Berkovich indenter) is the most common indenter used. Fig. 3 represents typical load and displacement data from a pointed indentation test.

Fig. 3

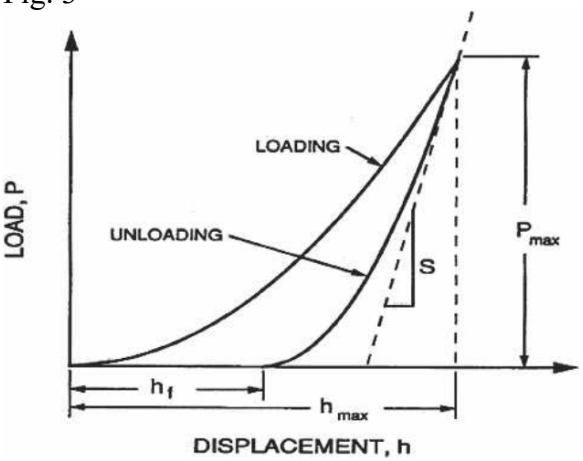

Fig. 3 – Force displacement curve generated with a pointed Berkovic indenter. Schematic representation of load versus indenter displacement data for an indentation experiment. The quantities shown are P_{max}: the peak indentation load; h_{max}: the indenter displacement at peak load; h_f: the final depth of the contact impression after unloading; and S: the initial unloading stiffness. (Oliver-Pharr 1992).

The principal parameters assessed with the nanoindentation technique are the Young modulus and the hardness (Vickers and/or Martens). Hardness and elastic modulus are measured as a function of indenter penetration depth and from the elastic recovery upon unloading. The easiest and most used method, even at the nano-scale, to evaluate these mechanical characteristics is the one by Oliver and Pharr, that can be applied to a large variety of indenters as long as they feature a regular geometry (Oliver-Pharr 1992).

Hardness can be defined as the measure of the resistance of a given material to permanent (plastic) deformation. Hardness is calculated with the formula:

$$H_{IT} = \frac{F_{max}}{A_r}$$

where *Fmax* is the maximum load and *Ar* represents the area of indentation. Indentation hardness H_{IT} can be then converted in Vickers hardness, H_V, with the formula:

$$HV = 1.854 * \frac{F}{D2}$$

where *F* is the applied load, measured in kilograms-force, and *D2* is the imprint area, measured in square millimetres.

Martens Hardness (HM) is calculated as the ratio between the applied force and the superficial area of the indenter (As) below the point of first contact. The value is expressed in GPa with the following formula:

$$HM = \frac{F}{A_s}$$

The elastic modulus, or Young modulus, is defined as the ratio between the applied strain and the consequent deformation under uniaxial load condition and it is normally measured in Pascal (N/m^2).

In the method introduced by Oliver and Pharr, the indentation modulus (E_{IT}) is calculated based on the slope of the unloading curve from the load displacement data:

$$S = \frac{dP}{dh}$$

From this value, the Young modulus (E) can then be determined with the present formula:

$$E_{IT} = \frac{E_i}{1 - v_i^2} + \frac{E}{1 - v^2}$$

Where v and E are the Poisson's coefficient and the Young's modulus of the material, respectively. Instead, v_i and E_i are the Poisson's coefficient and the Young's modulus of the diamond indenter.

As previously anticipated, nanoindentation technique has massively contributed to reveal the significance of dentinal microstructure on its mechanical behaviour. By allowing the separate assessment of the morphological components of the tissue, it has demonstrated the structural dependence of dentin mechanical properties. However, due to the influence of the dentinal microstructural variations on the mechanical properties measured, investigations with nanoindentation systems have to consider important issues such as the age of the tooth (young teeth have less mineral content and have bigger tubules), testing sites (dentin near the pulp has lower mechanical properties due to the increased tubule numbers and diameter) (Pashley 1985), less mineralized intertubular dentin (Kinney 1996), the decreased thickness of the highly mineralized peritubular cuff (Arends 1989), the direction of the load (whether it is perpendicular or parallel to tubules) (Poolthong 1998), and whether the test is performed on hydrated or dehydrated dentin (Angker 2004).

1.2 Ageing and endodontic treatment-related modification of Dentin

1.2.1 Aging of Dentin: microstructural, mechanical and chemical changes

Within the field of dentistry, the importance of ageing has become of greater interest in recent years due to its impact on the practice of restorative dentistry.
One of the most evident changes that occur in the microstructure of the dentinal tissue with ageing, is the reduction in tubule diameter due to the deposition of minerals within the lumens (Fig. 4 and 5). This process begins in the third decade of life and results in an increase in mineralization (Ten Cate 2008). As soon as the majority of tubule lumens have been filled, the tissue becomes transparent and is commonly known as "sclerotic." Transparency occurs when the tubule lumens become filled with minerals, decreasing the amount of light scatter off of the lumens. Specifically, the tubules are filled with a mineral phase; this filling is most likely a passive chemical precipitation (Natusch 1989). It has been reported that the mechanical properties of the deposits that fill the tubule are between the values reported for the peritubular and intertubular dentin. This suggests that the deposits may be a somewhat less dense form of mineral that forms by precipitation of apatite on the peritubular dentin (Balooch 2001).

Fig. 4

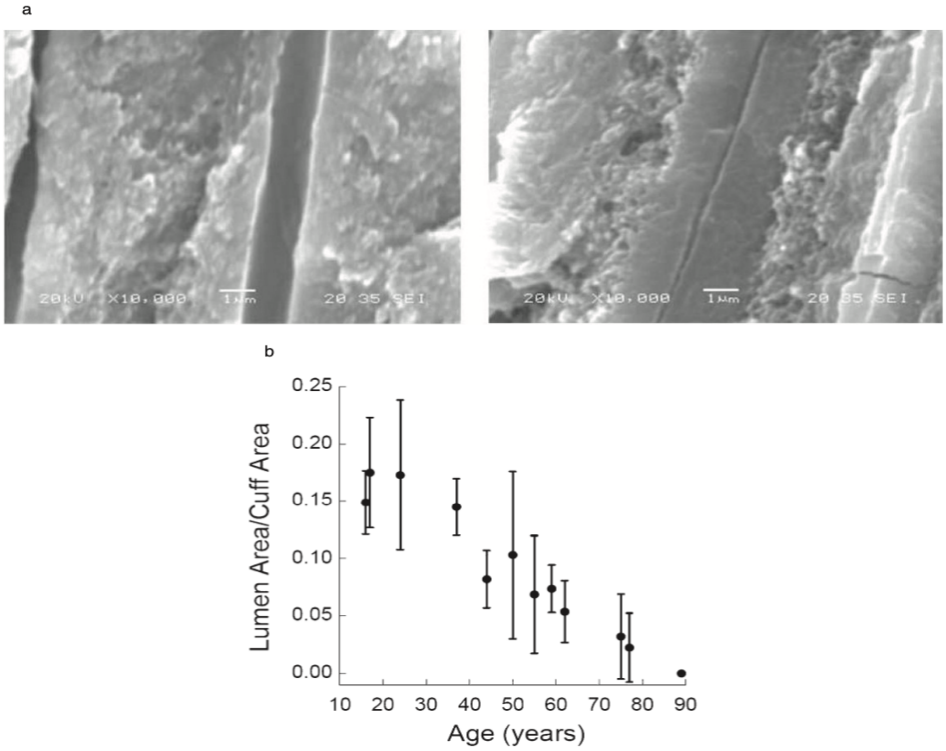

Fig. 4. Age-related changes of the microstructure of dentin. (a) SEM micrographs showing the lumen size of third molars coronal dentin of young (left; age = 20) and old (right; age = 67) patients. (b) Modification of average lumen size in adult human third molars. (Arola 2012).

Fig. 5

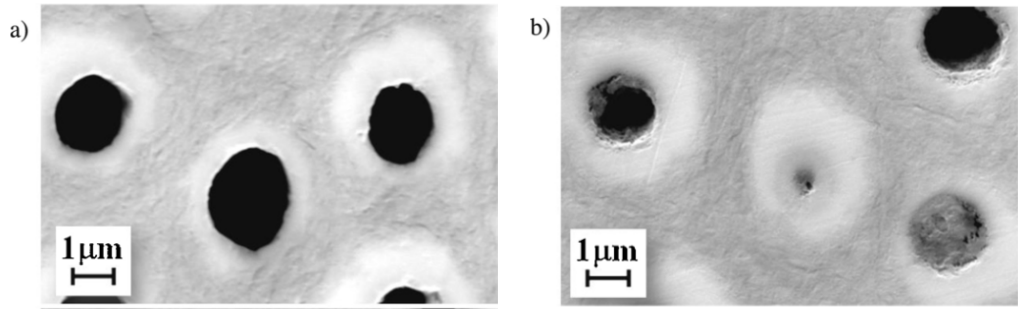

Fig. 5. Micrographs of dentinal tubules from outer dentin. (a) Young donor; (b) old donor. (Montoya 2015).

Besides the increased amount of mineral content, another important change is represented by the size of the mineral crystallites. They are smaller in transparent dentin than in normal dentin. For this phenomenon, two possible explanations have been suggested. First, a smaller mean size might result from the dissolution of minerals from the intertubular matrix into the tubule lumens. On the other hand, a reduction of the mean crystallite size could also be caused by precipitation of new, but smaller, crystallites within the matrix (Kinney 2005).

Dentinal sclerosis has an apico-coronal progression (Micheletti 1998). The total obliteration of tubules for coronal dentin might occur near the age of 70 (Tronstad, 2008). Even though sclerosis begings in the root, it is in the deep coronal dentin that the lowest relative collagen content and highest mineral to collagen ratios can be appreciated. In fact, there is a trend towards a decreasing difference in mineral concentration between normal and transparent dentin with approach to the root apex (Kinney 2005).

Macroscopically, a significant hypermineralization of the pulpal region compared to the outer region, between transparent and normal dentin, has also been observed (Fig. 6) (Kinney 2005). It is likely that the high density of filled tubule lumens in dentin near the pulpal tissue can explain much of this hypermineralization, although additional accretion of mineral into the intertubular matrix can not be excluded.

Fig. 6

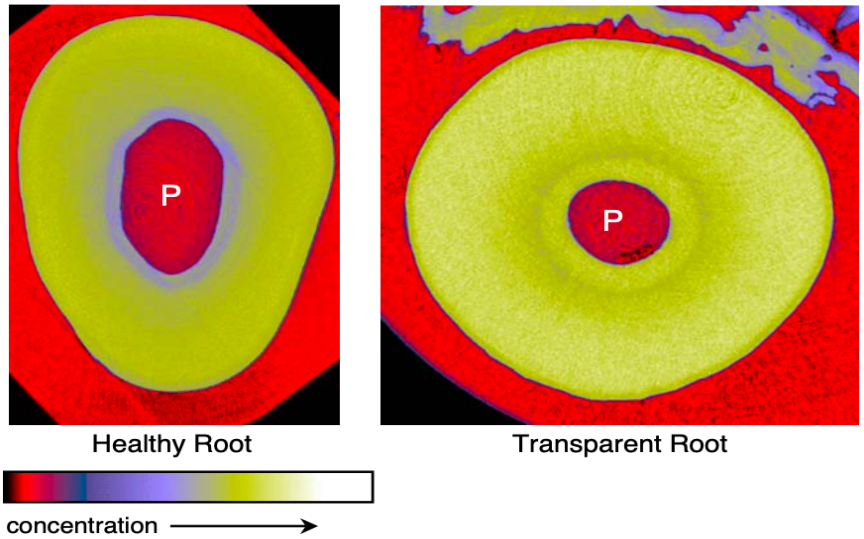

Fig. 6. Two-dimensional X-ray tomographic slices for normal and transparent root dentin. The pulp is indicated with a ‘‘P’’ in both examples. The colour scale for mineral concentration is shown at the bottom. The region immediately surrounding the pulp of normal dentin is less mineralized than the interior dentin. In contrast, the dentin near the pulp of the transparent root is hypermineralized to the extent that the mineral concentration is actually greater than the surrounding interior dentin (From Kinney 2005).

In the radicular region, sclerosis begins in the outer portion of tubules (Thomas 1994) and continues toward the pulp. Moreover, these modifications start in the mesial and distal sides of the root and then progress toward the lingual and buccal areas. Within the sclerotic tissue, the amount of remaining hollow lumens keeps decreasing with age (Schroeder 1993).

Thus, considering the type of the deposited matter within the lumens, dentin ageing leads to an increase in mineral content (Porter 2005). In general, older teeth have a higher mineral-to-collagen ratio compared to young ones and this also accounts for an increased hardness (Montoya 2005). However, the increased mineralization associated with transparency appears not only to be a result of the filling of the tubule lumens or changes in the mineralization of the peri-tubular cuff (Balooch 2001). Additional alterations may also take place in the mineralization of the intertubular dentin matrix (Giachetti 2002). As a matter of fact, whatever mechanism causes the deposition within the lumens might also lead to nucleation within the intertubular dentin matrix. However, a passive precipitation within the intertubular dentin matrix may not lead to any changes in the elastic properties (Kinney-Habelitz 2003). Whether there is dissolution or passive precipitation, the alterations would have to be small, since prior studies were unable to detect changes in the net mineral concentration within the intertubular matrix (Weber 1974).

Nevertheless, within the intertubular matrix, important modifications take place in the collagenous microstructure. Changes in the collagen matrix, as the cross-linking of collagen, may contribute to the structural response.

Type I collagen, once synthesised, undergoes extensive post-translational modifications, resulting in a characteristic pattern of cross-links, which are tissue-specific rather than collagen type-specific (Robins 2007). These modifications of the collagenous matrix are important for both its structural and mechanical properties, evidenced by the severe dysfunction of the tissue when cross-linking is disrupted (Paschalis 2011). To date, the known primary naturally occurring cross-

links in collagen are dehydrodihydroxylysinonorleucine (deH-DHLNL), dehydrohydroxylysinonorleucine (deH-HLNL), pyridinoline (PYD) and deoxypyridinoline (DPD; lysyl analog of PYD). The first two are NaBH4-reducible (their reduced forms are referred to as DHLNL and HLNL, respectively), while the others are non-reducible compounds (Robins 2007).

PYD, discovered by Fujimoto et al. (Fujimoto 1977), in tendon collagen, is thought to be the main maturation product of the reducible crosslinks (Eyre 1980). This non-reducible crosslink may form spontaneously in fibrils by interaction between two reducible crosslinks (Eyre 1980). The kinetics of relatively slow crosslinking reactions would seem best studied in tissues with neglectable turnover of collagen, as dentin (Bhaskar1980). Therefore, analysis of dentin at different ages is a useful index of the rate of maturation of hydroxylysine-based crosslinks in collagen fibrils. It has been shown that, in human dentin, DPD crosslinks increased with age and became the predominant crosslinks while the two reducible residues, deH-DHLNL and eH-HLNL, diminished (Walters 1983). In cartilage collagen the natural rate of disappearance of reducible crosslinks and appearance of DPD crosslinks is much faster than in bone or dentin (Eyre 1981). Possibly, the mineral deposited within and around collagen fibrils of calcified tissues inhibits the spontaneous crosslinking reactions, preserving some of the reducible crosslinks from further chemical change.

The content of PYD, in mineralized tissues, can be assessed by Raman microspectroscopic analysis (Gamsjaeger 2017). Indeed, the identification of an amide I underlying peak ~1660 cm^{-1} in Raman spectra signifies the presence of PYD collagen cross-links in mineralized tissue samples. Moreover, the area underlying 1660 cm^{-1} peak significantly correlates with PYD content, and may, therefore, be used in the analysis of hard tissues by Raman microspectroscopy to describe the spatial distribution of PYD cross-link content (Gamsjaeger 2017).

As minerals and collagen are considered the hard and tough counterparts of the tissue (Marshall 1997), the ageing of the dentinal substrate necessarily implies changes to its mechanical behaviour (Arola 2007). Several studies have been performed toward understanding the influence of ageing on the mechanical behaviour of dentin. As said, the mineral concentration increases as dentin ages, due to the decreased organic content and deposition of minerals in the dentinal tubules, whereas the crystal size decreases. However, the elastic modulus and hardness seem not to significantly change (Kinney 2005, Zheng 2005). Zheng et al. (Zheng 2005) analysed the changes in hardness and Young's modulus of dentin with ageing and reported that dentin does not undergo a significant change in hardness or Young's modulus with age in the middle and inner dentin. However, they also found an increase of 16% in hardness and around 5% in Young's modulus within the outer dentin. Nevertheless, overall, coronal dentin becomes more brittle with mineral deposition. Montoya showed significantly higher hardness for old inner and outer dentin compared to the younger one (Montoya 2015). This transition causes a significant decrease of its buffer capacity, the consequence of which is a drecrease in the strength and energy required to fracture (Arola 2005). More specifically, in the coronal portion, a decrease in strength of nearly 20 MPa each 10 years of life, that begins once adulthood is reached, can be measured (Fig. 7a). A decrease of 50% in strength and a 75% in energy required to fracture in old (aged 55) compared to young dentin (aged 30) can be detected. Moreover, it appears that

there is also a reduction in the fatigue strength of dentin with age (Kinney 2005). In a study, limited to tissues from the crowns of third molars, the old dentin exhibited significantly lower fatigue life regardless of the magnitude of cyclic stress (Fig. 7b).

Fig. 7

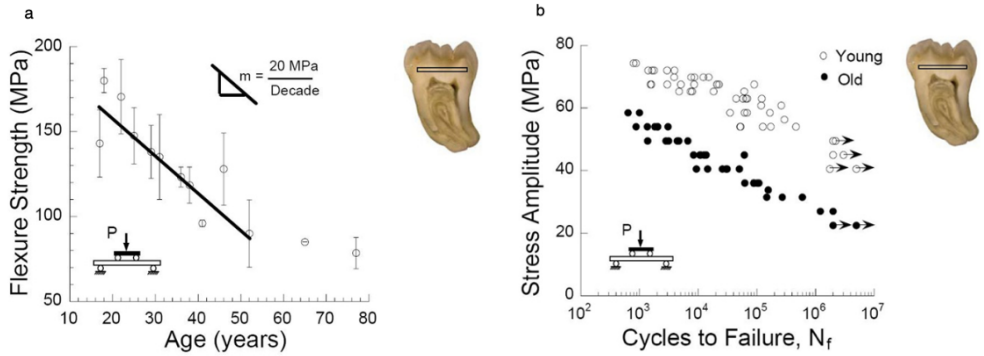

Fig. 7. Influence of patient age on coronal dentin strength. (a) Distribution of flexural strength in coronal dentin. A reduction of 20 MPa per decade of life can be noticed after adulthood. (b) Distribution of stress amplitude in coronal dentin of young and old patients. (Arola 2005).

Nonetheless, there is no evidence that the filling of the lumen is the only reason of these mechanical modifications. The dentin matrix may be another important factor. As a matter of fact, these modifications could also result from the age-related cross-linking of the collagen fibres (Miguez 2004).
Furthermore, dentin modifications due to endodontic treatment pulp removal could contribute to dentin sclerosis. A study, comparing the degree of apical translucency of vital versus root canal treated teeth, showed that, regardless of age, sclerotic dentin is more abundant in endodontically treated teeth (Thomas 1994). Despite these interesting findings, it must be pointed out that the greater level of sclerosis may take place before root canal treatment and be a result of pulp pathology and the related response of the odontoblastic processes.

1.2.2 Endodontic treatment-related modification of dentin.

It is generally accepted that root canal treated teeth are more fragile (Helfer 1972). Removing the pulp may contribute to dehydration and embrittlement of the dentinal tissue. However, it is not so clear that there are significant differences between vital and root canal treated teeth in terms of hydration (Papa 1994). An important aspect to take into account is rather the role of endodontic irrigants on chemical impoverishment of dentin and its influence on the physical properties. In endodontics, a broad variety of irrigants have been tested. NaOCl and EDTA are the most widely used (Fig. 8).

Fig. 8 – Word cloud representing irrigants used in endodontics

Fig. 8. From Dotto et al. (Dotto 2020). Word cloud representing the substances used in endodontics. The more a substance is used, the bigger it appears in the cloud.

Sodium hypochlorite (NaOCl) has the unique capacity to eradicate the complex intra-canal microbiota and to facilitate the removal of necrotic tissue and dentine debris from the root canal system. For this reason, NaOCl remains the most widely recommended irrigating solution in endodontics (Zehnder 2006). To date there is no consensus about the ideal NaOCl concentration. However, 0.5% to 5.25% solutions are the most widely used. Nonetheless, to maximise the tissue-dissolving and antimicrobial effects, hypochlorite solutions at higher concentration, even up to 10%, have been advocated (Matsumoto 1987). However, this concentration increase, not only affects the tissue dissolving and antimicrobial efficacy of the irrigant, but also its caustic potential (Baumgartner 1992, Hidalgo 2002). This happens because hypochlorite is a non-specific oxidising agent (Dychdala 1991). For instance, NaOCl is also commonly used to deproteinize hard tissues for biomedical applications (Johnson 2000). Sodium hypochlorite is known to fragment long peptide chains and to chlorinate protein terminal groups; the resulting N-chloramines are then broken down into other species (Davies 1993). As a consequence of the nonspecific action of NaOCl on organic matter, the properties of hypochlorite, which are desired in endodontics, appear to be mutually inter-linked with the untoward effects.
NaOCl interferes with the dentinal inorganic components too. Indeed, it dissolves magnesium and phosphate ions, while increasing the amount of dentinal carbonate (Sayin 2007, Tsuda 1996). In addition, it was reported that NaOCl significantly altered the Ca/P ratio of the root dentin surface (Dogan 2001). As the degree of dentin mineralization may affect the hardness profile of the dentin structure (Arends 1992), changes in mineral content after NaOCl treatment could account for changes in dentin micro-hardness.

The impact of NaOCl on the dentine matrix has received relatively little attention in the endodontic scientific community (Oyarzun 2002). According to literature, the mechanical properties of dentin may undergo detrimental changes as a result of irrigation with sodium hypochlorite and that it is attributed to degradation in the dentin matrix (Pascon 2009). For example, it appears that a 2-h exposure of dentine to a more than 3% NaOCl solution significantly decreases the elastic modulus and flexural strength of human dentine compared to physiological saline (Grigoratos

2001, Sim 2001). Marending et al. further demonstrated a concentration-dependent effect of NaOCl on mechanical dentin properties. This is the result of the disintegration of the collagen matrix while the inorganic component, apparently, is left intact (Marending 2017). The finding that hypochlorite does not alter the mineral content is in accordance with previous studies (Di Renzo 2001, Driscoll et 2002). However, there is also evidence that treatments with different concentrations of NaOCl can decrease root dentin microhardness (Ari 2004, Slutzky-Goldberg 2004, Oliveira 2007, Pascon 2009). Even a 1% NaOCl concentration is capable of altering dentin microhardness and 0.6% is enough to significantly affect the elastic modulus (Dotto 2020).

Thus, even though contrasting results are also present in literature (Machnick 2003), the evidence that NaOCl weakens dentin is quite strong (Marending 2017, Pascon 2009, Dotto 2020).

Chelating agents, such as different concentrations of EDTA, are also suggested to improve chemomechanical debridement of root canals by facilitating the removal of the inorganic components of the smear layer produced by the endodontic procedures (Hulsmann 2003). However, it is important to notice that the mineral phase in dentin has a protective effect on collagen. As explained in the previous sections, collagen fibrils, in dentin, are embedded in hydroxyapatite crystals. Therefore, also the demineralizing agents, used for the removal of the inorganic matter, can favour the destructive effect of NaOCl (Di Renzo 2001, Oyarzun 2002). Hoshi et al demonstrated that NaOCl exposure up to 48 h did not produce significant chemical modifications in the organic matter of human dentin, unless the specimen was previously demineralized (Hoshi 2001). Furthermore, it has been reported that EDTA can significantly reduce the root dentin microhardness (Cruz-Filho 2011, Ari 2004, Sayin 2007, Akcay 2012) and the modulus of elasticity (Machnick 2003, Wang 2017).

Chlorhexidine (CHX) is another irrigant that can be used in endodontics thanks to its broad antibacterial spectrum and a lower cytotoxicity compared to NaOCl (Fedorowicz 2012), even though it is not able to dissolve pulp tissue (Zhang 2010). It has been reported that CHX too, at concentrations of 0.2% and 2% can decrease the Ca and P levels and the microhardness of root dentin (Oliveira 2007, Ari 2005).

Moreover, intracanal medicaments have also been found to affect the apparent strength of teeth. For instance, while calcium hydroxide has been shown to decrease the ultimate strength of teeth (Andreasen 2002), mineral trioxide aggregate (Hatibovic-Kofman 2002) and micro- or nano-particulate SiO2-Na2O-CaO-P2O5 bioactive glass (Marending 2009) did not appear to negatively impact the flexural strength of root dentin.

While it appears evident the influence of intracanal irrigants, their concentration and time of application on the mechanical properties of dentin, in general, studies on the mechanical characteristics of root canal treated dentin, seem to produce conflicting findings (Dotto 2020). This could be explained by two important factors that are often disregarded. In the evaluation of root canal treated teeth, the period of time following pulp removal is often relatively short, or in cases where the teeth have received treatment *in situ* and are removed later, the duration of time after pulp

removal is unknown. Also, the age of the patient at the time of extraction is either not controlled or unknown.

In addition to the aforementioned chemical products used in primary root canal treatments, gutta-percha and sealer solvents are commonly used in orthograde endodontic retreatments. These chemical agents, by dissolving gutta-percha and sealers, accelerate the removal of endodontic filling material. However, solvents may change the physical and chemical properties of dentin. It has been reported that the use of chloroform, xylene, halothane and other products, during the treatment, significantly decrease dentin microhardness (Rotstein 1999, Butt 2013, Ferreira 2017, Ferreira 2021, Nalci 2021). Chloroform seems to have the worst effect on hardness, probably because of its better demineralizing effect, due to its lower pH (Nalci 2021). It has been speculated that the shift of mechanical properties may also be caused by alteration of the organic components and the consequent enlargement of the intercrystalline spaces that lead to increased porosity and permeability of the dentinal tissue (Nelci 2021). On the other hand, other authors found that chloroform and halothane did not affect dentin hardness or the content of calcium or phosphorus (Kaufman 1997, Erdemir 2003, Khedmat 2015).

Furthermore, solvent-induced alterations in the dentin surface might also affect dentin interaction with bonding materials (Macchi 1992, Erdemir-Eldeniz 2004, Muniz 2005).

1.3 Bonding to tooth structure as a game changer

The development of techniques and materials capable of bonding to tooth structure can be considered one of the biggest breakthroughs in dentistry. This revolution did not only mean the introduction of new materials and products, but rather a drastic paradigm shift in dentistry. Everything, from conceptualization to finalisation, had to consider new rules and innovative protocols. As a matter of fact, today, the approach to a preparation for a direct or indirect restoration does not aim at retentive parameters, as in the past. Adhesion, basically, made it possible to restore teeth without the need of retentive cavities, allowing clinicians to spare sound tissue and to consider new restorative strategies.

The journey toward adhesion began in the early fifties, when the very first attempt to develop an adhesive system was made (Hagger 1951). Thereafter, in 1955, Michael Bonocore conducted a series of studies treating enamel with 85% phosphoric acid for 30 seconds (s) in order to obtain an acid decalcification of its structure. The study, first of all, showed a remarkable surface area increase of the acid-etched enamel. Furthermore, the author placed a few resin drops on the etched area and proved how they kept unaltered much longer than those in contact with untreated enamel (Buonocore 1955). This paper is still considered one of the milestones of adhesive dentistry. The formation of resin interdigitations into the microporosities created by phosphoric acid dissolution of hydroxyapatite is still the basic mechanism that allows the micro-mechanical bonding of resin-based materials to enamel.
In 1958 Buonocore and Quigley reported an histological study of the adhesion area, where it was possible to detect, within the dentinal tissue, a 3-10 μm zone with

altered chromatic characteristics (Buonocore 1958). This transient zone, composed both by resin and dentinal tissue, will later be known as hybrid layer (HL) and considered the pillar of adhesion to dentin (Nakabayashi 1982). The first demonstrations of the presence of a HL between resin and dentin represented the first step in one of the most studied topics in dental research.

However, bonding to dentin is a more complicated procedure. As said, adhesion to dentin relies on the formation of the HL, a structure composed by demineralized collagen fibrils enveloped by a resin matrix (Nakabayashi 1982). Due to the relevant content of organic matter, mainly type I collagen, and water of this specific tissue, this procedure is not as straightforward as for enamel, that is almost entirely composed of hydroxyapatite (90 % vol), and strictly depends on the respect of operative protocols. Moreover, dentin is composed of a dense network of tubules that run from the pulp to the dentin-enamel junction (DEJ) and cementum-dentin junction (CDJ). These tubules, that contain odontoblastic processes, are delimited by peri-tubular dentin that is highly mineralized, while inter-tubular dentin (the portion of dentinal tissue between tubules) is far less mineralized. Therefore, for these reasons, dentin can be considered as a less homogeneous substrate than enamel, explaining the difference in bonding potential.
A thorough understanding of the structure and composition of dentin, and the changes that occur in its structure during adhesive procedures, is essential to appreciate the processes underlying the resin infiltration of this tissue.

1.3.1 Bonding systems and adhesion strategies to dentin

As resin monomers themselves are not capable of infiltrating mineralized tissues, traditionally, bonding systems consisted of an acid, primer and adhesive. These components can be found in separated bottles or all together, and can be carried in a single, two or three clinical application steps.
Several adhesive systems classifications have been proposed over the years. In 2003 Van Meerbeck et al. suggested one according to the way adhesives interact with the dental substrate, dividing them in two categories: etch and rinse (E&R) and self etc (SE) technique (Van Meerbeek 2003).

In the E&R approach, an acid, usually 30-40% phosphoric acid (H3PO4), is applied to the dentin surface and rinsed off, removing the smear layer, defined as the debris produced by cutting instruments, demineralizing the superficial dentin, and exposing the collagen matrix. Thus, a 5-10 μm deep mineral-free network of collagen suspended in rinse-water is created. Then, a resinous material, incorporated in a volatile liquid carrier, such as acetone or alcohol, is applied to the demineralized dentin. The carrier penetrates the moist dentin surface and carries the resinous material into the collagen matrix and dentinal tubules. The dentin is then air dried to evaporate the carrier, leaving the resinous material behind. The volatile liquid/resinous material is known as the primer. An unfilled or lightly filled resin is then applied to the dentin surface and light cured. This material, known as the adhesive, co-polymerizes with the resin already in the collagen matrix, locking it onto the dentin surface (Nakabayashi 1982), and providing a hydrophobic surface for co-polymerization with hydrophobic restorative resin materials. The resin infiltrated collagen matrix is commonly referred to as the hybrid layer. With most

products, the hybrid layer is between 2 and 5 μm in thickness (Van Meerbee2k 2003). To achieve a stable and long-lasting bond, ideally, the water contained in the mineral-free collagen matrix should be completely replaced by adhesive monomers (Van Meerbee2k 2003). However, a complete infiltration of monomers into the wet and demineralized dentin is difficult to achieve. This leads to incompletely infiltrated areas along the bottom of the HL made of denuded collagen fibrils surrounded by rinse-water (Frankerberger 2005). This has been confirmed by immunohistochemical labelling of the etched, resin-infiltrated dentin, after staining with anti-type I collagen antibodies. The study revealed a weak labelling of collagen fibrils at the top half of the HL, but an intense labelling in its deepest part (Breschi 2004). This means that resin envelopment of acid-etched collagen fibrils is more complete in the top half of the HL, rather than its bottom half.
To avoid an excessive dentin demineralization and the collapse of the collagenous fibrils it is mandatory not to etch the dentinal substrate for more than 15 s. A more extensive use of the etching would not allow resin monomers to infiltrate as deep as the front of demineralization (Mancuso 2021). This aspect is as important as the adequate etching water-rinsing and the subsequent moisture control. An extensive drying of the substrate may also lead to collagen collapse with consequent difficulties in resin diffusion (Stape 2021).

In the SE approach, also known as etch & dry, a separate acid-etching step is not required. Therefore, the smear layer is not removed, but rather partially dissolved. Thus, the smear layer becomes part of the adhesive interface (Perdigao 2021). In the SE 2 steps technique, etchant and primer are mixed in a one single product that does not require to be rinsed out after its application, but only dried. This step is then followed by a separate bonding application. Afterwards SE 1 step systems have been developed, featuring the acidic primer and resin together in a single solution. Regardless of the number of steps, with these products, the adhesive co-monomers, at the same time, demineralize and infiltrate the dentinal substrate, decreasing the discrepancy between the depth of demineralization and the depth of resin infiltration. Therefore, a more homogenous resin infiltration of demineralized collagen fibrils can be achieved when compared to E&R systems. Thus, the micro-mechanical retention takes place only in the first μm of the dentinal substrate, leaving behind the collagen fibrils protected by HA. Furthermore, these bonding systems feature the presence of functional monomers capable of chemically interacting with the HA present around the dentinal collagen fibrils. Therefore, the SE bonding mechanism can be considered both micro-mechanical and chemical (Saikaew 2022). Some authors reported a reduced amount of porosities, more homogenous resin infiltration and a better protection of collagen fibrils in SE adhesives compared to E&R technique (Nakabayashi 1996, Breschi 2003, Breschi 2004). Immunohistochemical labelling with anti-type I collagen antibodies confirmed these findings by presenting a weak, uniform gold labelling of the HL (Breschi 2004).

Nowadays, there is an ongoing trend, in adhesive dentistry, that tends to simplify bonding procedures by reducing application steps and shortening clinical application time. Therefore, new adhesives have been created, as the Universal Adhesives (UA). As these products present the bonding together with the etching and primer, they are also known as Self Adhesive (SA) systems and they are generally used in SE mode (self-etch 1 step). However they can also be used in

E&R mode and, one of the advantages is that, with these products, the moisture control is less critical than when using traditional E&R systems.
These adhesives are known as universal because they contain functional monomers, as the 10-Methacryloyloxydecyl dihydrogen phosphate (10-MDP), capable to chemically bond to dentin tissue, as well as metal, ceramic and composite (Nagarkar 2019). The incorporation of these functional monomers into the universal adhesives makes them different from the majority of the SE 1 step systems. To date, 10-MDP is considered the most effective monomer capable of forming ionic bonds with the hydroxyapatite (HA) of not-etched dentin and to create hydrolytically stable MDP-Ca salts. These new-formed salts, bonding to HA, maintain the collagen fibrils protected over time, thus preserving the integrity of the HL, that appear shallower, and contributing to the longevity of the adhesive interface (Yoshida 2000, Hanabusa 2012, Fehrenbach 2021). This chemical process is known as nanostratification or nanolayering and may be responsible for the good clinical performances of the adhesives containing 10-MDP (Sebold 2022).

1.3.2 Bonding to root canal dentin

The restoration of heavily compromised root canal treated (RCT) teeth, with no residual coronal structure appears to benefit from the insertion of a radicular post to enhance core-retention and resistance to flexural stresses (Mannocci 2021, Barreto 2016). In the past years, metal cast posts and cores have been routinely used (Mannocci 2021, Barreto 2016). However, thanks to the drastic improvement of adhesive technologies over years and the consequent need to reduce clinical invasiveness towards sound structure, adhesive metal-free posts increased their popularity (Barreto 2016, Goracci 2004). However, bonding to root canal dentin presents several drawbacks.

First of all, a root canal has a very unfavourable configuration factor (C-factor) that does not allow an ideal management of the polymerization shrinkage. When resin based materials polymerize, individual monomer molecules join to form chains that grow and intertwine, and the mass undergoes volumetric shrinkage (Feilzer 1988). Resinous materials shrink from 2 to 7%, depending on the volume occupied by filler particles (Feilzer 1988). The force of polymerization contraction often exceeds the bond strength of dentin adhesives to dentin, resulting in gap formation along the surfaces with the weakest bonds (De Munck 2005, Feilzer 1988). Separation often occurs within the hybrid layer (Feilzer 1989). As said, the root canal system has an unfavourable geometry for resin bonding (Tay 2005). A quantitative measure of the geometry of the cavity preparation for bonding is given by the Configuration factor or C-Factor, defined as the ratio of bonded to un-bonded resin surfaces (Carvalho 1996). The greater the percentage of unbonded surfaces, the less stress is placed on the bonded surfaces from polymerization contraction. As a matter of fact, the unbonded surfaces allow plastic deformation or flow within the resin mass during polymerization (Carvalho 1996). In the root canal system, the ratio might be 100:1 (Carvalho 1996). Virtually every dentin wall has an opposing wall and there are minimal unbonded surfaces. To better appreciate how unfavourable is the C-factor inside a root canal, it is important to point out that any ratio greater than 3:1 has been proven unfavourable for bonding (Yoshikawa 1999). Because of this unfavourable geometry, interfacial gaps are virtually always present in bonded endodontic posts (Goracci 2005), and gap formation seems to increase with time (Roulet 1994).

In addition to the unfavourable geometry, as just discussed, there are several other factors that make bonding in the root canal system a challenge.

First of all, another important aspect to take into account is the difficult moisture control and strict management of adhesive procedures inside such a deep and narrow space. The delivery of the acid, in the more traditional adhesive systems, the uniform application of a primer and the removal of its volatile carrier and the distribution of the adhesive may be difficult if not impossible to manage in the apical one-third of a radicular post-space. A flaw in one of these steps may be detrimental for the long term success of the adhesive interface. For instance, an incomplete removal of the primer solvent, (acetone or alcohol) can adversely affect the bond (Tay 2002). On the other hand, a careless management of the etching procedure may prevent adhesives from infiltrating dentinal tissue to the full depth of demineralization. This phenomenon is maybe the most important factor in the strength and stability of the resin-dentin interface. When this does not happen, fluid movement between the hybrid layer and non-infiltrated dentin accelerates the degradation of the bond (De Munck 2005). The presence of water in this area can cause hydrolysis and plasticizing of the interface components. Plasticization is a process in which fluids are absorbed by the resins, causing swelling and consequent degradation of their mechanical properties (De Munck 2005). Hydrolysis can break the covalent bonds within collagen fibrils and the resin polymers (Hashimoto 2000). This process is also enhanced by enzymes, metalloproteinases (MMPs), that are released by bacteria (Santerre 2001) and by dentin itself (Pashley 2004). MMPs are normally present in the dentinal tissue and are slowly released over time (Pashley 2004). However, when collagen fibrils are demineralized but not fully enveloped in resin, the activity of the MMPs increases and the collagen degradation is faster. MMPs are also released by bacteria, along with other enzymes (Pashley 2004, Santerre 2001), but bacteria are not necessary for collagen degradation to occur (Pashley 2004).

An additional issue to consider is the endodontic environment and, specifically, the substances that interact with the dentinal substrate troughout the root canal treatment. It has been previously discussed why and how abundantly NaOCl is employed in this type of treatment. However, it may cause problems when used right before adhesive resins. As it is a strong oxidising agent, it leaves behind an oxygen rich layer on the dentin surface that results in reduced bond strengths (Morris 2001, Lai 2001), and increased microleakage (Yiu 2002). A possible solution to this problem is the application of a reducing agent, such as ascorbic acid and sodium ascorbate, to the dentin surface after sodium hypochlorite irrigation, that is able to reverse the negative effects of sodium hypochlorite (Morris 2001, Yiu 2002). For the same reason, hydrogen peroxide may lead to the same problem (Erdemir 2004). However, oxygen is only one of the many substances that inhibit the polymerization of resins. Lubricant and Calcium-chelating agents (Ari 2003) and substances used to dissolve endodontic material, such as chloroform and halothane, may also cause significant loss of bond strength (Erdemir-Eldeniz 2004). Eugenol contained in endodontic sealers is another substance that inhibits the polymerization reaction of resins and can interfere with bonding (Macchi 1992, Muniz 2005). Concerns have also been raised about calcium hydroxide (CaOH), that is sometimes placed, as a medicament, in the root canal system, between appointments, for its antimicrobial properties. However, the complete removal of

CaOH paste from the root canal system, before obturation, is quite demanding (Sevimay 2004, Kim 2002). Therefore, concerns have been expressed that residual traces of calcium hydroxide could prevent effective bonding, as it can act as a physical barrier, and the high pH may also neutralise the acid primer in self-etching adhesives.

Moreover, the removal of the debris produced by the canal shaping procedures and the post-space preparation may be complicated in such narrow spaces (Perdigao 2007). This is an important issue, considering that effective dentin bonding requires a surface that is devoid of any debris. However, the effects of all the previously described substances and the possible presence of debris can be minimised if proper and careful cleansing procedures of the post space are followed. Such procedures may be carried out employing alcohol or a detergent, together with mechanical brushing, to remove any sign of sealer or trace of debris (Schwartz 2006).

Another barrier to an effective bonding is represented by the difficult penetration of the curing light in the deep portion of the post space that may not allow a proper conversion of the resin cement monomers (Schwartz 2006).

1.4 Specific aim

For all the aforementioned reasons, root canal treated (RCT) dentin is far from being an ideal substrate for adhesive techniques (Maroulakos 2018). However, a limited amount of research has been published about bonding on radicular dentin. Most of the knowledge about adhesion to dentin has been published in the restorative field and relates to coronal dentin.

Moreover, only a few studies assessed the bonding capacity of radicular dentin of root canal re-treated (RCR-T) teeth, and none of them used specimens with a history of natural ageing and function (Guedes 2014, Pelegrine 2016, Pereira 2019). Seemingly, while the correlation between the micro-morphology of the radicular resin-dentin interface (R-DI) and its bond strength have been already investigated in RCT teeth (Barreto 2016, Bitter 2014, Erik 2020, Pelozo 2019), a gap in the literature exists regarding RCR-T teeth, where no evaluations of the R-DI micro-morphology have been performed.

Therefore, the purpose of the first section of this in vitro study was to assess the bond strength and the micro-morphologic characteristics, in terms of HL thickness and number of resin-filled dentinal tubules, of the adhesive interface between root canal dentin and two resin cements (SERc and SARc) in root canal treated and naturally aged re-treated teeth. The chemical and structural characteristics of these two specific substrates were also assessed. As for the mechanical properties, Martens and Vickers hardness, modulus of elasticity and plastic deformation were evaluated via nanoindentation analysis. As for the chemical properties, the mineral to matrix ratio, crystallinity, carbonate to phosphate ratio, differences in collagen structure and quality, collagen organisation, and structural organisation and relative amount of collagen were evaluated via Raman spectroscopy.
The tested null hypothesis were that (1) the quality of the substrate (freshly endodontically treated and aged root canal re-treated dentin) has no influence, in

terms of bond strength and micro-morphology of the adhesive interface, on the adhesion of resin cements; (2) the topography of the substrate (coronal versus apical half of the root canal post-space) has no influence, in terms of bond strength and micro-morphology of the adhesive interface, on the adhesion of resin cements; (3) the type of resin cement (SERc versus SARc) has no influence on the bond strength and micro-morphology of the adhesive interface; (4) each of the aforementioned mechanical and chemical property was not influenced by the type of substrate (freshly vs aged root canal treated dentin); (5) each of the aforementioned mechanical and chemical property was not influenced by the topography of the root canal (coronal vs middle vs apical third).

The influence of one year ageing on the bond strength of the intra-canal R-DI of RCT and RCR-T teeth was also assessed. The tested null hypothesis were that (1) after one year ageing the quality of the substrate (freshly endodontically treated and aged root canal re-treated dentin) has no influence, in terms of bond strength on the adhesion of resin cements; (2) after one year ageing the topography of the substrate (coronal versus apical half of the root canal post-space) has no influence, in terms of bond strength on the adhesion of resin cements; (3) after one year ageing the type of resin cement (SERc versus SARc) has no influence on the bond strength of the adhesive interface; (4) ageing does not affect the bond strength in RCT and RCR-T teeth.

A parallel investigation aimed at assessing the influence of donor age (over 60 vs under 20) on the bond strength of the adhesive interface between root canal dentin and two resin cements (SERc and SARc) in RCT teeth. The tested null hypotheses were: (1) the age of the patient has no influence on the bonding potential of root dentin; (2) SERc and SARc behave the same, in terms of bond strength, regardless of the age of the patient; (3) topography of the substrate (coronal versus apical half of the root canal post-space), regardless of the age of the patient, does not influence the intra-canal bond strength.

Materials and methods

2.1 Bond strength and morphological analysis of the radicular dentin adhesive interface: Freshly *vs* aged root canal treated radicular dentin.

2.1.1 Study design

The general description of the main materials used in this section of the study, their manufacturers and composition are listed in Table 1. This study was designed in 4 study groups, where the specimens were randomly allocated (www.randomizer.org) considering:

1) Status of the specimens: a) extracted vital teeth and b) teeth that were root canal treated at least 15 years earlier were collected to prepare samples for push-out, at time 0 (T0) and after 1 year ageing (T1), and confocal laser microscopy at T0;
2) Adhesive approach performed for fibre post cementation: a) Self-etch approach (Clearfil Universal Bond Quick + Clearfil DC Core Plus, Kuraray Noritake; Okayama, Japan) and b) self-adhesive approach (iCEM, Kulzer, Hanau, Germany).

2.1.2 Sample preparation

Permanent straight single rooted vital and root canal treated human teeth of similar length and anatomy, extracted for periodontal reason in patients aged between 45 and 55, were collected in accordance with the local ethics committee (Protocol number CS2/0187). A sample size of 16 per group was calculated with G*Power 3.1.4 (Kiel University, Kiel, Germany) considering alpha-error = 0.05 and ß = 0.95. For the endodontically treated teeth, specimens were collected only when precise information on the timing of the former endodontic treatment was present, and only teeth treated at least 15 years earlier were included in the study. Moreover, for the same group, teeth that did not have gutta-percha filling were also excluded from the study. Roots with cracks, resorption or immature apices were discarded. After debriding the root surface, specimens were stored in 0,1% thymol at 4°C. Roots were sectioned, perpendicularly to the long axis of the tooth, at the level of the cemento-enamel junction (CEJ) with a low-speed diamond saw (Isomet 1000, Buehler, Lake Bluff, IL), to obtain a root length of 15 mm. Teeth with oval-shaped canals were excluded.
For the vital extracted teeth, manual scouting and mechanical glide-path were obtained, respectively, with #8-10 K-Files (Dentsply Sirona, Ballantyne, NC, USA) and ProGlider (Dentsply Sirona) up to full working length (WL). WL was recorded when the file tip became visible at the apical foramen under 10X magnification (Pro Ergo; Carl Zeiss, Oberkochen, Germany). Final shaping was performed with ProTaper Next X1, X2 and X3 (Dentsply Sirona). Throughout the shaping

procedures, root canals were irrigated with 10% ethylenediamine tetraacetic acid (EDTA) (Tubuliclean; Ogna, Muggiò, Italy) alternated with 5% sodium hypochlorite (NaOCl) (Niclor 5; Ogna) delivered with a 2-mL syringe and a 22-gauge needle. In RCT specimens, gutta-percha was removed with the aid of a D-limonene and 1,2 dichloropropane-based solvent (G.P.R.; Ogna, Muggiò, Italy) and shaping procedures were carried out as previously reported. Root canals were then dried with sterile paper points and sealed with dedicated gutta-percha points (ProTaper Next conform fit; Dentsply Sirona) and endodontic cement (Pulp Canal Sealer EWT; Kerr, Sybron, Romulus, MI, USA) warm vertically compacted. Specimens were then stored in 100% humidity at 37° C.
Forty-eight hours (h) later 10 mm of coronal gutta-percha were removed with D.T. Light Post Universal and Finishing Drill #1 (VDW, Munich, Germany). Post spaces were thoroughly cleaned with 5mL of distilled water delivered with a 22-gauge needle. Tapered fibre posts (D.T. Light Post #1; VDW) were tried inside the canal to ensure they could reach the desired length without binding to root canal walls. RCT and RCR-T specimens were then randomly allocated in two groups according to the adhesive protocol used to cement the fibre posts (Table1). Cements were light-cured 60 s after insertion to allow their chemical setting. Light curing was performed using a LED lamp (Valo, Ultradent, USA) leaned against the post-head to standardise its distance from the root canal. After cementation the samples were stored for 24 h in 100% humidity at 37° C.

2.1.3 Push-out bond strength analysis and failure mode evaluation

Each specimen was then sectioned perpendicularly to its long axis with a 0.35 mm diamond saw (Micromet; Remet, Bologna, Italy) at slow speed with water cooling to obtain 6 slices (3 coronal and 3 apical), generating eight sub-groups (Table 2). The slices of the specimens that underwent ageing were kept immersed in artificial saliva and stored in an incubator at 38° C for one year (T1) prior to being submitted to a push-out test.
The push-out test was performed by applying an axial load (apical to coronal) to the post at a crosshead speed of 0.5 mm min^{-1} using an Instron Machine I (model 10/D; Sintech, MTS, Canton, MA, USA). The maximum failure load was recorded in Newtons (N). Push-out bond strength was calculated in megapascal (MPa) by dividing the failure load (N) by the area of the bonded interface (SL) estimated from the formula for calculating the lateral surface area of a truncated cone: $SL=\pi (R + r) [h^2 + (R - r)^2]^{0.5}$, where π represents the constant (3.14), R is the coronal post radius, r is the apical post radius and h is the slice thickness. The latter was measured using a digital calliper, while the radii were calculated with ImageJ 1.35 S software (National Institutes of Health, Bethesda, MD, USA) from photographs taken with a stereomicroscope (Discovery V 12, Carl Zeiss).

Material	Type	Composition	Post Treatment [manufacturer instruction (MI)]	Post Space Treatment (MI)
Clearfil Universal Bond Quick (C Universal bond) (bond) + Clearfil DC Core Plus (Clearfil DC) (paste) (Kuraray Noritake; Okayama, Japan).	Self-etch (SE)	Bond: Bis-GMA (10–25%), ethanol (10–25%), HEMA (2.5–10%), 10-MDP, hydrophilic amide monomer, colloidal silica, silane coupling agent, sodium fluoride, camphorquinone, water. Paste: Bis-GMA, TEGDMA, hydrophobic/hydrophilic aliphatic dimethacrylate, hydrophobic aromatic dimethacrylate, silanated barium glass filler, silanated colloidal silica, colloidal silica, aluminium oxide filler, CQ, accelerator, initiator.	Apply phosphoric acid [5 seconds (s)]; rinse and dry; apply bond, then dry by blowing mild air.	SE mode: apply bond with a rubbing motion (no waiting time); dry by blowing mild air and paper point until bond does not move; LED light cure (10 s); squeeze paste; insert the post and light cure paste (20 s).
iCEM (Kulzer, Hanau, Germany).	Self-adhesive (SA)	Acidified urethane and di-, tri-, multifunctional acrylate monomers; 41% filler by weight.	None.	Rinse the post space with water; dry with mild air and paper points, without over-drying; squeeze paste; insert the post and light cure paste (40 s).

Table 1 - Materials and techniques employed for fibre post cementation

Table 2

Treatment	Cement	Area	Sub-groups
RCT (n=16)	iCEM (n=8)	coronal (n=24)	TIC
		apical (n=24)	TIA
	Clearfil DC (n=8)	coronal (n=24)	TDC
		apical (n=24)	TDA
RCR-T (n=16)	iCEM (n=8)	coronal (n=24)	RIC
		apical (n=24)	RIA
	Clearfil DC (n=8)	coronal (n=24)	RDC
		apical (n=24)	RDA

Table 2 - Specimen sorted by type of endodontic treatment, luting cement and area of the post space. TIC (treatment-iCEM-coronal) group; (B) (treatment-iCEM-apical) group; TDC (treatment-Clearfil DC-coronal) group; TDA (treatment-Clearfil DC-apical) group; RIC group (re-treatment-iCEM-coronal); RIA (re-treatment-iCEM-apical) group; RDC (re-treatment-Clearfil DC-coronal) group; RDA (re-treatment-Clearfil DC-apical) group. The same scheme has been replicated for specimens that underwent 1 year ageing (T1).

After the push-out test, all samples were analysed with the stereomicroscope at 40X magnification by a single trained operator, to assess the type of failure. Failures were classified as follows: A, adhesive failure between dentin and resin cement; C, cohesive failure within resin cement, and M, mixed failures including cement and the dentin-cement interface. As adhesive failure between post and resin cement and cohesive failures within the post or within the dentin did not occur, they were not included in the classification. Within each group, failures were expressed as percentages.

2.1.4 Micro-morphologic evaluation of the adhesive interface through CLSM analysis

Four permanent vital and four root canal treated human first maxillary molars were selected as previously described. Specimens were endodontically treated and re-treated as shown before. The same materials and procedures were employed to cement fibre posts. Specifically, fibre posts were luted with Clearfil DC in the palatal canal and with iCEM in the disto-buccal canal of each specimen. Before insertion of the posts, the adhesive system (C Universal Bond) was labelled with 0.1% fluorescein (FNa; Sigma Aldrich, Steinheim, Germany) and the resin cements (Clearfil DC and iCEM) were labelled with 0.1% rhodamine isothiocyanate (RITC; Sigma Aldrich, St Louis, MO, USA). Both the dyes were added, by means of a simple mixing process, in a ratio of 0.1%. To perform the analysis, the roots were perpendicularly sectioned, below the CEJ into slices of less than 1 mm thickness. The slices were, then, sorted in coronal and apical to obtain 6 slices (3 coronal and 3 apical), generating the same eight sub-groups as seen in table 2. The slices were then positioned, with the coronal side upward, on a microscope slide for grinding and polishing. To this purpose, a series of silicon carbide abrasive papers (1200, 2400, 4000 grit) using running tap water as a lubricant, have been employed. The samples were kept humid during the whole study.
Confocal laser scanning microscopy (CLSM) imaging was performed using a Leica SP8 confocal system (Leica Microsystems) equipped with an argon ion and 561 nm DPSS lasers. Samples were imaged using a HCX PL APO 40x/1.25 NA oil immersion objective. Series of x-y-z images (typically 0.145*0.145*1 μm^3 voxel size) were collected. Laser power and detector gain were set on the control sample

and kept the same for all conditions of the experiment. Images were recorded at a random area of each sample.
Images were analysed with ImageJ 1.35 S software. Specifically, the thickness of the HL was recorded at four randomly chosen locations and a mean value was obtained. The number of dentinal tubules penetrated by resin cements and adhesive were counted.

2.1.5 Bond strength and morphological analysis of the radicular dentin bonding interface - Statistical analysis

After ascertaining the normality (Shapiro-Wilk test) and homoscedastic (modified Levene test) assumptions of the data sets, the radicular bond strength data were analysed with three-way analysis of variance to examine the effects of the age of the endodontic treatment (new treatment/re-treatment), cement employed and root area and the interaction of those three factors on micro push-out bond strength. The effect of one year ageing on push-out bond strength was also evaluated. Post-hoc pairwise comparisons were performed using the Tukey test. Chi-square test was used to analyse differences in the failure modes.
Evaluation of the data obtained from confocal microscope was performed comparing the samples of the different groups and subgroups with respect to the morphologic characteristics (hybrid layer thickness, number of tags penetrated with adhesive and resin cement) and calculating the mean values for each slice followed by statistical analysis using the three-way Anova test and Tukey post hoc analyses. For all tests, statistical significance was pre-set at $\alpha=0.05$. All statistical analyses were performed using Stata 12.0 (StataCorp, College Station, Texas, USA).

2.2 Nanoindentation analysis of radicular dentin

The samples used for the push-out test at T0 were also employed to assess the mechanical properties of RCT and RCR-T via nanoindentation analysis. More precisely, for each specimen, the first, the third and the fifth slice were selected, representative, respectively, of the coronal, middle and apical third of the post-space. All slices were embedded in acrylic resin (Technovit 4071, Heraeus Kulzer, Hanau, Germany) with the coronal side upwards and polished with ascending sandpapers till 2400 grit.

On each slice four areas of the dentinal tissue were identified: mesial, distal, buccal and lingual to the root canal, at a distance of 300 μm from the pulp-dentin interface. Each area was at least 1 mm^2 large. Twenty-five indentations were performed for each area, for a total of one hundred indentations for each slice.

Tests were carried out with the Nanoindenter FISCHERSCOPE HM2000 (Helmut Fischer, Dortmund, Germany), equipped with a diamond Vickers indenter (Indenter Vickers V DIA H2N) and characterised by a theoretical force resolution of ≤ 150 nN and a theoretical displacement resolution of ≤ 10 pm. The shape and hardness correction of the indenter were calibrated, through a built-in procedure in the software of the indenter, with a certified, traceable calibration standard, the BK7 glass. Input curve, represented in Fig. 9, is characterised by loading and unloading phases imposing a loading and unloading rate of 15mN/s. Before the onset of the

unloading step the maximum load value, reached in correspondence of the set nanoindentation depth, was held for 5 s.
Tests were performed in displacement control imposing a maximum value of indentation depth of 2000 nm.
The loading-displacement curves were then analysed by using the Oliver-Pharr method (Oliver-Pharr, 2004) in order to quantify the plastic and elastic micromechanical properties through the nanoindentation hardness and modulus.

Fig. 9

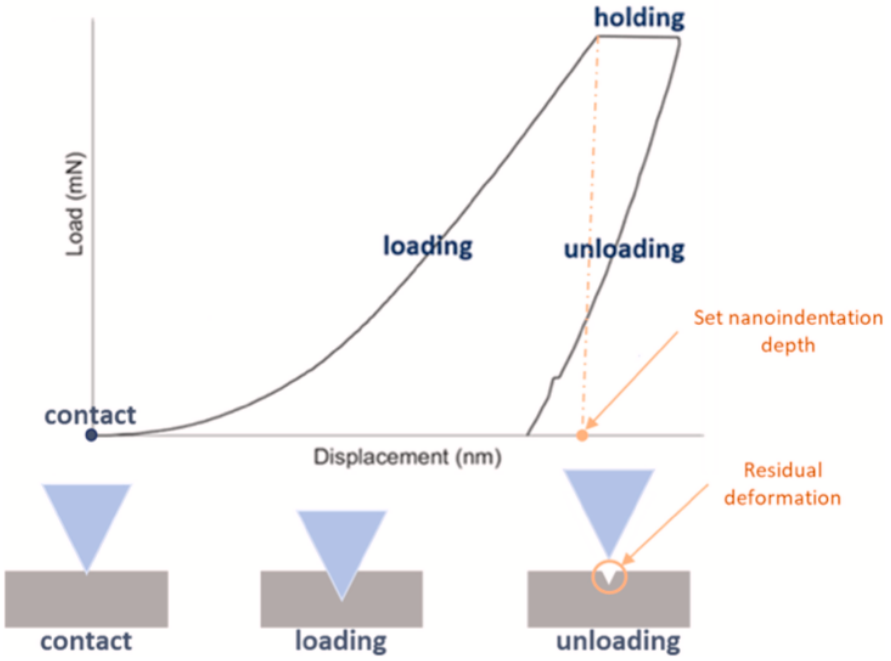

Fig. 9 - Scheme of the main phases of a nanoindentation test. Contact point: the set threshold is reached and the test starts; loading phase: the tip penetrates the sample until the set nanoindentation depth is reached; holding phase: the load reached in correspondence of the set nanoindentation depth is held for 30 s; unloading phase: the tip is subtracted from the sample. Image taken from Serino et al. (Serino2022).

2.2.1 Nanoindentation analysis of radicular dentin - Statistical analysis

The independent variables analysed were the nanoindentation modulus, nanoindentation hardness (Vickers and Martens) and the percentage of plasticization. For each slice 100 indentation points were selected on radicular dentin, performing 4 matrixes (5 x 5) in 4 different areas, that is 300 indentation per sample or rather 4800 indentations for each group. The outliers were identified and discarded through the modified Thompson Tau Test. Since the assumption of the normality of the data was violated, the obtained data were analysed through the Kruskal-Wallis test, a non-parametric test, with a significance level of 1%.

2.3 Raman spectroscopy for the analysis of radicular dentin

The same samples that underwent the mechanical analysis via nanoindentation test were also analysed with Raman spectroscopy, within 7 days, to assess the chemical properties. Between the two analyses the specimens were kept in 100% humidity.

Raman spectroscopy measurements were performed directly on the tooth surface, employing a portable modular spectrometer manufactured by BWTEK. The instrument is equipped with a monochromatic laser (λ: 1064 nm) and a BTC284N spectrometer (measurement range: from 100 cm^{-1} to 2500 cm^{-1} with resolution equal to 10.56 cm^{-1}) coupled with a CCD sensor. The instrument is connected to a compact microscope (BAC151) in order to allow the observation of the area under analysis and to focus the beam on the surface.

The following parameters were employed: laser power of 225 mW, integration time of 60 s, 12 repetitions for each area, 80× lens (analysed area with diameter of about 20 μm). For each sample, two measurements on two different points of the enamel surface were collected.

Raman spectra were collected to evaluate the intensity of the main bands at ~960 cm^{-1}, ~1070 cm^{-1}, ~1246 cm^{-1}, ~1450 cm^{-1} and ~1655 cm^{-1}. Indeed, regarding dentin, the main Raman bands are assigned to phosphates (PO_4^{3-}, 960 cm^{-1}), and carbonate (CO_3^{2-}, 1070 cm^{-1}) groups. The latter band is associated with the carbonate substitution in the hydroxyapatite lattice: carbonate can replace phosphate groups in the hydroxyapatite structure, and the Raman band at 1070 cm^{-1} corresponds to the symmetric stretching mode (ν_1) of CO_3^{2-} ions within the apatite lattice. Carbonate substitution affects HA in terms of solubility and reactivity. Changes in the intensities of these bands with respect to the ones in healthy enamel are correlated to the presence of carious lesions and they are attributed to demineralization-induced decrease of crystallinity. In addition, in Raman dentin spectra the bands at 1655 cm^{-1}, 1450 cm^{-1}, and 1246 cm^{-1} are attributable to the organic matrix and in particular to amide I, CH_2, and amide III groups, respectively.

In order to characterise the dentin, parameters such as the full width at half maximum of a single band (FWHM) and intensities ratios of selected peaks, can be extracted. In particular, the intensity of the phosphate peak (I_{960}), related to the mineral compound of the hydroxyapatite; the mineral to matrix ratio (I_{960}/I_{1660}), which provides information on the amount of the mineral and the organic component; crystallinity (1/ $FWHM_{960}$), which is related to the degree of order of the hydroxyapatite crystals; and carbonate-to-phosphate ratio (C/P) (I_{1070}/I_{960}), which give information on the carbonate incorporation in the hydroxyapatite lattice. Eventually, the intensity of the peaks of the organic matrix (I_{1655}, I_{1450}, I_{1246}) are used to evaluate the structural organisation and amount of collagen.

Fig. 10 shows a representative Raman spectrum of radicular dentin acquired with the main peaks investigated. Table 1 shows Raman bands and extracted parameters for radicular dentin. Absolute band intensities and relative peak intensities of select pairs of bands from the Raman spectrum were then assessed (Table 3).

Fig. 10

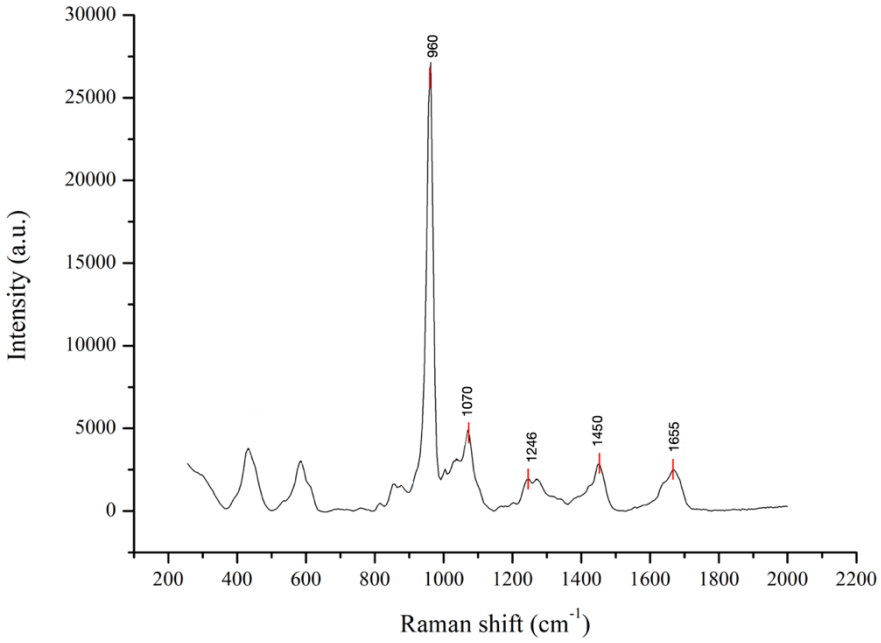

Fig. 10. Raman spectrum of radicular dentin without baseline. Highlights of the main peaks investigated.

Table 3

Band (cm^{-1})	Assignment	Extracted parameter (cm^{-1})	
960	Phosphate (PO_4^{3-})	I_{960}	
1070	Carbonate (CO_3^{2-})	I_{1070}	
1246	Amide III	I_{1246}	
1450	CH_2	I_{1450}	
1655	Amide I	I_{1655}	
		I_{960}/I_{1655}	Mineral to Matrix ratio (M/M)
		$1/FWHM_{960}$	Crystallinity
		I_{1070}/I_{960}	Carbonate to Phosphate ratio (C/P)
		I_{1655}/I_{1246}	Amide I/Amide III
		I_{1246}/I_{1450}	Amide III/CH_2

Table 3 – Raman peaks and extracted parameters of radicular dentin

In order to extract these parameters, after acquisition, Raman spectra were processed with Python software in the spectral range from 200 cm^{-1} to 2000 cm^{-1}. The baseline was detected with the software using the Asymmetric Least Squares Smoothing. Then, a standard normal variate (SVN) transformation was applied. This method allows the normalisation of the spectra by subtracting from the spectra its mean value and dividing it by its standard deviation.

After baseline removal, the number and the position of the peaks and the required Lorentzian shape of interest within specific Raman shift intervals were defined. In particular, the following intervals were processed:

- 800-1200 cm^{-1}, to extract the information of the following peaks: 960 cm^{-1} and 1070 cm^{-1} (Fig. 11);
- 1150-1800 cm^{-1}, to extract the information of the following peaks: 1246 cm^{-1}, 1450 cm^{-1} and 1655 cm^{-1} (Fig. 12).

Fig. 11

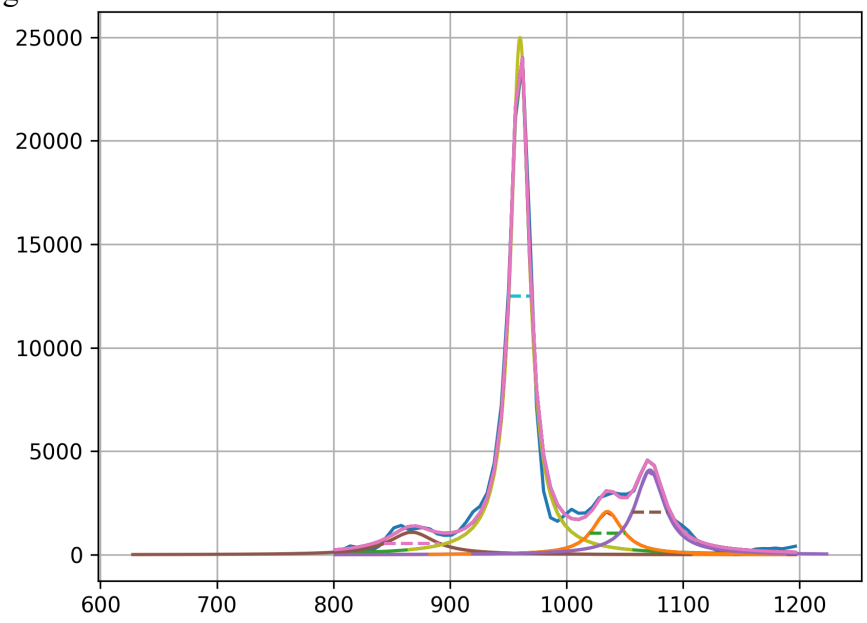

Fig. 11. Deconvolution and fitting of Raman spectra of dentin, from 800 cm^{-1} to 1200 cm^{-1}. Blue line: acquired spectrum without baseline; pink line: computed spectrum.

Fig. 12

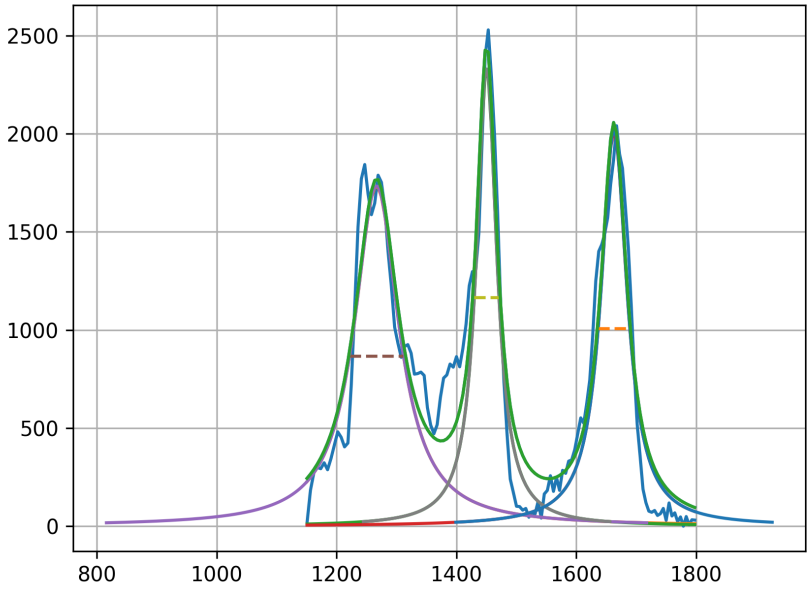

Fig. 12. Deconvolution and fitting of representative Raman spectra of dentin, from 1150 to-1800 cm^{-1}. Blue line: acquired spectrum without baseline; pink: computed spectrum.

At this point, the software, starting from the defined peaks, processed the spectra by performing a non-linear fitting, based on an incremental least-square optimization algorithm. This was done in order to minimise the error between the sum of all fitted base peaks and the original spectrum. At the end of the fitting, the software returned the fitted spectra as the sum of the fitted peaks and the parameters of each peak (such as centre, amplitude, width, etc., according to the fitting shape).

2.3.1 Raman spectroscopy - Statistical analysis

After ascertaining the normality (Shapiro-Wilk test) and homoscedastic (modified Levene test) assumptions of the data sets, Raman spectroscopy data were analysed with three-way analysis of variance (ANOVA) to examine the effects of the age of the endodontic treatment (new treatment *vs* old treatment) and root area (coronal *vs* middle *vs* apical) and the interaction of these two factors on the chemical and structural properties of radicular dentin. Post-hoc pairwise comparisons were performed using the Tukey test. Statistical significance was pre-set at $\alpha=0.05$. All statistical analyses were performed using Stata 18.0 (StataCorp, College Station, Texas, USA).

2.4 Bond strength and morphological analysis of the radicular dentin adhesive interface: Young *vs* aged radicular dentin.

Thirty-two permanent straight single rooted vital human teeth, of similar length and anatomy, were extracted from patients belonging to two different age groups: "under 20" and "over 60". The reason for the extraction was, respectively, orthodontic or periodontal. Teeth were collected in accordance with the local ethics committee (Protocol number CS2/0187). A sample size of 16 per group was calculated with G*Power 3.1.4 (Kiel University, Kiel, Germany) considering alpha-error = 0.05 and ß = 0.95. Roots with cracks, resorption or immature apices were discarded. After debriding the root surface, specimens were stored in 0,1% thymol at 4°C. Roots were sectioned, perpendicularly to the long axis of the tooth, at the level of the cemento-enamel junction (CEJ) with a low-speed diamond saw (Isomet 1000, Buehler, Lake Bluff, IL), to obtain a root length of 15 mm. Teeth with oval-shaped canals were excluded.

The materials and techniques employed for the canal scouting , shaping, irrigation procedures and root canal filling were the same as those previously reported. Specimens were then stored in 100% humidity at 37° C.
Forty-eight hours (h) later, fibre posts were cemented. RCT and RCR-T specimens were then randomly allocated in two groups according to the adhesive protocol used to cement the fibre posts (Fig 13).The materials used and the protocol carried out for post-space and fibre post preparation were the same as previously described (Tab. 1, page 33). After cementation the samples were stored for 24 h in 100% humidity at 37°C.

Fig. 13

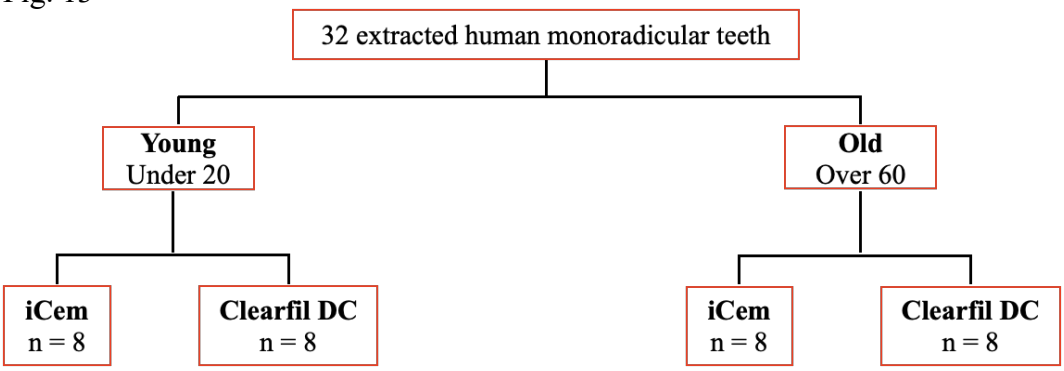

Fig. 13 - Schematic presentation of study groups and subgroups.

Each specimen was then sectioned perpendicularly to its long axis with a 0.35 mm diamond saw (Micromet; Remet, Bologna, Italy) at slow speed with water cooling to obtain 6 slices (3 coronal and 3 apical), generating eight sub-groups. Push-out bond test and failure mode evaluation were then conducted as previously reported.

2.4.1 Bond strength and morphological analysis of the radicular dentin adhesive interface. Young *vs* aged radicular dentin - Statistical analysis

After ascertaining the normality (Shapiro-Wilk test) and homoscedastic (modified Levene test) assumptions of the data sets, the radicular bond strength data were analysed with three-way analysis of variance to examine the effects of the age of the patient (Under 20/Over 60), cement employed and root area and the interaction of those three factors on micro push-out bond strength. Post-hoc pairwise comparisons were performed using the Tukey test. Chi-square test was used to analyse differences in the failure modes. For all tests, statistical significance was pre-set at $\alpha=0.05$. All statistical analyses were performed using Stata 12.0 (StataCorp, College Station, Texas, USA).

Results

3.1 Bond strength and morphological analysis of the radicular dentin adhesive interface: Freshly *vs* aged root canal treated radicular dentin.

3.1.1 Bond strength of the radicular dentin adhesive interface at time 0 (T0): Freshly *vs* aged root canal treated radicular dentin.

Bond strength data were expressed as means and standard deviations (SD) and summarised in Table 4. Results of the three-way ANOVA showed a significant difference for the variable "endodontic treatment" (RCT/RCR-T) ($p=0.01$) and "root area" (apical/coronal) ($p=0.003$) as well as for the interaction between the cement employed and the root area ($p=0.023$). The factor "cement" had no effect on the push-out bond strength ($p>0.05$). Tukey post hoc test showed that bond strength is significantly higher in freshly endodontically treated teeth compared to retreated teeth, independently from the cement employed and the root area considered. In addition, root coronal dentin showed bond strength values significantly higher than root apical dentin.

Table 4

	iCEM RCT	Clearfil DC RCT	iCEM RCR-T	Clearfil DC RCR-T
C	12.35 (± 2.49) ^{a,1}	10.85 (± 3.07) ^{ab,1}	10.53 (± 3.33) ^{ab,1}	9.33 (± 3.43) ^{b,1}
A	9.86 (± 3.18) ^{a,2}	9.70 (± 2.4) ^{a,1}	8.48 (± 2.37) ^{a,1}	9.88 (± 1.61) ^{a,1}

Table 4 – Bond strength values (expressed in MPa) according to different groups. Values are expressed as mean (± SD). C and A stand, respectively, for coronal and apical. Within each line, different superscript letters indicate statistically significant differences. Within each column different superscript numbers indicate statistically significant differences ($p < 0.05$).

3.1.1.1 Failure mode of the radicular dentin adhesive interface at time 0 (T0): Freshly *vs* aged root canal treated radicular dentin.

Failure modes distribution of the debonded specimens, expressed as percentages of the total number of specimens tested, are summarised in Table 5. Statistical analyses showed a predominance of adhesive failures between dentine and resin cement in all groups ($p<0.05$), followed by mixed failures.

Table 5

	TIC	TIA	TDC	TDA	RIC	RIA	RDC	RDA
Adhesive	75	14.28	66.67	28	68	57.14	86.20	47.62
Cohesive	10.71	35.71	20	44	20	23.8	10.34	23.80
Mixed	14.28	50	6.6	28	12	19.04	3.44	28.57

Table 5 – Failure modes after Push-out test. Values are expressed as percentage (%). TIC (treatment[RCT]-iCEM-coronal), TIA (treatment[RCT]-iCEM-apical), TDC (treatment[RCT]-Clearfil DC-coronal), TDA (treatment[RCT]-Clearfil DC-apical), RIC (re-treatment[RCR-T]-iCEM-coronal), RIA (re-treatment[RCR-T]-iCEM-apical), RDC (re-treatment[RCR-T]-Clearfil DC-coronal), RDA (re-treatment[RCR-T]-Clearfil DC-apical).

3.1.2 Morphological analysis of the radicular dentin adhesive interface at time 0 (T0): Freshly *vs* aged root canal treated radicular dentin.

The hybrid layer thickness was significantly influenced by the factors "endodontic treatment" (RCT/RCR-T) ($p=0.03$) and "resin cement" (Clearfil DC/iCEM) ($p=0.001$). The factor "root area" had no effect on the thickness of the hybrid layer ($p>0.05$).
The hybrid layer thickness of freshly devitalized teeth proved to be significantly higher than that of retreated teeth. Further, post hoc test showed that iCEM cement produced a hybrid layer thinner than that of Clearfil DC (Table 6). Representative samples of HL formation and filled tubules are shown in Fig. 14. The number of dentinal tubules penetrated with resin cement and adhesive was significantly affected by the root area considered. Coronal slices showed a significantly higher number of penetrated dentinal tubules than apical portions ($P<0.05$) (Table 7). Representative images of filled tubules are shown in Fig. 15.

Table 6

	iCEM RCT	Clearfil DC RCT	iCEM RCR-T	Clearfil DC RCR-T
C	2.04 (±0.98) ^{a,1}	3.45 (±1.20) ^{a,1}	1.27 (±0.79) ^{b,1}	3.27 (±1.21) ^{a,1}
A	1.72 (±1.26) ^{ab,1}	3.34 (±1.48) ^{a,1}	0.94 (±0.56) ^{b,1}	2.55 (±1.70) ^{ac,1}

Table 6 – HL thickness (expressed in μm) according to different groups. Values are expressed as mean (± Standard Deviation). C and A stand, respectively, for coronal and apical. Within each line, different superscript letters indicate statistically significant differences. Within each column different superscript numbers indicate statistically significant differences ($p < 0.05$).

Fig. 14

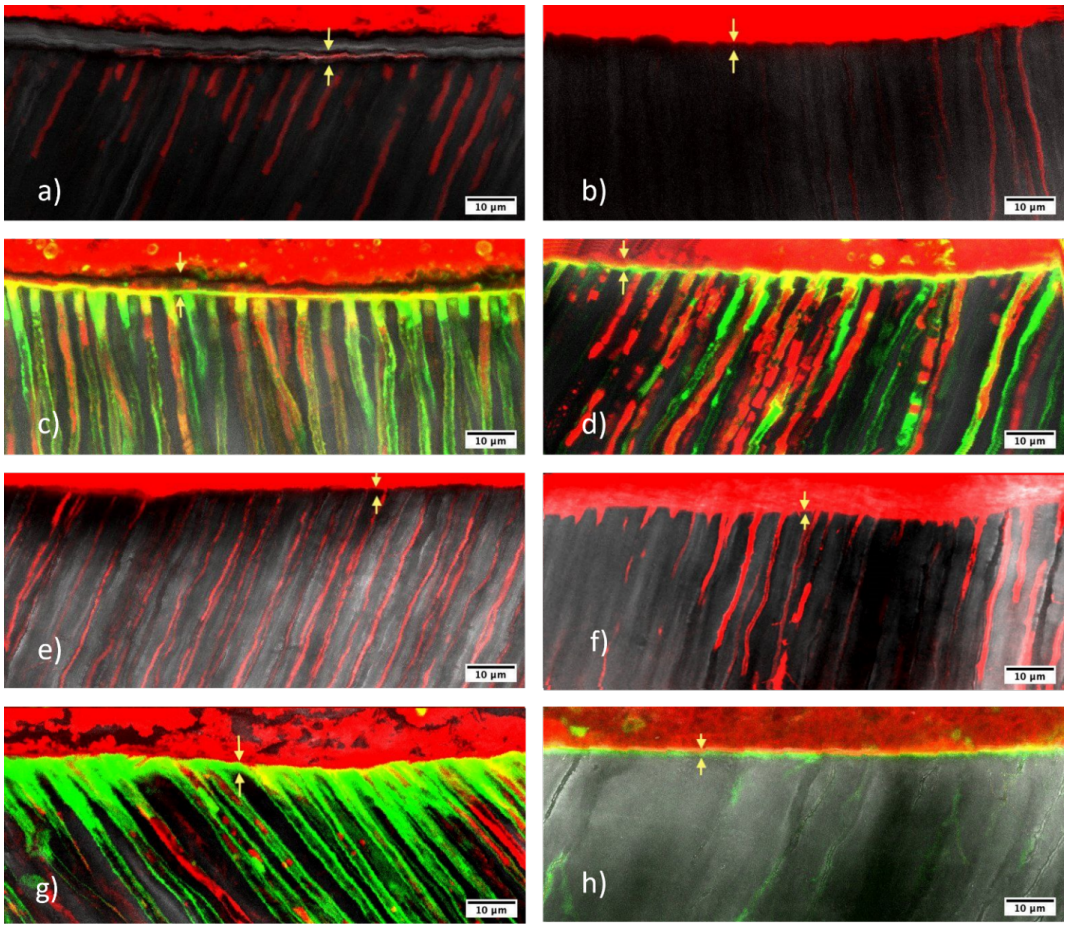

Fig. 14 – Representative images of HL thickness for the different groups. (A) TIC (treatment-iCEM-coronal) group; (B) TIA (treatment-iCEM-apical) group; (C) TDC (treatment-Clearfil DC-coronal) group; (D) TDA (treatment-Clearfil DC-apical) group; (E) RIC group (re-treatment-iCEM-coronal); (F) RIA (re-treatment-iCEM-apical) group; (G) RDC (re-treatment-Clearfil DC-coronal) group; (H) RDA (re-treatment-Clearfil DC-apical) group.

Table 7

	iCEM RCT	Clearfil DC RCT	iCEM RCR-T	Clearfil DC RCR-T
C	64.76 (±16.45) ^{a,1}	61.05 (±18.1) ^{a,1}	62.66 (±16.06) ^{a,1}	64.6 (±12) ^{a,1}
A	48.27 (±27.46) ^{a,1}	64.07 (±12) ^{a,1}	52.47 (±22.79) ^{a,1}	50.62 (±20.43) ^{a,1}

Table 7 – Number of tubules penetrated by adhesive and resin cement. Values are expressed as mean (± SD). C and A stand, respectively, for coronal and apical. Within each line, different superscript letters indicate statistically significant differences. Within each column different superscript numbers indicate statistically significant differences ($p < 0.05$).

Fig. 15

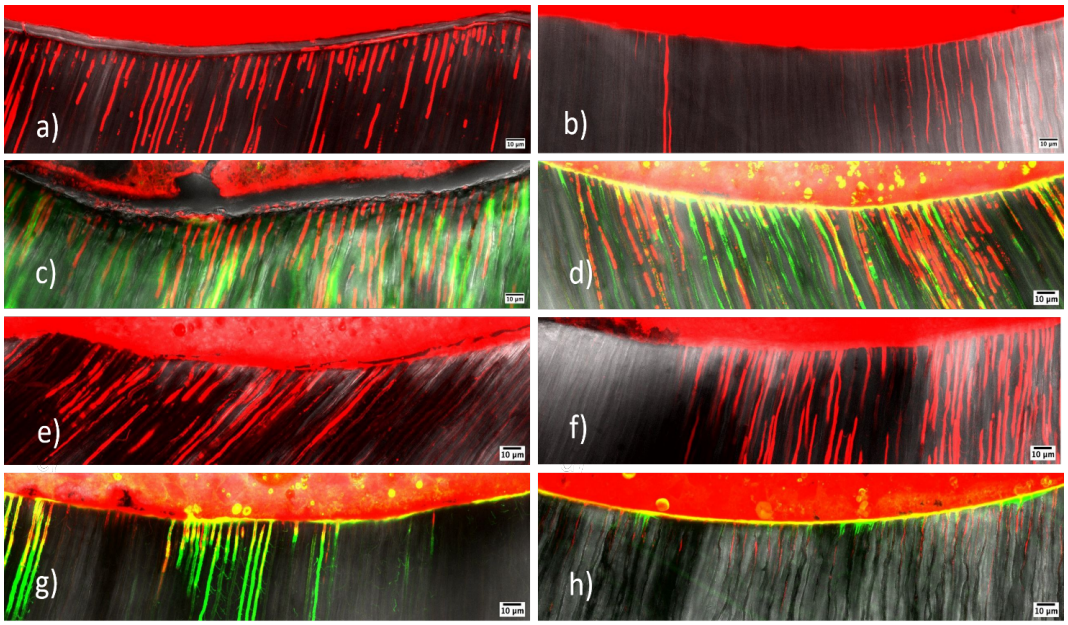

Fig. 15 – Representative CLSM images of resin cement-infiltrated dentinal tubules. (A) TIC (treatment-iCEM-coronal) group; (B) TIA (treatment-iCEM-apical) group; (C) TDC (treatment-Clearfil DC-coronal) group; (D) TDA (treatment-Clearfil DC-apical) group; (E) RIC group (re-treatment-iCEM-coronal); (F) RIA (re-treatment-iCEM-apical) group; (G) RDC (re-treatment-Clearfil DC-coronal) group; (H) RDA (re-treatment-Clearfil DC-apical) group.

3.1.3 Bond strength of the radicular dentin adhesive interface after one year ageing (T1): Freshly *vs* aged root canal treated radicular dentin.

Results of the three-way ANOVA showed, overall, significantly lower bond strength values for the samples tested after one year ageing (T1) compared to the ones assessed at time zero (T0) (p=0.000). Bond strength data were expressed as means and standard deviations (SD) and summarised in Table 8 and 9.

Table 8

	iCEM RCT		Clearfil DC RCT		iCEM RCR-T		Clearfil DC RCR-T	
	C	A	C	A	C	A	C	A
T0	12.35 [1] (± 2.49)	9.86 [1] (± 3.18)	10.85 [1] (± 3.07)	9.70 [1] (± 2.4)	10.53 [1] (± 3.33)	8.48 [1] (± 2.37)	9.33 [1] (± 3.43)	9.88 [1] (± 1.61)
T1	5.97 [2] (± 2.21)	6.82 [2] (± 2.50)	6.40 [2] (± 2.00)	7.67 [2] (± 2.40)	6.86 [2] (± 2.42)	7.80 [1] (± 2.02)	6.72 [2] (± 2.02)	8.48 [2] (± 1.80)

Table 8 – Bond strength values (expressed in MPa) according to different groups. Values are expressed as mean (± SD). C and A stand, respectively, for coronal and apical. Within each column different superscript numbers indicate statistically significant differences ($p < 0.05$).

After one year ageing, significant differences were reported for the variables "cement" and "root area". Bond strength values, overall, were higher in the samples where posts were luted with Clearfil DC compared to the ones treated with iCEM (p=0.021) and in the apical half compared to the coronal portion of the post-space (p=0.000). There was no statistically significant difference between freshly and aged root canal treated specimens (p=0.1) (Table6).

Table 9

	iCEM RCT	Clearfil DC RCT	iCEM RCR-T	Clearfil DC RCR-T
C	5.97 (± 2.21) [a, 1]	6.40 (± 2.00) [a, 1]	6.86 (± 2.42) [a, 1]	6.72 (± 2.02) [a, 1]
A	6.82 (± 2.50) [a, 1]	7.67 (± 2.40) [ab, 2]	7.80 (± 2.02) [ab, 2]	8.48 (± 1.80) [b, 2]

Table 9 – Bond strength values (expressed in MPa) according to different groups. Values are expressed as mean (± SD). C and A stand, respectively, for coronal and apical. Within each line, different superscript letters indicate statistically significant differences. Within each column different superscript numbers indicate statistically significant differences ($p < 0.05$).

3.1.3.1 Failure mode of the radicular dentin adhesive interface at T1: Freshly *vs* aged root canal treated radicular dentin.

Failure modes distribution of the debonded specimens, expressed as percentages of the total number of specimens tested, are summarised in Table 10. Statistical analyses showed a predominance of adhesive failures between dentine and resin cement in all groups ($p<0.05$), followed by mixed and cohesive failures. Pearson chi2 test showed no statistical correlation between groups and type of fracture (p=0.322).

Table 10

	TIC	TIA	TDC	TDA	RIC	RIA	RDC	RDA
Adhesive	68	68	70	77	72	66	72	68
Cohesive	16	8	30	11	14	10	14	8
Mixed	19	24	0	12	14	24	14	28

Table 10 – Failure modes after Push-out test. Values are expressed as percentage (%). TIC (treatment[RCT]-iCEM-coronal), TIA (treatment[RCT]-iCEM-apical), TDC (treatment[RCT]-Clearfil DC-coronal), TDA (treatment[RCT]-Clearfil DC-apical), RIC (re-treatment[RCR-T]-iCEM-coronal), RIA (re-treatment[RCR-T]-iCEM-apical), RDC (re-treatment[RCR-T]-Clearfil DC-coronal), RDA (re-treatment[RCR-T]-Clearfil DC-apical).

3.2 Mechanical properties of radicular dentin

Nanoindentation analysis showed that the elastic modulus of freshly root canal treated radicular dentin (RCT) was significantly higher ($p<0.01$) (Fig.16).

Fig. 16

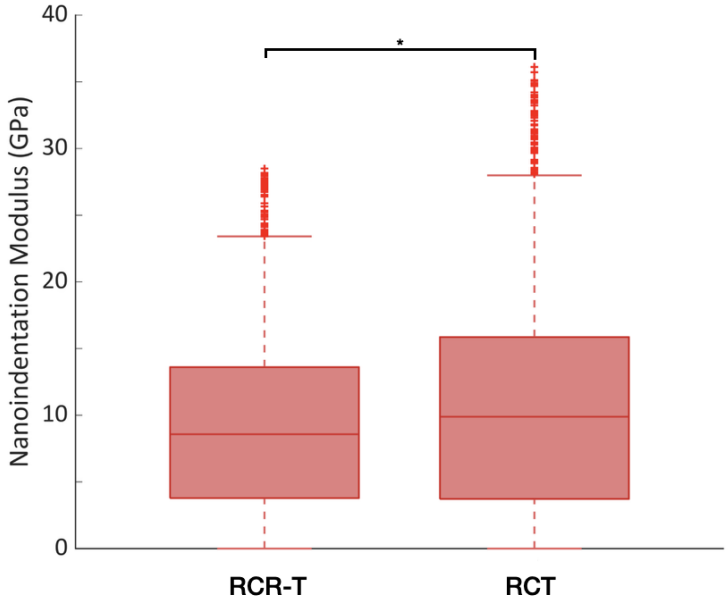

Fig. 16. Young modulus of radicular dentin. The asterisk indicates a statistically significant difference ($p<0.01$).

Considering the topography, in both groups the apical third showed lower values of elastic modulus compared to the middle and coronal portion. In the group RCR-T the difference between coronal and apical third is very close to statistical significance (Fig. 17).

Fig. 17

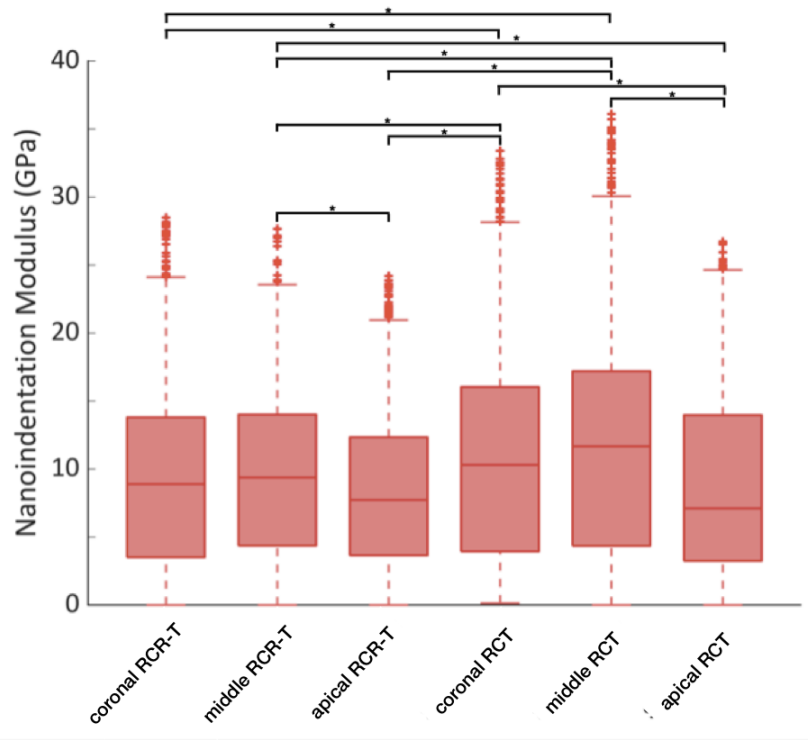

Fig. 17. Young modulus of radicular dentin. The asterisk indicates a statistically significant difference ($p<0.01$).

Hardness, both Vickers (Fig. 18) and Martens (Fig. 19), were found significantly higher in the specimens with a fresh endodontic treatment (RCT) ($p<0.01$).

Fig. 18

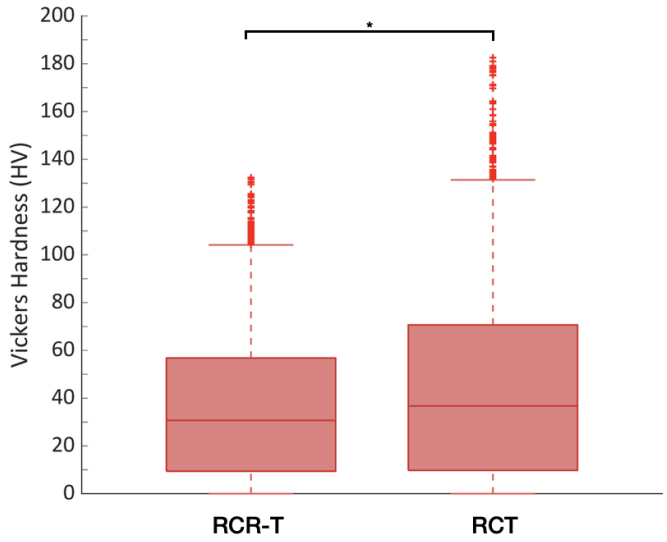

Fig. 18. Vickers Hardness of radicular dentin. The asterisk indicates a statistically significant difference ($p<0.01$).

Fig. 19

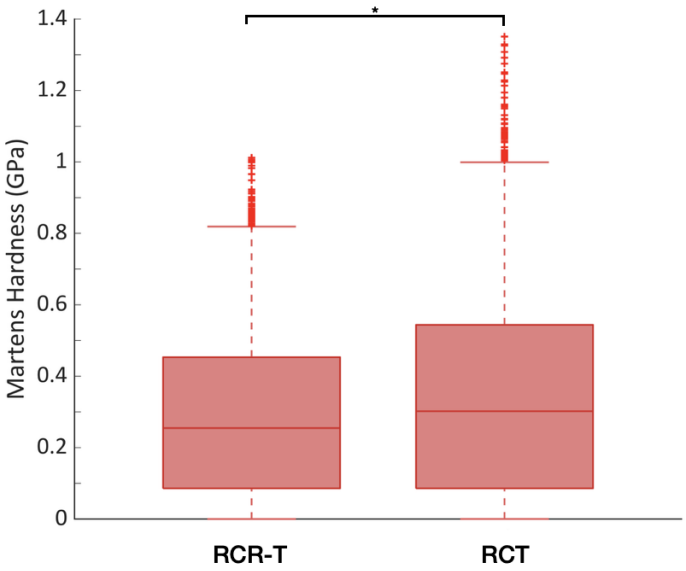

Fig. 19. Martens Hardness of radicular dentin. The asterisk indicates a statistically significant difference ($p<0.01$).

The topographical analysis of Martens hardness is quite comparable to Vickers'. For both the tissues analysed, a trend of higher hardness values was registered in the middle section of the radicular portion investigated. Indeed, Vickers and Martens hardness were significantly higher in the middle section of the RCT group ($p<0.01$). Vickers hardness was significantly higher also in the middle portion of the RCR-T group ($p<0.01$). In this group, the trend is respected for Martens hardness too, even though the difference was not statistically significant ($p>0.01$). Furthermore, in the specimens with a fresh endodontic treatment (RCT) the apical third showed Vickers and Martens hardness significantly lower than the middle and coronal portions ($p<0.01$) (Fig. 20 and 21).

Fig. 20

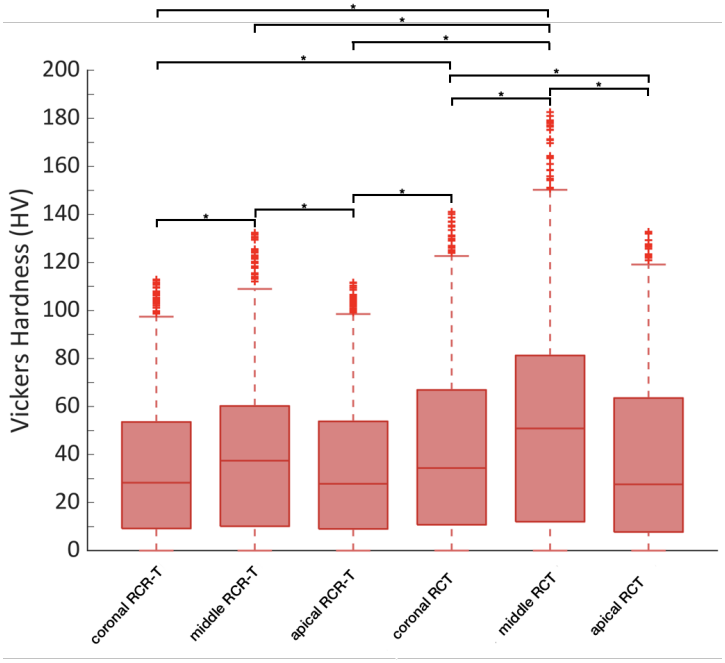

Fig. 20.Vickers hardness of radicular dentin. The asterisk indicates a statistically significant difference ($p<0.01$).

Fig. 21

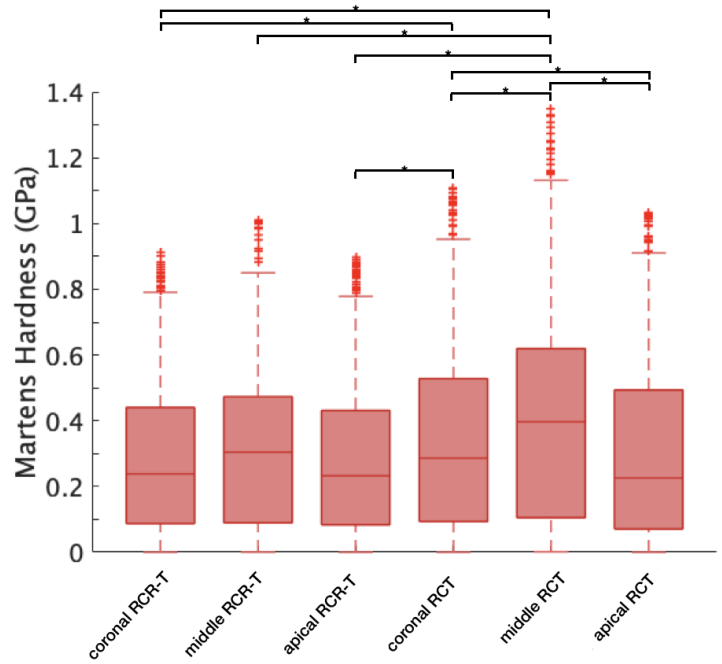

Fig. 21. Martens hardness of radicular dentin. The asterisk indicates a statistically significant difference ($p<0.01$).

The statistical test pointed out that that specimens with aged endodontic treatment (RCR-T) showed a degree of plasticization significantly higher compared to teeth with a recent treatment (RCT) ($p<0.01$). However, as evident in Fig. 22, the values were so close that this may be considered not scientifically meaningful.

Fig. 22

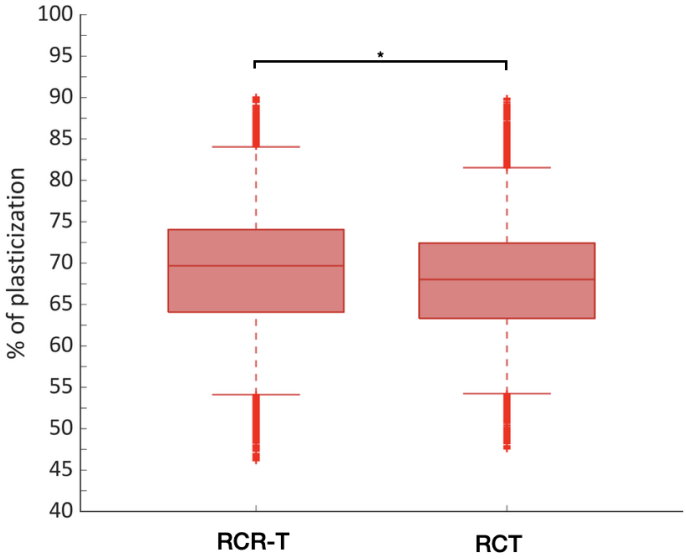

Fig. 22. Degree of plasticization of radicular dentin. The asterisk indicates a statistically significant difference ($p<0.01$).

In the RCR-T group, the degree of plasticization was significantly different among subgroups ($p<0.01$): the middle third showed the highest value, while the apical third the lowest (Fig. 23). Conversely, in the group with a fresh root canal treatment (RCT), the middle third showed the lowest value compared to the other anatomical counterparts ($p<0.01$) (Fig. 23).

Fig. 23

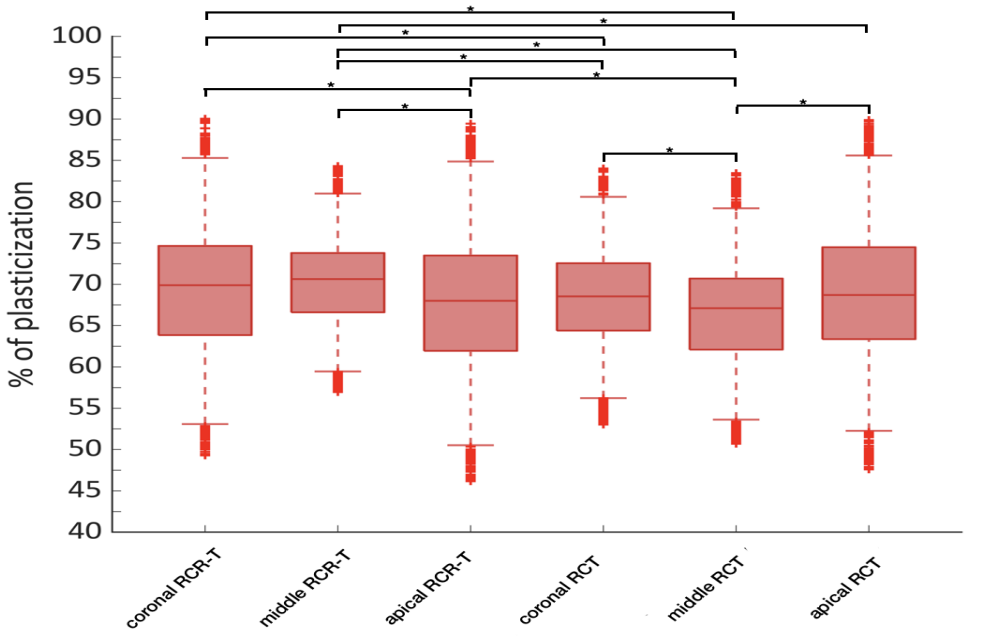

Fig. 23. Degree of plasticization of radicular dentin. The asterisk indicates a statistically significant difference ($p<0.01$).

Therefore, root canal dentin with an aged endodontic treatment showed significantly lower mechanical characteristics (Young modulus, hardness and percentage of plasticization) compared to the dentinal tissue with a recent root canal treatment ($p<0.01$) (Fig. 16, 18, 19, 22). However, as stated before, the difference in terms of degree of plasticization was not scientifically consistent (Fig. 22). Moreover, considering the topography of the radicular section analysed, all the mechanical properties investigated are significantly different, between RCT and RCR-T groups, in the coronal and middle thirds, but never in the apical portion (Fig. 17, 20, 21, 23).

Table11 reports mean and standard deviation of each mechanical property tested via nanoindentation analysis.

Table 11 – Means and standard deviations of the mechanical properties

		Elastic modulus (GPa)		Vickers hardness (GPa)		Martens hardness (GPa)		% Plasticization	
		Mean and SD	Median and 25°-75° percentile	Mean and SD	Median and 25°-75° percentile	Mean and SD	Median and 25°-75° percentile	Mean and SD	Median and 25°-75° percentile
RCT	coronal	10.63 (7.26) [a]	10.3 (3.93-16.04) [a]	41.17 (33.47) [a]	34.39 (10.75-66.92) [a]	0.33 (0.26) [a]	0.28 (0.093-0.528) [a]	68.30 (6.62) [a]	68.53 (64.38-72.55) [a]
	middle	11.53 (7.68) [a]	11.67 (4.35-17.2) [a]	52.02 (41.26) [b]	50.88 (12.10-81.27) [b]	0.39 (0.29) [b]	0.39 (0.10-0.61) [b]	66.66 (6.83) [b]	67.10 (62.09-70.69) [b]
	apical	8.74 (6.35) [b]	7.10 (3.25-13.98) [b]	37.17 (32.85) [c]	27.60 (7.74-63.57) [c]	0.29 (0.25) [c]	0.22 (0.07-0.49) [c]	68.86 (8.85) [a]	68.70 (63.35-74.49) [a]
	overall	10.31 (7.22) [1]	9.897 (3.72 -15.86) [1]	43.47 (36.61) [1]	36.76 (9.77-70.68) [1]	0.33 (0.27) [1]	0.30 (0.08-0.54) [1]	67.97 (7.61) [1]	68.03 (63.31-72.42) [1]
RCR-T	coronal	9.23 (6.14) [cd]	8.90 (3.51-13.81) [cd]	34.20 (27.45) [d]	28.35 (9.24-53.59) [d]	0.28 (0.21) [d]	0.23 (0.08-0.44) [d]	69.35 (8.46) [c]	69.88 (63.84-74.64) [c]
	middle	9.50 (5.86) [c]	9.374 (4.35-14.01) [c]	38.64 (29.58) [e]	37.48 (10.13-60.25) [e]	0.30 (0.22) [d]	0.30 (0.08-0.47) [d]	70.28 (5.75) [c]	70.62 (66.6-73.7) [c]
	apical	8.53 (5.64) [bd]	7.80 (3.74-12.45) [bd]	34.40 (28.41) [cd]	28.24 (9.29-54.26) [cd]	0.27 (0.22) [cd]	0.23 (0.08-0.43) [cd]	67.71 (9.36) [ad]	68 (61.93-73.48) [ad]
	overall	9.09 (5.90) [2]	8.65 (3.86-13.61) [2]	35.79 (28.57) [2]	30.8 (9.59-56.89) [2]	0.28 (0.21) [2]	0.25 (0.08-0.45) [2]	69.07 (8.16) [2]	69.67 (64.07-74.05) [2]

Table 11. Means and SD; Median, 25° and 75° percentile of the mechanical properties values. Within each column different superscript letters or numbers indicate statistically significant differences ($p < 0.001$). Between groups only the same sub-groups are to be compared.

3.3 Assessment of Chemical properties of radicular dentin by Raman Spectroscopy

This section reports the results obtained by Raman spectroscopy. The values extracted by the Raman spectra are shown as box plots. The box plots represent the dispersion of the data of each parameter: the boxes include 50% of values (from 25 to 75%) and are spitted by the median line, while the bars connect the maximum and the minimum values.

The chemical analysis of the inorganic content of radicular dentin showed a significantly higher mineral to matrix ratio (M/M), calculated as the ratio between the intensities of the phosphate and amide I peaks, in the freshly root canal treated tissue (p=0.001) (Fig. 24). This value, generally, was found higher in the coronal third of the post-space compared to the middle and apical portion. In the apical third, the M/M, overall, presented the lowest values. Moreover, between the two groups investigated [freshly root canal treated (RCT) *vs* aged root canal treated (RCR-T)] significant differences were found in the coronal third (p=0.018), but not in the middle and apical one (p>0.05) (Fig. 25).

Fig 24

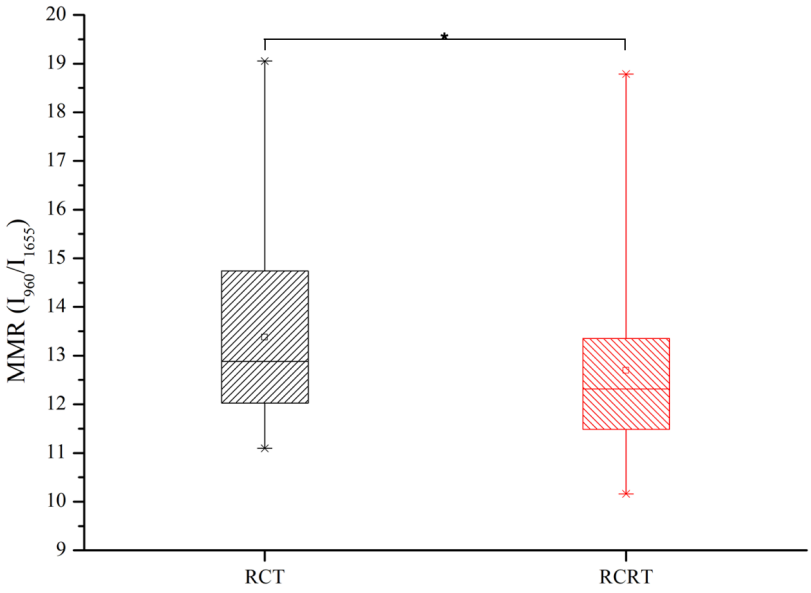

Fig. 24: Mineral to matrix ratio (I_{960}/I_{1655}) for RCT and RCRT groups The asterisk indicates a statistically significant difference (P<0.05). The box plots represent the dispersion of the data of the mineral to matrix ratio, expressed as a percentage: the boxes include 50% of the values (from 25 to 75%) and are divided by the median line, the bars connect the maximum and the minimum values.

Fig. 25

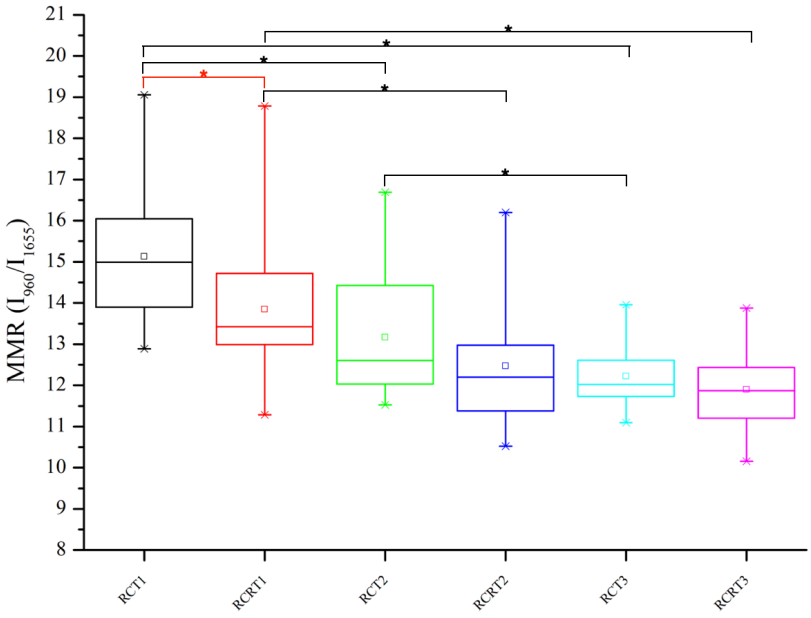

Fig. 25: Mineral to matrix ratio (I_{960}/I_{1655}) for coronal (1), middle (2), and apical (3). The asterisk indicates a statistically significant difference ($P<0.05$). The red bar highlights the difference between groups. The black bar highlights differences within groups. The box plots represent the dispersion of the data of the mineral to matrix ratio, expressed as a percentage: the boxes include 50% of the values (from 25 to 75%) and are divided by the median line, the bars connect the maximum and the minimum values.

The intensity of the phosphate peak found within the two substrates investigated was comparable (p>0.05) (Fig. 26). The only significant differences could be appreciated between the topographical sections of the group with a fresh endodontic treatment (RCT): in the apical third of the post space the phosphate peaks intensity was significantly higher compared to the coronal (p=0.004) and middle (p=0.002) sections (Fig. 27).

Fig. 26

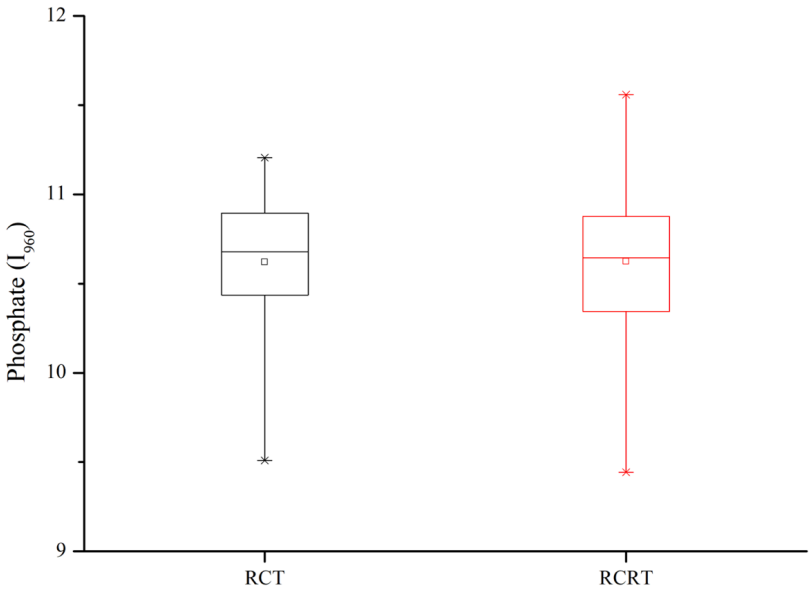

Fig. 26: Intensity of the phosphate peak (I_{960}) for RCT and RCRT groups. The asterisk indicates statistically significant difference (P<0.05). The box plots represent the dispersion of the data of the intensity of the phosphate peak, expressed as a percentage: the boxes include 50% of the values (from 25 to 75%) and are divided by the median line, the bars connect the maximum and the minimum values.

Fig. 27

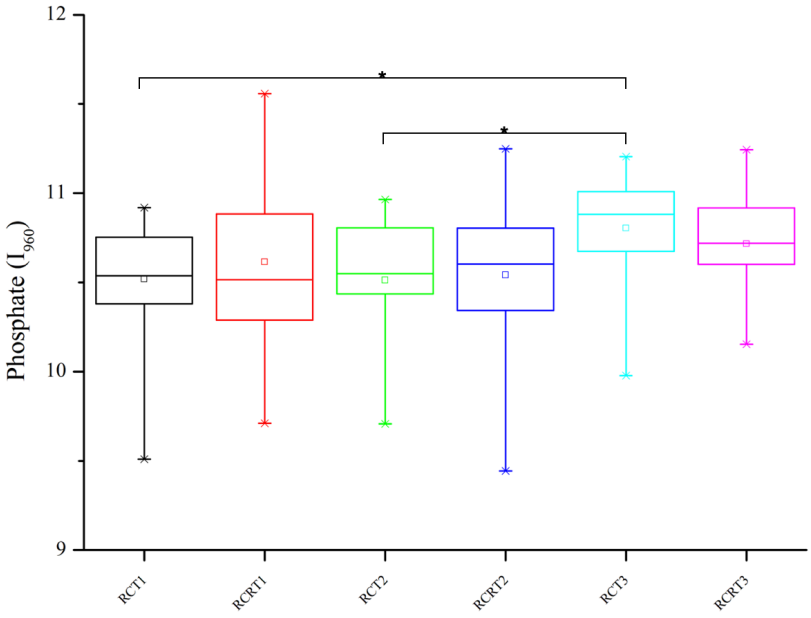

Fig. 27: Phosphate peak intensity (I_{960}) for coronal (1), middle (2) and apical (3). The asterisk indicates a statistically significant difference ($P<0.05$). The box plots represent the dispersion of the data of the intensity of the phosphate peak, expressed as a percentage: the boxes include 50% of the values (from 25 to 75%) and are divided by the median line, the bars connect the maximum and the minimum values.

Regarding the carbonate/phosphate ratio, in the tissue with an aged endodontic treatment (RCR-T) a significantly higher value was found compared to the RCT group (p=0.001) (Fig. 28). Analysing the canal topography, this ratio, in the RCR-T group, was higher in the middle (p=0,001) and apical (p=0,027) portions compared to the coronal section (Fig. 29). The difference in C/P ratio, between the two groups, was statistically significant in the middle (p=0.0001) and apical (p=0.016) third of the post space, but not in the coronal one (Fig. 29).

Fig. 28

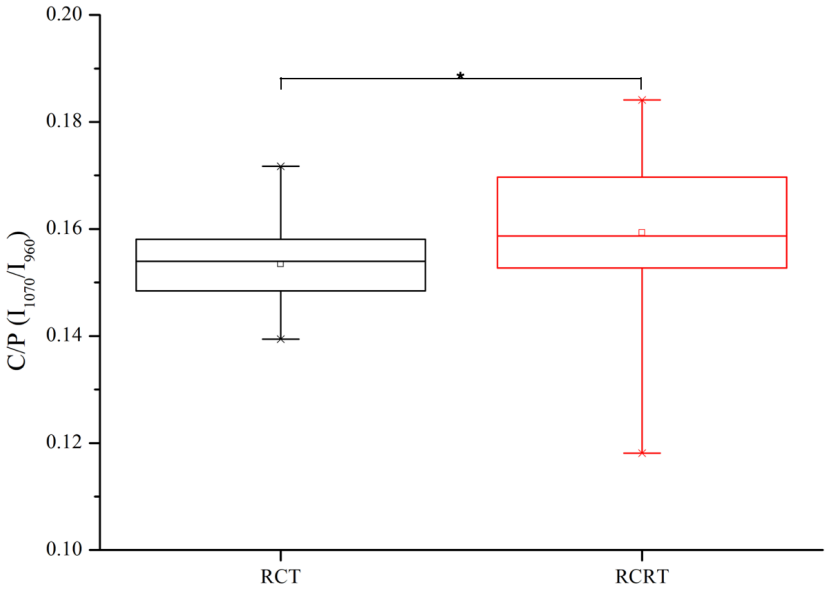

Fig. 28: Carbonate to phosphate ratio (I_{1070}/I_{960}). The asterisk indicates a statistically significant difference ($P<0.05$). The box plots represent the dispersion of the data of the carbonate to phosphate ratio, expressed as a percentage: the boxes include 50% of the values (from 25 to 75%) and are divided by the median line, the bars connect the maximum and the minimum values.

Fig. 29

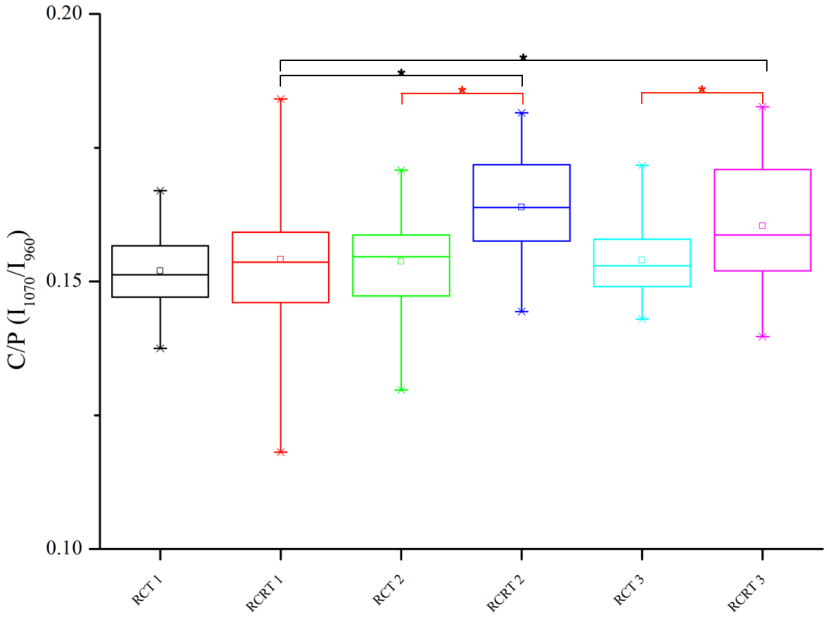

Fig. 29: Carbonate to phosphate ratio (I_{1070}/I_{960}) for coronal (1), middle (2) and apical (3). The asterisk indicates a statistically significant difference ($P<0.05$). The red bar highlights differences between groups. The black bar highlights differences within groups. The box plots represent the dispersion of the data of the carbonate to phosphate ratio, expressed as a percentage: the boxes include 50% of the values (from 25 to 75%) and are divided by the median line, the bars connect the maximum and the minimum values.

The analysis of crystallinity did not reveal any significant difference between groups (Fig. 30) and sub-groups ($p>0.05$).

Fig. 30

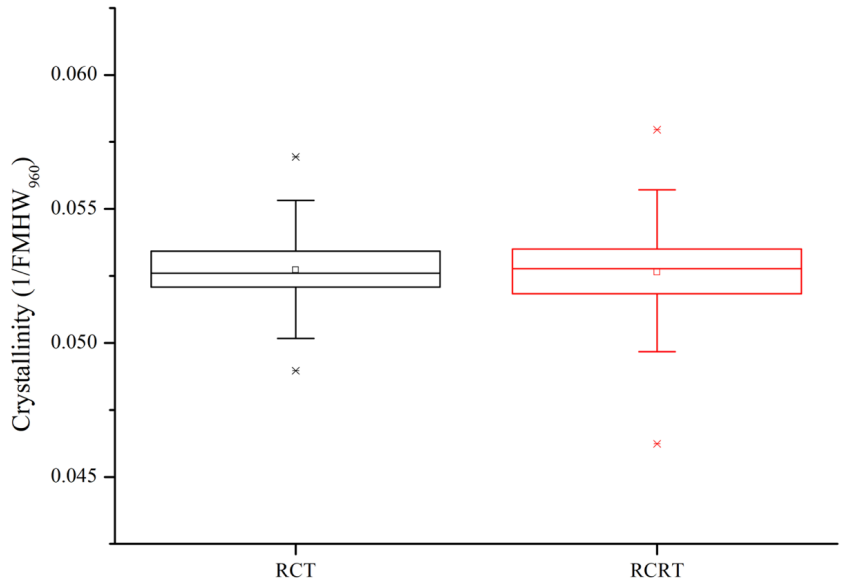

Fig 30: Crystallinity $(1/FWHM_{960})$ - The box plots represent the dispersion of the data of the crystallinity, expressed as a percentage: the boxes include 50% of the values (from 25 to 75%) and are divided by the median line, the bars connect the maximum and the minimum values.

Table 12 reports means and standard deviations (SD) of the peak intensities and extracted parameters belonging to the mineral phase of radicular dentin.

Table 12 – Peak intensities and extracted parameters for mineral components.

		M/M ratio		I Phosphate		C/P ratio		Crystallinity	
		Mean and SD	Median and 25°-75° percentile	Mean and SD	Median and 25°-75° percentile	Mean and SD	Median and 25°-75° percentile	Mean and SD	Median and 25°-75° percentile
RCT	coronal	15.12 (1.61) [a]	15.13 (13.92-16.23) [a]	10.52 (0.32) [a]	10.56 (10.39-10.75) [a]	0.15 (0.006) [a]	0.15 (0.147-0.156) [a]	0.05 (0.001) [a]	0.052 (0.051-0.053) [a]
	middle	13.16 (1.40) [b]	12.60 (12.03-12.62) [b]	10.51 (0.34) [a]	10.54 (10.37-10.8) [a]	0.15 (0.005) [a]	0.155 (0.151-0.158) [a]	0.05 (0.001) [a]	0.052 (0.052-0.053) [a]
	apical	12.22 (0.72) [c]	12.05 (11.73-12.62) [c]	10.80 (0.30) [b]	10.88 (10.66-11.01) [b]	0.15 (0.007) [a]	0.152 (0.149-0.158) [a]	0.05 (0.001) [a]	0.053 (0.052-0.053) [a]
	overall	13.37 (1.71) [1]	12.87 (12.02-14.74) [1]	10.62 (0.35) [1]	10.67 (10.43-10.89) [1]	0.15 (0.006) [1]	0.153 (0.148-0.158) [1]	0.05 (0.001) [a]	0.052 (0.052-0.053) [a]
RCR-T	coronal	13.84 (1.72) [d]	13.57 (12.87-14.78) [d]	10.61 (0.43) [a]	10.6 (10.27-10.88) [a]	0.15 (0.01) [a]	0.152 (0.144-0.158) [a]	0.05 (0.002) [a]	0.052 (0.051-0,054) [a]
	middle	12.46 (1.52) [b]	12.2 (11.26-13.01) [b]	10.54 (0.39) [a]	10.6 (10.32-10.82) [a]	0.16 (0.009) [b]	0.164 (0.157-0.172) [b]	0.05 (0.001) [a]	0.052 (0.051-0.053) [a]
	apical	11.90 (0.93) [bc]	11.87 (11.18-12.58) [bc]	10.71 (0.29) [ab]	10.72 (10.57-10.92) [ab]	0.16 (0.01) [b]	0.158 (0.153-0.17) [b]	0.05 (0.001) [a]	0.053 (0.052-0.053) [a]
	overall	12.69 (1.61) [2]	12.37 (11.49-13.4) [2]	10.62 (0.38) [1]	10.65 (10.35-10.88) [1]	0.16 (0.01) [2]	0.158 (0.152-0.169) [2]	0.05 (0.001) [a]	0.052 (0.052-0.053) [a]

Table 12 - Means and SD; Median, 25° and 75° percentile of the peak intensities of radicular dentin mineral components. Within each column different superscript letters or numbers indicate statistically significant differences ($p < 0.05$). Between groups only the same sub-groups are to be compared.

Within the organic content of radicular dentin, the tissue with aged endodontic treatment (RCR-T) featured a significantly lower amide I/amide III peaks ratio (p=0.034) (Fig. 31), whereas no significant differences were found between anatomical subgroups (p>0.05).

Fig. 31

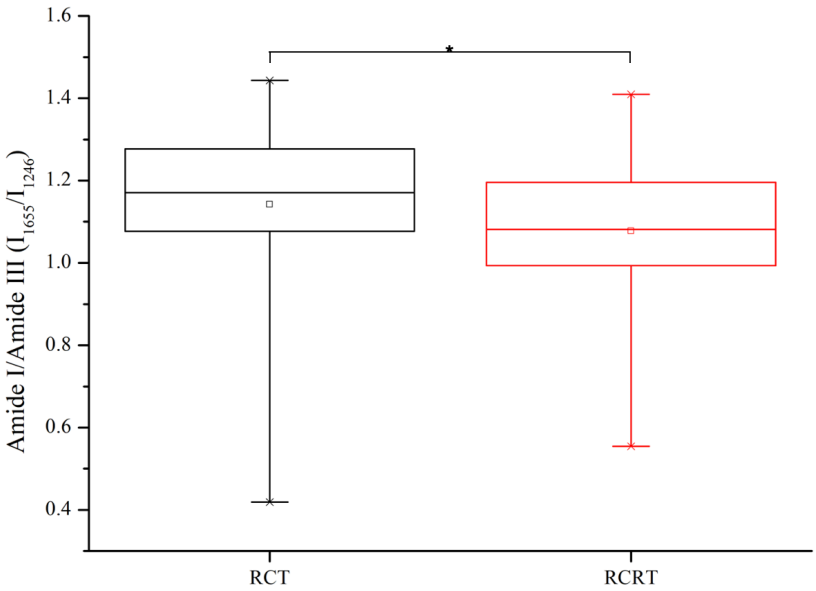

Fig 31: Amide I/Amide III (I_{1655}/I_{1246}) - The asterisk indicates a statistically significant difference (P<0.05). The box plots represent the dispersion of the data of the Amide I to Amide III ratio, expressed as a percentage: the boxes include 50% of the values (from 25 to 75%) and are divided by the median line, the bars connect the maximum and the minimum values.

The amide I/CH_2 ratio was also found altered by endodontic treatment. Indeed, in the group RCR-T, this value was significantly lower compared to the group with a fresh root canal treatment ($p=0.001$) (Fig. 32). Furthermore, this value, for both the groups, was significantly higher in the middle and apical section of the post space compared to the coronal portion ($p<0.05$) (Fig. 33). The difference in amide I/CH_2 ratio between the tissues analysed was statistically significant in the middle ($p=0.025$) and apical ($p=0.014$) third, but not in the coronal one ($p=0.284$) (Fig. 33).

Fig. 32

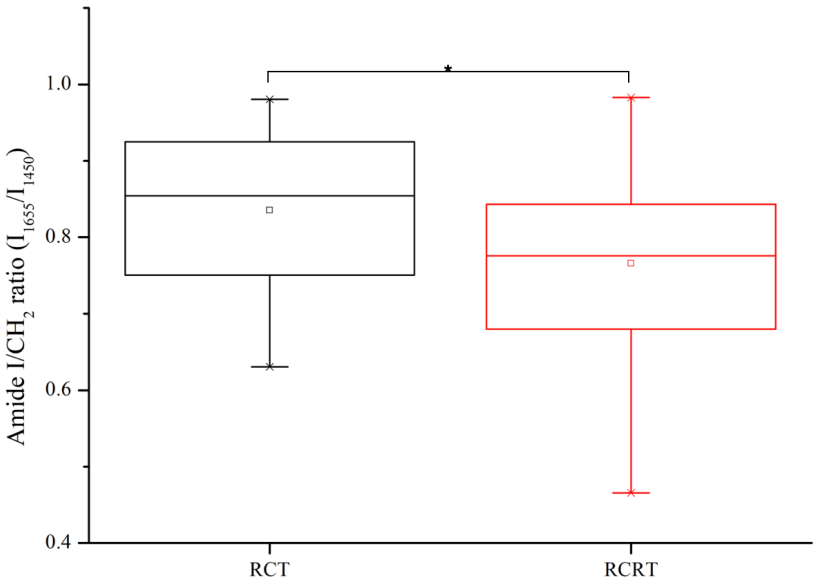

Fig. 32: Amide I/CH_2 ratio (I_{1655}/I_{1450}) – The asterisk indicates a statistically significant difference ($P<0.05$). The box plots represent the dispersion of the data of the Amide I to CH_2 ratio, expressed as a percentage: the boxes include 50% of the values (from 25 to 75%) and are divided by the median line, the bars connect the maximum and the minimum values.

Fig. 33

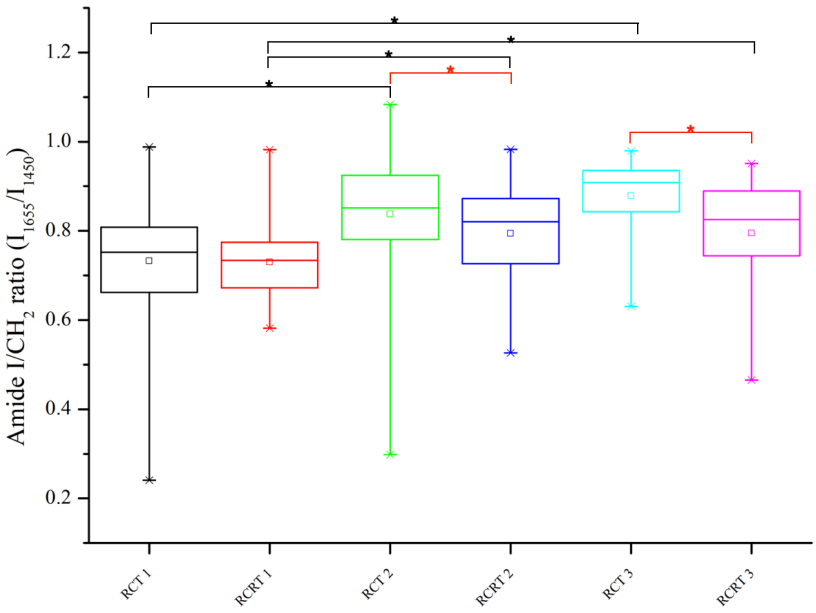

Fig. 33: Amide I/CH_2 (I_{1655}/I_{1450}) ratio for coronal (1), middle (2) and apical (3). The asterisk indicates a statistically significant difference ($P<0.05$). The red bar highlights differences between RCT and RCRT groups. The black bar highlights differences within groups. The box plots represent the dispersion of the data of the Amide I to CH_2 ratio, expressed as a percentage: the boxes include 50% of the values (from 25 to 75%) and are divided by the median line, the bars connect the maximum and the minimum values.

The intensity of the peak of amide III was significantly higher in the specimens with aged endodontic treatment (RCR-T) compared to the group with a fresh root canal therapy ($p=0.005$) (Fig. 34). The topographical analysis showed no significant differences between the anatomical areas ($p>0.05$) (Fig. 35). However, the difference in amide III intensity between the coronal and apical third of the RCT group is close to the significance threshold ($p=0.054$) (Fig. 35). A similar difference was found, in the coronal third, between RCT and RCRT group ($p=0.059$) (Fig. 35).

Fig. 34

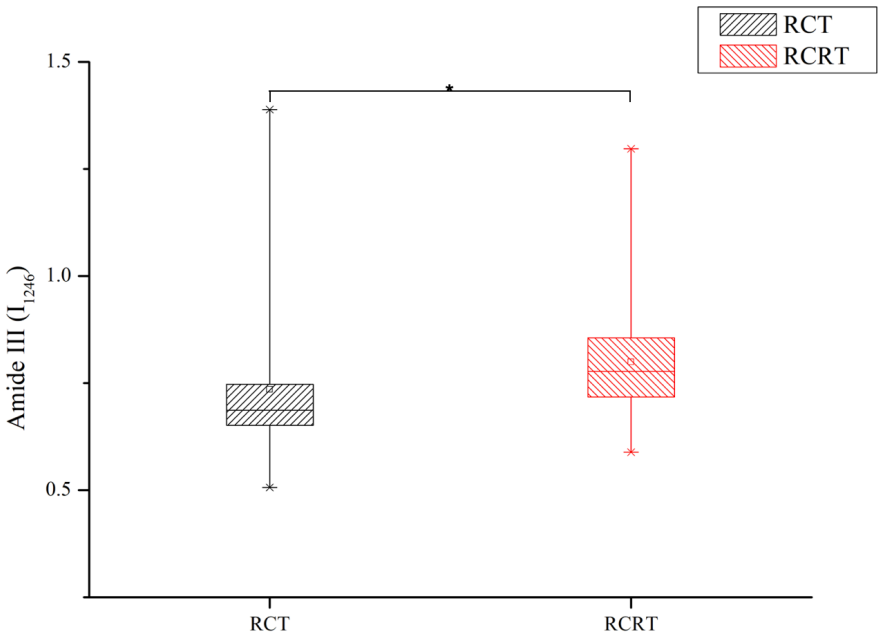

Fig 34: Intensity of Amide III (I1246)- The asterisk indicates a statistically significant difference (P<0.05). The box plots represent the dispersion of the data of the Amide III intensity, expressed as a percentage: the boxes include 50% of the values (from 25 to 75%) and are divided by the median line, the bars connect the maximum and the minimum values.

Fig. 35

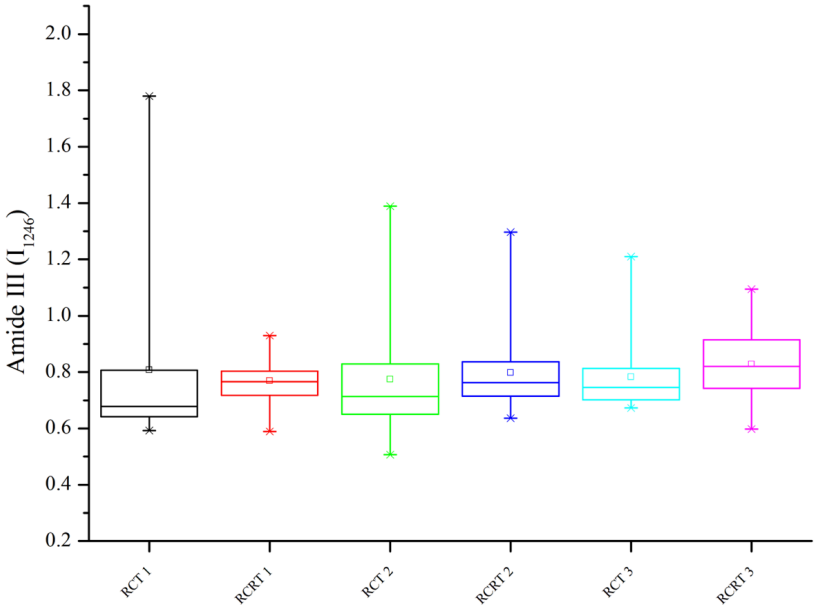

Fig 35: Amide III intensity (I_{1246})– No significant difference was found between subgroups (P>0.05). The box plots represent the dispersion of the data of the Amide III intensity, expressed as a percentage: the boxes include 50% of the values (from 25 to 75%) and are divided by the median line, the bars connect the maximum and the minimum values.

Also the intensity of the CH_2 peak was found significantly higher in the group with an aged endodontic therapy (p=0.0001) (Fig. 36), whereas no significant differences could be appreciated between anatomical subgroups (p>0.05).

Fig. 36

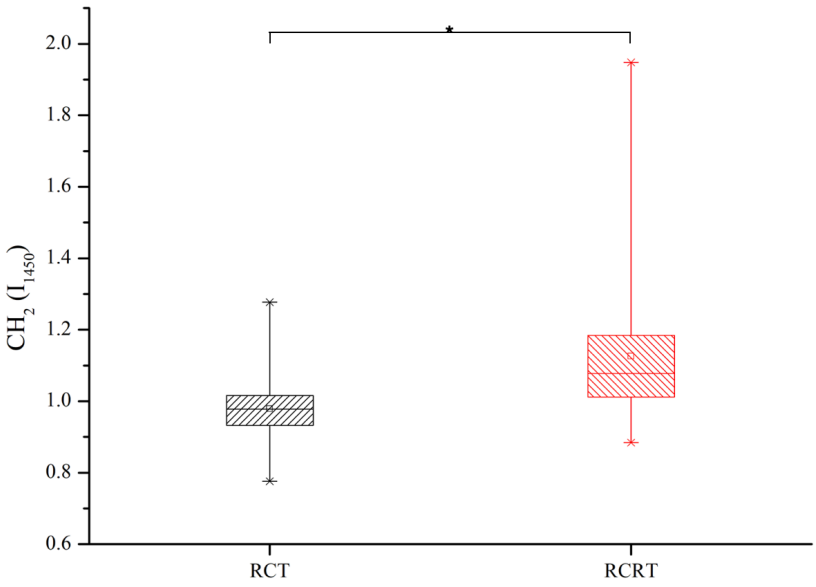

Fig. 36: CH_2 intensity (I_{1450}) – The asterisk indicates a statistically significant difference (P<0.05). The box plots represent the dispersion of the data of the CH_2 intensity, expressed as a percentage: the boxes include 50% of the values (from 25 to 75%) and are divided by the median line, the bars connect the maximum and the minimum values.

Likewise, in the RCR-T group, a significantly higher intensity of the amide I peak could be appreciated compared to the group with a recent endodontic therapy (p=0.005) (Fig. 37). Overall, amide I value was significantly higher in the middle and apical third compared to the coronal one (p<0.05), and in the apical third compared to the middle section (p<0.05) (Fig. 38), even though the difference between the apical and middle third in the RCR-T group is only close to statistically significance (p=0.052) (Fig. 38). Between the groups analysed (RCT *vs* RCR-T), amide I peak intensity was significantly different exclusively in the coronal third (p=0.025) (Fig. 38).

Fig. 37

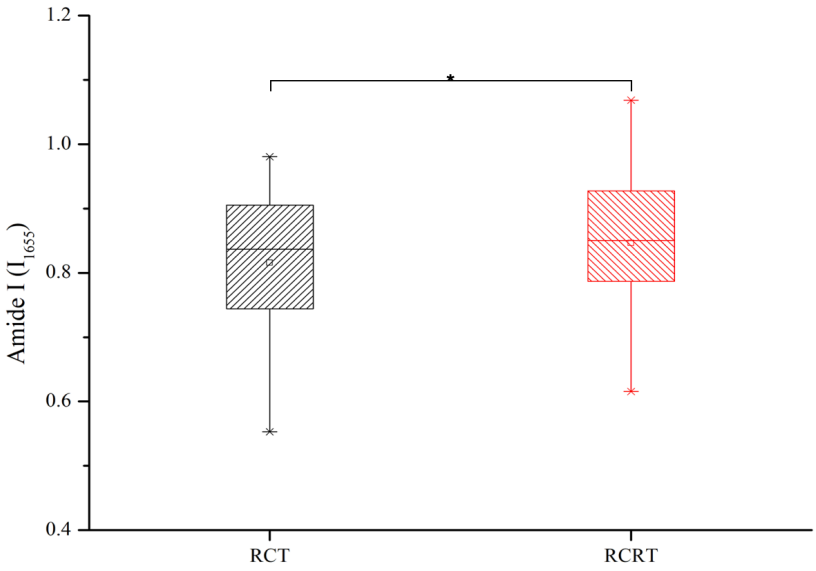

Fig. 37: Amide I intensity (I_{1655}) – The asterisk indicates a statistically significant difference ($p<0.05$). The box plots represent the dispersion of the data of the Amide I intensity, expressed as a percentage: the boxes include 50% of the values (from 25 to 75%) and are divided by the median line, the bars connect the maximum and the minimum values.

Fig. 38

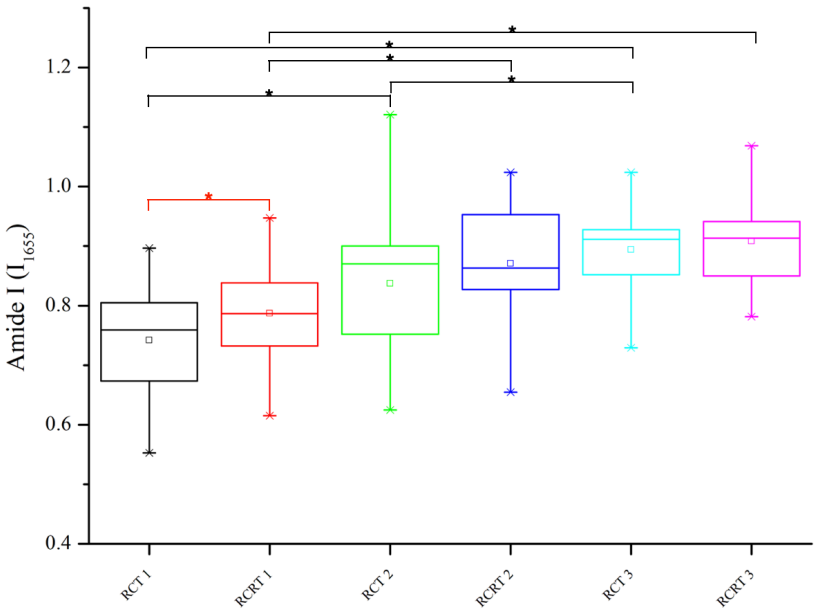

Fig. 38: Amide I intensity for coronal (1), middle (2) and apical (3). The asterisk indicates a statistically significant difference ($P<0.05$). The red bar highlights differences between groups. The black bar highlights differences within groups. The box plots represent the dispersion of the data of the Amide I intensity, expressed as a percentage: the boxes include 50% of the values (from 25 to 75%) and are divided by the median line, the bars connect the maximum and the minimum values.

Table 13 reports means and standard deviations (SD) of the peak intensities belonging to the organic phase of radicular dentin.

Table 13 – Peak intensities and extracted parameters of organic components.

		Amide I/ Amide III		**Amide I/CH_2**		**Amide I**		**Amide III**		**CH_2**	
		Mean and SD	Median and 25°-75° percentile	Mean and SD	Median and 25°-75° percentile	Mean and SD	Median and 25°-75° percentile	Mean and SD	Median and 25°-75° percentile	Mean and SD	Median and 25°-75° percentile
RCT	coronal	1.08 (0.19) [a]	1.086 (1.049-1.129) [a]	0.75 (0.07) [a]	0.752 (0.706-0.793) [a]	0.72 (0.07) [a]	0.732 (0.668-0.778) [a]	0.69 (0.15) [a]	0.665 (0.623-0.684) [a]	0.96 (0.08) [a]	0.954 (0.911-1.021) [a]
	middle	1.15 (0.22) [a]	1.21 (1.034-1.338) [a]	0.85 (0.10) [b]	0.864 (0.790-0.933) [b]	0.81 (0.09) [b]	0.857 (0.745-0.878) [b]	0.72 (0.17) [a]	0.681 (0.637-0.740) [a]	0.96 (0.13) [a]	0.948 (0.921-1) [a]
	apical	1.17 (0.18) [a]	1.213 (1.128-1.294) [a]	0.88 (0.08) [b]	0.912 (0.849-0.936) [b]	0.88 (0.06) [c]	0.910 (0.846-0.926) [c]	0.77 (0.13) [a]	0.736 (0.688-0.801) [a]	1.01 (0.10) [a]	0.994 (0.971-1.025) [a]
	overall	1.14 (0.20) [1]	1.213 (1.128-1.294) [1]	0.83 (0.10) [1]	0.853 (0.750-0.925) [1]	0.81 (0.1) [1]	0.836 (0.741-0.905) [1]	0.73 (0.15) [1]	0.687 (0.651-0.750) [1]	0.98 (0.10) [1]	0.977 (0.930-1.02) [1]
RCR-T	coronal	1.02 (0.13) [a]	1.021 (0.935-1.092) [a]	0.72 (0.08) [a]	0.725 (0.664-0.774) [a]	0.77 (0.08) [d]	0.778 (0.714-0.822) [d]	0.76 (0.09) [a]	0.753 (0.711-0.847) [a]	1.08 (0.13) [a]	1.065 (0.986-1.156) [a]
	middle	1.09 (0.21) [a]	1.138 (1.017-1.246) [a]	0.77 (0.12) [c]	0.814 (0.677-0.843) [c]	0.85 (0.1) [be]	0.860 (0.821-0.929) [bc]	0.80 (0.14) [a]	0.762 (0.702-0.846) [a]	1.11 (0.15) [a]	1.075 (1.012-1.176) [a]
	apical	1.08 (0.19) [a]	1.146 (1.005-1.194) [a]	0.80 (0.13) [c]	0.827 (0.748-0.907) [c]	0.90 (0.07) [ce]	0.911 (0.843-0.940) [ce]	0.88 (0.30) [a]	0.830 (0.735-0.920) [a]	1.17 (0.26) [a]	1.097 (0.996-1.227) [a]
	overall	1.06 (0.18) [2]	1.081 (0.989-1.195) [2]	0.76 (0.12) [2]	0.784 (0.679-0.843) [2]	0.84 (0.1) [2]	0.851 (0.786-0.927) [2]	0.82 (0.20) [2]	0.778 (0.717-0.867) [2]	1.12 (0.19) [2]	1.077 (1.011-1.184) [2]

Table 13: Means and SD; Median, 25° and 75° percentile of the peak intensities of radicular dentin organic components. Within each column different superscript letters or numbers indicate statistically significant differences ($p < 0.05$). Between groups only the same sub-groups are to be compared.

3.4 Bond strength of the radicular dentin adhesive interface: Young *vs* aged radicular dentin.

Results of the three-way ANOVA showed, overall, significantly lower bond strength values for the young radicular dentin compared to the old one (p=0.000). The variables "cement" (p=0.559) and "root area" (p=0.817) did not significantly influence the bond strength values, while the interaction between "age" and "cement" had a significant impact (p=0.006). Bond strength data were expressed as means and standard deviations (SD) and summarised in Table 14.

Table 14

	iCEM Young	Clearfil DC Young	iCEM Old	Clearfil DC Old
C	6.95 (± 1.86) $^{a, 1}$	7.74 (± 3.21) $^{ab, 1}$	10.42 (± 3.09) $^{b, 1}$	9.55 (± 3.30) $^{b, 1}$
A	6.66 (± 3.67) $^{a, 1}$	8.66 (± 2.92) $^{ab, 1}$	10.34 (± 4.03) $^{b, 1}$	9.39 (± 2.81) $^{b, 1}$

Table 14 – Bond strength values (expressed in MPa) according to different groups. Values are expressed as mean (± SD). C and A stand, respectively, for coronal and apical. Within each line, different superscript letters indicate statistically significant differences. Within each column different superscript numbers indicate statistically significant differences ($p < 0.05$).

3.4.1. Failure mode of the radicular dentin adhesive interface: Young *vs* aged radicular dentin.

Failure modes distribution of the debonded specimens, expressed as percentages of the total number of specimens tested are summarised in Table 15, where a predominance of adhesive failures, between dentine and resin cement, can be appreciated in all groups. However, Pearson chi2 test showed a statistical correlation between groups and type of fracture (p=0.322). Indeed, in the apical sections a much higher presence of mixed fracture was detected.

Table 15

	YIC	YIA	YDC	YDA	OIC	OIA	ODC	ODA
Adhesive	68.96	51.85	81	60	77	47	71	33
Cohesive	13.79	7.40	15	17	13	20	23	26
Mixed	17.24	40.76	4	23	10	33	6	41

Table 7 – Failure modes after Push-out test. Values are expressed as percentage (%). YIC (Young-iCEM-coronal), YIA (Young-iCEM-apical), YDC (Young-Clearfil DC-coronal), YDA (Young-Clearfil DC-apical), OIC (Old-iCEM-coronal), OIA (Old-iCEM-apical), ODC (Old-Clearfil DC-coronal), ODA (Old-Clearfil DC-apical).

Discussion

4.1 Bond strength and morphological analysis of the radicular dentin adhesive interface: Freshly *vs* aged root canal treated radicular dentin.

The challenge of effective bonding to root canal dentin is well described (Maroulakos 2018). Seemingly, the micro-morphology of the radicular resin-dentin interface (R-DI) and its bond strength have been already investigated in RCT teeth (Bitter 2014, Barreto 2016, Pelozo 2019, Erik 2020). Nonetheless, a gap in the literature exists regarding RCR-T teeth, where no evaluations of the R-DI micro-morphology have been performed. Furthermore, few studies investigating the bond strength in RCR-T teeth were conducted by treating and re-treating vital specimens (Guedes 2014, Pelegrine 2016, Pereira 2019), and one of them was even carried-out on bovine teeth (Guedes 2014). However, this procedure implies that the contribution of natural ageing is excluded from the factors that may affect this specific substrate and there is no correspondence with a clinical scenario. Otherwise, in the RCR-T specimens collected in this study for bond-strength and R-DI morphologic analysis, the former endodontic treatment was executed at least 15 years earlier, simulating a clinical environment.

Based on the present results, the first null hypothesis should be substantially rejected since the bond strength of freshly endodontically treated dentin was significantly higher than that obtained in naturally aged and devitalized, at least 15 years earlier, teeth undergoing endodontic re-treatment ($p=0.01$). This is in agreement with the literature (Guedes 2014, Pelegrine 2016, Pereira 2019), even though Pelegrine et al. showed that only SERc was affected by the substrate, markedly in the apical portion, and no significant difference was registered for the SARc (Pelegrine 2016). It is generally accepted that debris and smear layer produced by RCR-T procedures may act as an obstacle between resins and the dentinal surface, being difficult to eradicate from the post space (Guedes 2014). Guedes et al. demonstrated that the solvents used to remove gutta-percha might be detrimental towards the adhesive procedures (Guedes 2014), since they could penetrate dentinal structure enough to withstand the debriding action of the post space preparation. The softened gutta-percha itself could easily be compacted into dentinal tubules, where it cannot be removed, thus hampering the bond strength of resin cements (Horvath 2009). Moreover, it has been reported that gutta-percha solvents can alter the chemical structure (organic and inorganic composition) of dentin, and that any changes in these components can affect the adhesion of restorative materials (Rotstein 1999). In such regard, it can be expected that the solvent used in the present study may have contributed to the worse bonding performance of the re-treated specimens.

As stated before, aged dentin is a less favourable substrate for adhesion (Lopes 2011, Perdigao 2013) and root canal treatment further accelerates the ageing process (Yan 2019). One of the main and most evident structural modifications that takes place with ageing is the gradual reduction of tubules lumen, due to the accumulation of minerals in peri-tubular dentin (Montoya 2015). After an adequate

number of lumens have been filled, the tissue appears transparent, as the amount of light scatter off of the lumens decreases, and dentin that has undergone this change is known as “sclerotic” (Kinney 2005). Besides the increased amount of mineral content, another important change is represented by the size of the mineral crystallites. They are smaller in transparent dentin than in normal dentin (Kinney 2005). Thus, because of the nature of the deposited material filling the lumens, dentin ageing leads to an increase in mineral content (Porter 2005). In general, older teeth have a higher mineral-to-collagen ratio compared to young ones and this also accounts for an increased hardness (Montoya 2015). Within the intertubular matrix, the most important modifications take place in the collagenous microstructure. Type I collagen, once synthesised, undergoes extensive modifications, resulting in a characteristic pattern of cross-links (Robins 2007). These changes in the collagen matrix may increase dentinal fragility, contributing to the structural response (Yan 2019).

Therefore, it can be speculated that the aforementioned age-related organic and inorganic structural changes may render the interaction of the dentinal substrate with adhesive resins less favourable.

In the present study, the luting cement employed had a significant influence on bond strength only when considering the coronal half of the post-space, where SARc was superior to SERc irrespective of the canal treatment ($p=0.023$). Pelegrine et al. reported similar results, but only in the apical third of the re-treated specimens (Pelegrine 2016). In SARc, the methacrylate monomers modified by carboxylic or phosphoric acid-groups, can condition the dentinal substrate without any etching or bonding pre-treatment. These systems do not require smear layer removal, that is rather modified and infiltrated (Ferracane 2011). Besides the micro-mechanical interlocking SARc also interact with calcium ions creating a chemical bond (Ferracane 2011). Moreover, they are less sensitive to humidity, which may be relevant in areas where moisture control is difficult, such as the post-space (Maroulakos 2018). These properties may explain the positive bond strength performance of SARc. Nonetheless, other reports showed different results. Pereira et al. discouraged the use of SARc in re-treated teeth even though the difference with SERc was not significant (Pereira 2019). The heterogeneity of outcomes may be explained, besides the differences in methodologies, by the vastness of luting cements available on the market.

Comparisons have often to be made between studies testing different materials. The heterogeneous group of cements available on the market differs in terms of composition, delivery system, setting reaction, setting time and pH (Radovic 2008, Miotti 2020). The bond strength of the SARc used in this study (iCEM) had never been tested in radicular dentin before.

The lack of agreement persists when analysing the impact of root area on bond strength. Data obtained in the present investigation reflect the trend according to which adhesion is less effective in the apical portion of the post space ($p=0.003$) (Perdigao 2007, Guedes 2014, Maroulakos 2018). This may be coherent with the increased difficulty to remove debris from the deep area of the root canal, and the decreased number of dentinal tubules available for resin infiltration in this region (Maroulakos 2018). However, these drawbacks may be compensated by the better match between the canal and the post diameter in the apical area. Indeed, the fibre post retentive strength is the result of chemical bonds, micro-mechanical interlocking and sliding friction (Goracci 2005). Moreover, the better polymerization of resin cements in the coronal third, due to the proximity of the curing light, is counterbalanced by the higher polymerization shrinkage (Pulido

2016). Coherently, other studies reported the highest bond-strength values in the apical portion (Pelegrine 2016, Yuanli 2021), while in other reports the root region had no significant influence (Goracci 2004, Pelegrine 2016). A limitation of the present study is that the post-space was divided in only two halves: coronal and apical. This has been done in order to simplify procedures.
The failures analysis, assessed after the push-out test, revealed how most fractures were detected between the dentinal surface and the luting cement, namely adhesive failures. These findings are confirmed by other authors (Bitter-Paris-Mueller 2009, Ferracane 2011, Guedes 2014, Pereira 2019). Guedes et al. further investigated adhesive failures through confocal microscopy and reported how the separation occurred between the HL and the resin cement (Goracci 2004). The present study does not add data in this context since the CLSM analysis of failures was not a target of the study.
CLSM was rather used to investigate the micro-morphology of the R-DI. As stated by Bitter et al., CLSM is advantageous for the visualisation of more detailed information with respect to both penetration and distribution of resin cement and adhesive (Bitter-Paris-Mueller 2009). Moreover, in the same study, confocal microscopy, compared to scanning electron microscopy (SEM), provided comparable results in terms of HL thickness (Bitter-Paris-Mueller 2009). In the present investigation, Fluorescein and Rhodamine were used as dyes as they are easy to distinguish and do not diffuse one into the other (Bitter-Paris-Mueller 2009). However, drawbacks of CLSM are the difficulty to standardise the dye powder incorporation into the resin and the fact that the dye does not form any covalent bond with the resin. This may lead, respectively, to a not uniform dye distribution and to dye leaching into the hydrophilic dentinal tissue (D'Alpino 2006). As CLSM images of the present study showed homogeneous fluorescence, a uniform distribution of the dye could be assumed. An additional issue is represented by the possible negative effect of the dyes on polymerization and adhesive strength (D'Alpino 2006). However, this does not represent a limitation for the present study as the bond strength analysis was performed on different samples.
CLSM showed that RCR-T dentinal substrate, compared to RCT one, produced a significantly less thick HL ($p=0.03$) regardless of the luting cement it interacts with. As stated before, there are no studies evaluating the R-DI morphology in re-treated specimens to compare these data with. However, these results seem coherent with the abovementioned effect that natural ageing and endodontic treatment have on dentin (Guedes 2014, Montoya 2015, Yan 2019). HL thickness was also influenced by the type of luting cement employed. SERc, compared to SARc, produced a significantly thicker HL, regardless of the canal treatment ($p=0.001$). This is in line with the literature. As a matter of fact, SARc only interact superficially and do not produce a considerable HL (Ferrari 2001, Al Assaf 2007, Bitter-Paris-Pfuertner 2009, Bitter 2014). It must be pointed out that, due to this only superficial morphological interaction, the HL detection and measurement in CLSM images is challenging when evaluating SARc and its reliability and reproducibility could be questioned. Bitter et al. detected hybridization of dentin only sporadically (Bitter-Paris-Pfuertner 2009). Yet, it is important to have a visualisation of the micro-morphologic characteristics of the dentin-adhesive interface, and CLSM imaging has proven to provide a reliable estimation of HL thickness (Bitter-Paris-Mueller 2009).
Conversely, the root topography had no significant influence on the HL morphology, even though, as expected, a tendency of higher values in the coronal

region was registered ($p>0.05$). These results are supported by other authors (Bitter-Paris-Pfuertner 2009, Bitter 2014).
In this study, the aged endodontically re-treated substrate did not significantly affect resin tags formation. It may be speculated that the age- and treatment-related changes in inter-tubular dentin interfere more than the tubular characteristics in terms of resin infiltration (Gwinnett 1993). In all groups, resin tags were consistently represented and produced in comparable numbers by the luting cements tested. However, the ability of SARc to penetrate dentinal tubules is questioned by literature. Pelegrine et al., in a SEM investigation, stated that the tested SARc (RelyX U200, 3M ESPE, Seefeld, Germany) did not show any resin tag (Pelegrine 2016). Similar data were also obtained in a CLSM analysis in which the same luting cement was tested (Bitter-Paris-Pfuertner 2009). Therefore, such deep disagreement with the results of the present study may be mainly attributed to the different type of SARc used (iCEM). Resin tag formation was significantly influenced by the root region considered. It has been thoroughly demonstrated how the number of dentinal tubules decreases toward the apex (Garberoglio 1976). Coherently, significantly less resin-filled tubules are found in the apical portion of a post-space (Bitter 2014, Pelegrine 2016). The present study did not deviate from this assumption.
A final consideration is reserved to the irrigation protocol used in the present study. In general, endodontic irrigants do have an impact on the chemo-mechanical properties of dentin and on its bonding potential (Lai 2001, Hayashi 2005, Hi 2008, Dotto 2020). As a matter of fact, NaOCl irrigation may lower the resin-dentin bond strength values of RCT dentin (Morris 2001). When NaOCl is used for root canal irrigation, there might be some reactive free radicals which can cause the incomplete polymerization of monomers (Lai 2001). On the other hand, the possible adverse effects of EDTA on adhesion seem to depend on the bonding system used (Hayashi 2005). The removal of the smear layer is a disadvantage for adhesion when SERc systems are used (Hayashi 2005). This has to be taken into account when investigating adhesion on RCT teeth. Therefore, as the use of calcium chelating agents is an important step in Endodontics, excessive demineralization caused by endodontic irrigation should be avoided when SERc are to be used (Hayashi 2005). In the present study, a relatively short application of NaOCl and EDTA was performed, simulating endodontic irrigation in ordinary practice. Yet, resin tags and HL formation may have been also influenced by the irrigation protocol employed, since the demineralization and the deproteinization facilitate the penetration of the resin tags into the dentinal tubules (Hayashi 2005).
A limitation of the study is that, for the RCR-T group, it was not possible to gather precise information on the former endodontic treatments, performed at least 15 years earlier. These data, as the type of sealer used or irrigation protocol, are of importance as these factors could affect the structure and properties of root dentin.
Within the limitations of the present study, it can be concluded that resin cement bond strength potential is significantly hampered in an aged root canal treated substrate. However, the same substrate did not entirely interfere with hybridization, as resin tags formation was not affected by dentin condition.
Moreover, the present study demonstrated how iCEM SARc showed similar bond strength values compared to SERc.

4.1.1 Bond strength of the radicular dentin adhesive interface after one year ageing (T1): Freshly *vs* aged root canal treated radicular dentin.

The following study aimed to evaluate the differences, expressed in MPa, of bond strength to root dentin using different adhesive cementation protocols on different substrates: freshly (RCT group) *vs* aged (RCR-T group) root canal treated radicular dentin. The null hypothesis were that the quality of the dentinal substrate (RCT *vs* RCR-T) (1), the topography of the substrate (coronal *vs* apical half of the radicular post-space) (2) and the different adhesive approaches (self-etch *vs* self adhesive resin cement) (3) are unable to affect the immediate (T0) and over time (T1) bond strength and that one year ageing (T1) does not affect the bond strength in RCT and RCR-T teeth (4).

The results of the immediate bond strength were already discussed in the previous paragraph. Based on the data obtained from this study, the adhesive approach chosen and the topography of the post space, over time, affected the bond strength in a statistically significant way, while the quality of the substrate did not have any significant influence. Overall, one year ageing produced a significant drop of the bond strength. Therefore, the null hypothesis has to be partially rejected. Although the long-term direct water storage and the subsequent submission of root slices to a dislodging load during the push-out test do not reproduce clinical ageing and functional loads, this protocol has been widely used to test the bonding longevity of fibre posts (Leitune 2010, Zhou 2013, Shafiei 2016).

As said, in the present study, one year ageing in artificial saliva, overall, significantly influenced the bonding performances of the tested materials, which lead to the rejection of the fourth null hypothesis, as the bond strength significantly decreased after artificial storage. This finding is in accordance with existing literature (Bitter 2008, Marchesi 2013, Shafiei 2016, Angeloni 2017, Shafiei 2017, Comba 2019, Nogueira 2023). These results may partially be attributed to the degradation of both the resin and collagen matrix during the accelerated ageing used in this study via direct artificial saliva exposure of the sliced specimens. Indeed, sectioning the bonded roots before water storage can cause the formation of microcracks in polymer networks, as a consequence of water sorption, resulting in gaps at the post-adhesive interface (Hashimoto 2002, Radovic 2007) and hydrolytic degradation of collagen fibres (Montanari 2011). In the present study, the drop of the over time bond strength was more evident in the freshly root canal treated group (RCT) as the difference in immediate bond strength between RCT and RCR-T was statistically significant, while it was not after one year storage. One of the reasons for the lower degradation of the bonding performance of aged root canal treated radicular dentin (RCR-T) may be attributable to the increased presence, in this substrate, of cross-linked collagen fibres (Yan 2019). As a matter of fact, the cross-linking of amino acids creates a more stable collagenic net, with new stable covalent intra-molecular and inter-molecular peptide bonds and this results in reduced molecular mobility, which is mandatory to allow the collagenolytic activity of the enzymes (Liu 2011, Tezvergil-Mutluay 2012, Mazzoni 2014). More specifically, these irreversible conformational changes reduce potential cleavage sites of MMPs, decreasing the enzymatic biodegradation of collagen fibrils (Scheffel 2014). Coherently, the application of artificial cross-linkers prior bonding procedures has been proven to increase the long term bond strength of resin cements to radicular dentin (Mazzoni 2013, Shafiei 2016, Comba 2019, Souza 2021). However, self-

etch and self-adhesive luting systems may not be aggressive enough to demineralize beyond the smear layer and to expose the collagen fibrils or endogenous enzymes within the underlying intact dentin (Monticelli 2008). Therefore, the drop in push-out bond strength of the tested materials observed over time in the present study could be caused by other factors, such as water sorption of the hydrophilic components of these cements, or the degradation of ester bonds of the resin monomer components, rather than activation of MMPs.

Other authors, however, reported that ageing does not significantly affect the long-term bond strength of resin cement to radicular dentin (Santana 2014). In one study, where ageing did not interfere with the bonding performance of a self-adhesive cement, it was suggested that the long-term water storage may be not enough to negatively affect the SARc network as the collagen fibres could be completely covered by the resin phase, being inaccessible to the water needed to produce collagenolysis (Montanari 2011, Santana 2014). Some other authors, interestingly, obtained significantly higher bond strength values after one year ageing (Josic 2022). These findings were justified by a possible hygroscopic expansion of the luting cements and fibre posts produced by the penetration of water molecules during the 12 months storage of the root slices in artificial saliva (Chai 2004, Vichi 2008). According to this theory, the volumetric expansion of composite cement and fibre posts may increase the frictional resistance between the material and the canal walls, resulting in its greater resistance towards the axial forces applied during the push-out test (Goracci 2005). Although this phenomenon would not match with the general lower push-out values obtained after one year ageing, it may help to explain the better performance of the self-adhesive cement (SARc). As a matter of fact, this material shows peculiar water sorption and solubility characteristics (Mazzitelli 2009). The acidic monomers, with hydrophilic characteristics, present in the SARc can absorb more water than the more traditional multi-step composite cements, and this would lead to their higher net expansion and more intimate contact to root canal walls (Park 2014). Nonetheless, other authors reported no significant differences of long-term bond strength between SERc and SARc (Shafiei 2016). However, it is important to stress that the bonding performances of self-adhesive resin cements depend on the chemical interaction between the acidic monomers and the hydroxyapatite of dentin (Sarkis-Onofre 2014) and are less sensitive to the operative technique since they do not require dentin pre-treatment, which implies that their behaviour is more material-dependent than technique-dependent (Lorenzetti 2019). Therefore, to discuss the performances of self adhesive resin cements compared to other systems, the wide product-specific differences in bond strengths of these materials inside the root canal has to be always taken into account (Marchesi 2013, Bitter 2017). Indeed, one of the most relevant product-specific features is the different sorption characteristics of the various SARc (Marghalani 2012), whose importance has been just described.

In the present investigation, after one year ageing, the bond strength values in the coronal section of the post-space were found significantly lower than the values registered in the apical half. Even though elements with oval canals were not included in the present study, it can be hypothesised that the better match between fibre post diameter and canal shape in the apical third may have favoured, on the long term, the higher bond strength values registered in this anatomical region. As a matter of fact, the fibre post retentive strength is the sum of chemical bonds, micro-mechanical interlocking and sliding friction (Goracci 2005). Furthermore,

the better polymerization of resin cements in the coronal third, due to the proximity of the curing light, may be unfavourably counterbalanced by a higher polymerization shrinkage (Pulido 2016). This factor could also explain the higher bond strength values, on the long term, in the apical region of the root canal. Nonetheless, other authors reported that the root region did not have any influence (Pala 2014, Shafiei 2016, Josic 2022), suggesting that the establishment of a proper balance between the different components of the cements and an efficient polymerization initiation and propagation, would solve the issue of differences in the quality of polymerization in different root regions (Josic 2022). Nonetheless, other reports showed higher bond strength values for the coronal sections of the post space (Santana 2014).

It has been reported that the use of posts, adhesive and resin cement combination from the same manufacturer could prevent potential incompatibilities between the materials and increase the chemical affinity and potential of each system (Bitter 2013). To date, no specific posts are recommended for the adhesive systems employed in the present study. However, as the vast majority of the failures for both the cements were detected at the dentin/cement interface, the compatibility between the fibre post and the adhesive materials employed can be assumed.

The main limitation of the present study is that the intraoral conditions were not fully simulated. Only a single load test on bonded root slices, without thermomechanical cycling, was employed to assess bond strength. Furthermore, only two luting systems were investigated. Therefore, the current results can not be generalised. Considering these limitations, although it would seem logical to assume that multi-step composite cements would exhibit a more durable bond strength to root canal dentin compared to the simplified self-adhesive composite cements, the immediate and over-time results of the present study emphasise that the use of simplified single-step luting systems, such as SARc, may be a reasonable option when adhesion is required in anatomical constraints with a heavily modified substrate, such as the post-space of RCT aged teeth. This observation is in agreement with existing literature (Kurtz 2003, Goracci 2005, Radovic 2008, Radovic-Mazzitelli 2008, Mazzoni-Marchesi 2009, Mazzitelli 2010, Sarkis-Onofre 2014). However many factors such as thermal changes, that determine expansion and contraction forces, occlusal loads, enzymatic degradation, salivary enzymes, and bacterial collagenolytic activity may affect the adhesive stability (De Munck-Van Meerbeck 2005, Schwartz 2005). Therefore, further studies are needed to assess the long-term performances of these cements.

4.2 Chemical and mechanical properties of freshly and aged root canal treated radicular dentin.

Radicular fracture is a serious threat for the long term survival of root canal treated teeth. One of the reasons for this clinical scenario may be an increased fragility of the dentinal tissue, which has been often linked to the presence of a root canal treatment (Kinney 2005, Porter 2005). However, to date, there are no exhaustive explanations regarding the mechanisms that lead to the brittleness of the endodontically treated dentin.

Nonetheless, several factors contributing to the weakening of the root canal treated tissue have been proposed (Kishen 2006). First of all, the amount of radicular dentin removed by the endodontic treatment, modifying the geometrical and structural properties of the tooth, may affect its resistance to fracture (Sathorn 2005, Tang 2010). Furthermore, the use of endodontic irrigants and medications may lead to a demineralization of the dentinal substrate (Marshall 1995, Doyon 2005). Moreover, the bacterial collagenolytic activity may further deteriorate the organic matrix of dentin, contributing to its weakening (Mayrand 1985). Other hypotheses focus on the possible dehydration of endodontically treated dentin and consequent change in its mechanical properties (Kishen 2006, Kishen 2007). In any case, these factors do not entirely explain why endodontically treated teeth are weaker and more prone to fracture (Kishen 2006).

Since dentin is the most abundant histological dental tissue, it may be expected that the reason for the greater fragility of root canal treated teeth is to be found in the alterations to its structure (Kinney 2005, Porter 2005). However, to date, very few studies regarding the properties of root canal treated radicular dentin with a real ageing and function in the oral cavity have been published. Therefore, the aim of this section of the present study was to assess how the endodontic treatment, over time, may influence the chemical and mechanical properties of radicular dentin.

Regarding the chemical properties, in the present study, the freshly endodontically treated teeth (RCT) showed a significantly higher mineral-collagen ratio compared to those with an aged endodontic treatment (RCR-T). Conversely, Yan et al. reported no significant differences between vital and root canal treated tissue (Yan 2019). It has been shown how sodium hypochlorite can increase the mineral-collagen ratio within the radicular dentin (Hu 2010, Gu 2017). Sodium hypochlorite can deteriorate the dentinal collagenic fibres it comes into contact with (Padmakumar 2022), leaving a mineral layer characterised by increased fragility and lower resistance to flexural fracture (Gu 2017, Wang-Feng 2017). It has been quantified that the superficial collagen loss after only 2 minutes of 2.5% NaOCl action is as 40%, reaching a plateau of 60% between 6 and 10 minutes of application (Ramirez-Bommer 2018). However this would only partially explain our results. Sodium hypochlorite seems to act at a depth of only 25-30 µm (Gu 2017, Ramirez-Bommer 2018), that may be increased as deep as 85 µm when a NaOCl-EDTA-NaOCl alternate irrigation is performed (Ramirez-Bommer 2018). In the present study, the dentinal substrate analysed with Raman microscopy also includes areas far from this zone of action. Although it seems unlikely that the action of irrigants can have repercussions even at a distance, some authors have demonstrated that the loss of collagen even for only 1 µm of depth can significantly influence flexural

rigidity and resistance (Gu 2017). Furthermore, in our study, sodium hypochlorite was used, with the same methodology and concentrations, also in the group with an aged root canal treatment, where the mineral-collagen ratio was lower. It could be hypothesised that the use of endodontic solvents and the intrinsic characteristics of dentin with a primary endodontic treatment (RCR-T) could have mitigated the effects of hypochlorite.

Compared to vital dentin, endodontically treated dentin, at the same age, seems to appear more sclerotic, a typical characteristic of older dentin (Thomas 1994). It also seems well established that the age factor is directly proportional to the mineral-collagen ratio (Montoya 2015). It would therefore be expected that elements with aged endodontic treatment would have an increased mineral-collagen ratio. All the samples included in this study, however, belong to the same age group (45-55) and no analyses on the occlusion degree of the dentinal tubules were performed. Considering the dentinal tissue of the aged endodontically treated teeth (RCR-T) as sclerotic would therefore be a speculation. For these reasons, no assumptions will be made in this regard.

Furthermore, in the present study, the intensity of the phosphate peak, that can be assumed as a representation of the amount of hydroxyapatite, was found comparable between freshly and aged root canal treated teeth. Similar results were obtained between vital and endodontically treated dentinal tissue by Zelic et al. (Zelic 2014). Considering that the mineral-collagen ratio is the ratio between the phosphate (960 cm^{-1}) and the amide I (1660 cm^{-1}) peaks, and having found, in the present study, that the peak intensity of phosphate between the two groups was similar, it can be assumed that the amide I peak intensity was higher in the aged endodontically treated dentinal tissue (RCR-T). This aspect will be further discussed in a following section. It has been suggested that the dentinal phosphate content may be affected by the use of ethylenediaminetetraacetic acid (EDTA), an endodontic irrigant with calcium-chelating properties (Ramirez-Bommer 2018). Ten minutes exposition to this agent may cause a 60% phosphate loss in a 19 µm layer (Ramirez-Bommer 2018). The phosphate loss front increases considerably when the use of EDTA follows that of hypochlorite (Ramirez-Bommer 2018). However, calcium-chelating agents seem not to have any effect on the content of carbonate (Gandolfi 2018, Bergmans 2004).

Indeed, another important aspect of the chemical analysis performed in the present investigation, is the carbonate-phosphate ratio. This value was found significantly higher for the specimens with an aged endodontic treatment. This points to the fact that, in this tissue, over time, an accumulation of carbonate may take place. This element replaces phosphate in the pure structure of hydroxyapatite. This ratio, therefore, indicates the degree of carbonate incorporation into the hydroxyapatite matrix (Salehi 2013). The results of this study are in agreement with those of a recent article (Karteva 2022). In the aged root canal treated dentinal tissue, unlike the vital one, in addition to the increase in carbonate, the presence of weaker apatitic structures has been reported, such as octacalcium phosphate (OCP), dicalcium phosphate dihydrate (DCPD) and tricalcium phosphate (TCP) (Zelic 2014, Karteva 2022). These elements can undermine the overall structural strength of the dentin (Suchanek 1998, Charriere 2001, Johnson 2011). It has been hypothesised that the presence of these calcium-poor compounds can be attributed to the dehydration and tissue re-crystallization that would occur following the removal of the dental pulp

and the consequent endodontic treatment (Karteva 2022). The hydroxyapatite crystals of dentin are covered by a hydrated layer with a composition similar to that of DCPD and OCP (Dorozhkin 2014, Rey 2007). This layer can be easily destroyed by dehydration and this would result in increased crystallinity and secondary precipitation of DCPC and OCP (Karteva 2022). We can hypothesise that a similar mechanism could explain the increased carbonate/phosphate ratio obtained in the present study. Furthermore, in the present research, the carbonate-phosphate ratio in the group with aged endodontic treatment (RCR-T) was found significantly higher in the apical and middle thirds of the radicular post-space compared to the coronal. This may perhaps suggest an apico-coronal progression of the carbonate deposition within the radicular dentin tissue. Regarding the use of sodium hypochlorite, conflicting results are reported about its effect on the carbonate-phosphate ratio (Hu 2010, Padmakumar 2022). However, it seems well established that this substance can remove, in addition to the organic matrix, also the phosphate, magnesium and carbonate ions from dentin (Sakae 1988).

Lower carbonate content within a mineral structure corresponds, theoretically, to increased crystallinity (Xu 2009). Although in the present study the elements with old endodontic treatment showed an increased presence of carbonate, their crystallinity did not differ from that detected in the recently endodontically treated samples. Results obtained on cortical bone have shown how increased crystallinity can correspond to better stiffness and resistance to fracture and lower ductility of the tissue (Yerramshetty 2008). To date no data have been published regarding the crystallinity of dentin with a real endodontic history with which we could compare our results.

Nonetheless, it has also been suggested that the mineral content, alone, is not determinant to the mechanical properties of dentin (Kinney-Marshall 2003). Indeed, the changes in the mineral component due to the endodontic treatment are accompanied by those that occur in the organic structure. The latter, certainly, has an important role in terms of the mechanical responses of the tissue. To assess the chemical properties of the dentinal organic matrix, protein structure is one of the main factors to take into account. Amide bands, given the repetition of the peptide unit of proteins, have been widely used to study their secondary structure and their conformational transitions (Ager 2005).

In the present study, it has been found that the tissue with old endodontic treatment (RCR-T) showed significantly higher levels of amide I compared to the one with a recent endodontic treatment (RCT), and whose chemical properties can be therefore considered closer to those of vital dentin. This result is quite surprising if we consider that aged endodontically treated dentin show changes similar, if not more evident, to those that take place in old dentin (Yan 2019) which, unlike bone tissue, does not undergo collagenic turnover and is therefore expected to have less collagen. On the other hand, however, other authors had already found, highlighting the controversy, how amide I peaks increased with age, both in dentin and in bone tissue (Ager 2005, Ager 2006). A possible explanation could be a hypothetical lower water content in the dentin tissue with old endodontic treatment. Ager et al. reported an increase in amide I intensity in dehydrated dentinal tissue. This would be due to a greater interaction of collagen fibres caused by elongation and intrafibrillar movement which would lead to an increase in intercollagenic hydrogen bonds (Lakshmi 2003, Ager 2005). Conversely, the presence or absence

of mineral (even intra-fibrillar) may not have a direct effect on the intensity of the amide I peak (Ager 2006). Another phenomenon that could explain the increase in amide I in endodontically treated tissue, in addition to dehydration, could be the increase, in this substrate, in cross-links between collagen fibres (Yan 2019).

According to some authors, hydrated collagen fibres have also a better structural organisation, while dehydration causes structural disorders and mechanical stress. Dehydration would lead to the loss of adsorbed or chemically bound water molecules, leading to the destabilisation of the quaternary structure of collagen molecules and lowering the degree of organisation of the fibrils (Bella 1995, Price 1997, Orgel 2000). This could be one of the reasons why, in our study, the amide I/amide III and amide I/CH2 ratios were higher in recently endodontically treated teeth (RCT). A significant reduction in the amide I/amide III ratio is linked to an alteration of the secondary structure of collagen (Xu 2012). The amide I/CH2 ratio is also an indication of structural modifications (Salehi 2013).

The possible effects of endodontic irrigants on the collagenous matrix have been previously discussed. There is evidence that calcium-chelating agents can also have an influence on collagen conformation. Indeed, some authors have shown how EDTA and citric acid can significantly alter the levels of amide III found at the dentin level, indicating a certain conformational rearrangement of the proteins (Taddei 2017, Gandolfi 2018). A decrease in the amide III peak may indicate a disorganisation in the secondary structure of the protein unit that forms collagen fibres (Bet 2001), modifying the natural architecture between the organic and mineral components of dentin (Prestes 2013). In the present study, however, the lowest amide III peak levels were found in the newly endodontically treated group.

The results obtained in the present investigation seem to indicate that endodontic treatment, over time, does not lead to a quantitative loss of organic material (the peaks of amide I, amide III and CH_2 were even more intense in this tissue), but instead leads to a worse organisation and quality of the collagen itself (lower amide I/amide III and amide III/CH_2 ratios). However, there is a lack of studies in the literature that analyse the chemical properties of the organic matrix in elements treated endodontically and extracted after years of function. Therefore, it is difficult to compare our results with those of other authors.

As previously anticipated, it is commonly accepted that endodontically treated teeth are more prone to fracture than vital ones (Tang 2010). Nonetheless, the scientific evidence that endodontically treated dentin has compromised mechanical properties and is therefore more fragile and weaker than vital dentin does not seem to be that strong. To date, only a few authors have focused on the mechanical properties of dentin with a real endodontic history. As a consequence, it is difficult to compare our results with existing literature.

In this regard, in the present study, it was found that the dentinal tissue endodontically treated 15 years earlier (RCR-T) had a significantly lower Young's modulus than that of dentin with a recent endodontic treatment. These results find support in the literature (Carter 1983, Huang 1992). Other authors have instead concluded that the elastic modulus and hardness of intertubular dentin do not change before and after root canal treatment (Cheron 2011). In cortical bone, Young’s modulus has been directly correlated to crystallinity, which indicated a

more mature and elongated crystal status (Yerramshetty 2008). However, in the present investigation, no differences were found, between the two groups, in terms of crystallinity.

Furthermore, aged root canal treated radicular dentin, in the present study, showed also significantly lower values of both Vickers and Martens hardness. Other authors did not report any difference in hardness between the two substrates (Lewinstein 1981, Cheron 2011). However, in the study by Cheron et al. (Cheron 2011), which reported no difference in elastic modulus and hardness between the two tissues, the age of the root canal treatment is not known. This could explain the difference of those data compared to the results of the present research, where only elements with an endodontic history of at least 15 years were taken into consideration. Nonetheless, decreased tissue hardness has been attributed to an increase in carbonate (Seyedmahmoud 2017). This would be in line with what was found in the present study and could help explain why tissue with aged endodontic treatment had significantly lower hardness. This characteristic may also vary on the basis of the location of the measurement. The hardness value decreases if the indentations are performed close to the pulp space. This may be explained by the higher number of widely open dentinal tubules near the pulp, which provides less resistance for the testing indenter (Pashley 1985, Oliveira 2007). In this study, the dentin hardness of the samples was measured at a depth of 300 µm from the pulp-dentin interface, at the mid-root level, and the obtained hardness measurements were in line with the literature (Ari 2004, Aslantas 2014). It has also been reported that hardness and elastic modulus of dentin is dependent exclusively on the mineral content rather than collagen (Balooch 1998). Accordingly, another study showed that after the removal of dentin collagen using NaOCl, the dentin had relatively unchanged hardness and elastic modulus, while an opposite outcome was seen when the dentin was etched before (Marshall 2001).

Specimens with aged root canal treatment, in the present study, showed also a significantly higher degree of plastic deformation upon nanoindentation than the freshly treated ones. This would mean that they are more easily subject to irreversible deformations. However it has to be pointed out that this difference was significant only from a statistical point of view. Considering the box-plots shown in Fig. 22, it is evident that the values are comparable and the difference is not scientifically relevant. These data are conflicting with what has already been demonstrated in a previous study where 50% of dentin samples from teeth with endodontic treatment had shown greater plastic deformation compared to vital dentin (Huang 1992).
It is noteworthy to highlight that, in the present study, the elastic modulus, Martens and Vicker's hardness showed the lowest values in the apical third of the post-space for both the RCT and RCR-T groups. In the RCT group even the degree of plasticization was found the highest in this anatomical section. These data seem to point, generally, toward lower mechanical properties of the root apical third. Since, as previously reported, in the apical third of both groups higher amide I peaks and amide I/CH_2 ratio were registered, which indicate better quality and organisation of the collagen, it can be assumed that the lower mechanical properties of the apical third are more to be attributable to the mineral component rather than the organic one and, more precisely, to the mineral-matrix ratio, that showed overall the lowest values in the apical third, and to the carbonate-phosphate ratio which showed the same pattern in the RCR-T group.

It has already been explained how the use of endodontic irrigants such as NaOCl and EDTA can negatively influence the mechanical properties of dentin such as hardness, elastic modulus and flexural strength (Grigoriatos 2001, Sim 2001, Pascon 2009, Ari 2004 , Cruz-Filho 2011, Ackay 2012, Machnick 2003, Wang 2017). This could help to shed light on the results obtained in the present study. In the samples analysed, these irrigants were used in the same modalities and quantities both for endodontic treatment (RCT group) and retreatment procedures (RCR-T group). That said, the specimens with old root canal treatment had probably already been subjected, during the primary treatment, to irrigation procedures of which, however, we have no information. It can be easily hypothesised that this tissue has therefore been subjected over the years to at least a double exposure to chemical agents and this could be a contributing factor to the impoverishment of its mechanical properties.
Other studies attributed the decline of mechanical properties of dentin, such as increased fragility (Yan 2017, Miguez 2004), to the increase, over the years, in the cross-linking of collagen fibres (Walters 1983). Yan et al. demonstrated how this phenomenon is accelerated in endodontically treated teeth, suggesting that it is the main cause of the loss of mechanical resistance of these elements (Yan 2019).
Some authors, instead, attributed the change of mechanical properties of endodontically treated dentin to dehydration (Bertassoni 2012, Shemesh 2018, Rodig 2018). Indeed, Young's modulus and hardness of hydrated and dehydrated dentin have been analysed via nanoindentation (Bertassoni 2012). A statistically significant difference was found regarding hardness: dehydrated dentin had an average hardness value of 1.43 (0.12) GPa while hydrated dentin had 0.88 (0.11) GPa (Bertassoni 2012). Similar results have also been obtained by previous studies: Guidoni et al. reported a decrease in the elastic modulus of dry dentin by approximately 35% and in hardness by almost 30% (Guidoni 2006).
However, this remains a controversial topic. One one hand, some studies do report a loss of moisture in the dentinal tissue of endodontically treated teeth (Kishen 2005, Soares 2007, Yan 2011). On the other hand, however, it is unclear whether and how the mechanical resistance of endodontically treated dentin is associated with changes in the water content within this tissue (Kahler 2003, Bajaj 2006, Kishen 2007). Furthermore, in our study, no data were collected regarding the exact state of hydration of the samples investigated, which were, however, always stored at 100% humidity. Therefore, considering the aged endodontically treated dentin (RCR-T group) as a less hydrated tissue compared to the recently treated one (RCT group) would be a speculation.

Nonetheless, the degree of change of the mechanical, and consequently, of the chemical properties, seems to be specific for each individual and therefore probably also determined by factors such as systemic health conditions and use of drugs, eating habits, trauma history or chewing habits. For example, Yan et al. demonstrated how the same type of tissue, in patients of exactly the same age, demonstrated significant differences in terms of flexural resistance (Yan 2019).

An important aspect of our results, going into the specifics of canal topography, is that the differences between the two tissues under examination, for all the mechanical properties analysed, were found to be statistically significant for both the coronal and the middle third of the post-space , but not for the apical one. This could perhaps suggest a corono-apical progression, within the root, of the structural

breakdown, over the years, of the endodontically treated tooth. On the chemical side of the analysis, a similar trend could be detected for the amide I peak (I_{1655}) and the mineral-collagen ratio (I_{960}/I_{1655}) that have been found significantly different in the coronal third of the radicular post-space, but not in the middle and apical thirds. As no significant difference was registered for the mineral content (I_{960}) between the two groups in any of the anatomical sections analysed, it can be hypothesised that the differences in mechanical properties between the two groups in the coronal and middle thirds could be linked to the amide I peak.

This study has several limitations. First of all, the initial state of the pulp of the endodontically treated teeth is not known. The presence of any endodontic infections and/or state of pulp inflammation may have had an influence on the chemical properties of the dentin. Likewise, for obvious chronological reasons, we are not aware of the irrigation protocol used for the primary root canal treatment of elements treated at least 15 years earlier. A precise knowledge of the quantities, quality, concentration and timing of application of each chemical agent used in the primary endodontic treatment would have been an important information for a clearer understanding of the chemical and structural changes.
Another limitation in the mechanical analysis methodology is that the nanoindentation performed in the present study did not allow the distinction between peri- and intertubular dentin. Furthermore, as mentioned before, the nanoindentation procedures were performed in a dry environment. Any dehydration during the measurements could have caused the collapse of the collagen fibres and therefore affected the outcomes (Marshall 1998, Saeki 2001). The results we obtained must therefore be evaluated taking these factors into account.

The decreased resistance of root canal treated teeth has certainly multifactorial causes. One of the determining factors remains the loss of tooth substance caused by previous pathologies (caries and trauma) and by root canal shaping and conservative-prosthetic procedures (Reeh 1989, Sedgley 1992). However, taking into account the limitations of the present study, the results presented in this investigation demonstrate how endodontic treatment, per se, may produce a chemical and structural breakdown of the radicular dentinal tissue over the years. It can be hypothesised that the increase in carbonate (carbonate-phosphate ratio), and therefore the increase of weaker apatitic structures, on one hand and the decrease in collagen quality and organisation (amide I-amide III and amide I-CH_2 ratio) on the other, may be linked to the lower mechanical properties of aged root canal treated radicular tissue, such as lower elastic modulus and hardness. These important chemical and mechanical changes could indeed play a significant role in the survival of the endodontically treated teeth. The null hypotheses 4) and 5) can therefore be rejected.
However, the present work is the first to assess the chemical and structural properties of root dentin subjected to years of function after endodontic treatment. Therefore, further studies are necessary to better understand the correlation of chemical properties with mechanical ones and their role in the increased fragility of endodontically treated teeth.

4.3 Bond strength of the radicular dentin adhesive interface: Young *vs* aged radicular dentin.

The increase of life expectancy (Mayhew 2015) and the public health improvement (Muller 2017) have led to a higher demand for the restoration of elderly patients' teeth. However, ageing of the dentinal tissue is related to important chemical and structural modifications, such as the increase of mineral deposition within the tubules and branches that fenestrate into inter-tubular dentin and the cross-linking of the collagen fibres (Montoya 2015, Yan 2019, Weerakoon 2022). A dentinal tissue that has undergone these changes, generally known as "sclerotic dentin", may influence the behaviour of adhesive systems.

For instance, this physiological hypermineralization makes the aged dentin less sensitive to acid etching compared to normal dentin (Ding 2009, Lopes 2011, Weerakoon 2022). Therefore, since dentin adhesive strategy involves mainly micro-mechanical interlocking on inter-tubular dentin, combined with resin tags formation in the dentinal tubules, it would be reasonable to expect that the traditional etch-and rinse strategies on old hypermineralized dentin would be less effective (Lopes 2011). However, only a very few in vitro studies compared the effect of dentin age on dentin bond strength (Tagami 1993, Burrow 1994, Giannini 2003, Ozer 2005, Brackett 2008, Oliveira 2012). The majority of these reports did not find any significant effect of dentin age on adhesion (Tagami 1993, Burrow 1994, Giannini 2003, Brackett 2008, Oliveira 2012). To the best of the author's knowledge only one investigation has been published regarding the influence of dentin age on the bond strength of resin cements on root canal dentin (Limeira 2019).

The results of this study showed that age affected the bond strength of fibre posts cemented to root canals, leading to the rejection of the first null hypothesis. Self-etch (SERc) and self-adhesive (SARc) resin cements obtained higher bond strength values in the aged group over the young one, even though for the SERc the difference was not statistically significant. However, bond strength was not significantly influenced by the type of luting strategy and this leads to the acceptance of the second null hypothesis. Other authors obtained opposed results in radicular dentin, where the self-adhesive resin cement performed best in the young group and showed, generally, lower bond strength values compared to the self-etch approach. The authors attributed this result to the low diffusion of SARc in the dentinal substrate and to the poor formation of a hybrid layer and resin tags (Limeira 2019). Ozer et al. reported the better performance of one of the SERc tested on aged coronal dentin (Ozer 2005).

The rationale of using self-etch and self-adhesive approaches is based on the fact that these systems do not require any acid etching of the dentinal substrate. This may be a reasonable option on aged dentin tissue, particularly in anatomical sections where etching procedures and moisture control would be particularly challenging such as the post space of root canals (Rodrigues 2017). Self-etching adhesive systems are characterised by a simultaneous partial demineralization of the dentinal substrate and infiltration of resin monomers into the dentinal matrix, resulting in the creation of a thin and irregular hybrid layer containing scattered apatite crystals (Ubaldini 2018). Additionally, the acidic functional monomer

present in the SERc may interact with calcium ions present in the residual hydroxyapatite and form insoluble calcium salts (Ikemura 2003, Ubaldini 2018). Differently from Self-etch systems, Self-adhesive resin cements do not require any bonding agent (Mine 2014). As for SERc, these systems produce micromechanical retention and chemical interaction between acidic groups and hydroxyapatite. However, self-adhesive resin cements interact only superficially with dental hard tissues (De Munck 2004).

Therefore the chemical structure of the dentin matrix may have an influence on the bond strength of self-etching and self-adhesive systems as even small chemical changes may influence the tissue interaction behaviour and bonding efficacy (Nakajima 2005, Yoshihara 2011, Feitosa 2014). It has been reported that old intertubular dentin is characterised by higher Ca concentration and increased Ca:P ratio compared to young dentin (Xu 2014). Simultaneously, on peritubular dentin, a deposition of a less dense form of mineral, composed by apatite precipitation, takes place (Balooch 2001). These aged-induced tissue morphological and chemical alterations may affect the action of acid etching or resin monomer infiltration and therefore adversely affect the adhesion process (Giannini 2003, Lopes 2011, Xu 2014). At the same time, in our study, the hypothetical increased mineral content of the aged dentinal tissue may have determined the better performances of SERc and SARc in this group, as these systems chemically interact, via ionic bond formation, with the mineral matter of dentin (Fehrenbach 2021).

In the present study, the topography of the post-space did not have a significant influence on the bond strength irrespective of the luting materials used and the age group. Therefore, the second null hypothesis can be accepted. Conversely Limeira et al. reported a mean bond strength decreasing significantly from coronal to apical in the young group, but not in the old one (Limeira 2019).

One of the limitations of this study is that only two luting systems were tested. Future studies need to investigate the performances of different adhesive approaches on young and old root canal treated dentin. Therefore, the results obtained in the present research can not be generalised. A second limitation is that the intraoral conditions were not fully simulated. Only a single load test on bonded root slices, without thermomechanical cycling, was employed to assess bond strength. Within the limitations of the present study, it can be suggested that self-adhesive resin cements are not an ideal choice in young radicular dentin. The higher amount of organic content of this type of tissue may not favour the bonding mechanisms of SARc. These systems, on the contrary, seem to be an appropriate solution on substrates rich in mineral phase, as aged dentin. In general, as aged sclerotic dentin appear to be more resistant to demineralization (Ding 2009, Lopes 2011, Weerakoon 2022), probably as a result of the increased content of the mineral phase, it seems reasonable in this type of tissue to prefer adhesive approaches that do not contemplate the etching of dentin in order to aim for micromechanical interlocking. Van Meerbeek et al., already in 1994, showed the hybrid resin layer formed in sclerotic dentin to be much thinner, exhibiting few or no tubule tags, as compared to normal occlusal dentin (Van Meerbeek 1994). Therefore, in aged dentinal tissue it may be appropriate to choose materials that are rather capable to chemically interact with the substrate, as self-etch and self adhesive systems.

References

- Ager JW III, Nalla RK, Breeden KL, Ritchie RO (2005) Deep-ultraviolet Raman spectroscopy study of the effect of aging on human cortical bone. J Biomed Opt 10:034012:1–7.
- Ager JW, Nalla RK, Balooch G, Kim G, Pugach M, Habelitz S, Marshall GW, Kinney JH, and Ritchie RO, J. Bone Miner. Res. 21, 1879–1887 (2006).
- Akcay I, Sen BH. The effect of surfactant addition to EDTA on microhardness of root dentin. J Endod 2012;38:704–7.
- Akkus, A.; Yang, S.; Roperto, R.; Mustafa, H.; Teich, S.; Akkus, O. A Portable Fiber-Optic Raman Spectrometer Concept for Evaluation of Mineral Content within Enamel Tissue. J. Clin. Exp. Dent. 2017, 9, e238–e241.
- Al-Assaf K, Chakmakchi M, Palaghias G, Karanika-Kouma A, Eliades G. Interfacial characteristics of adhesive luting resins and composites with dentine. Dent Mater 2007; 23: 829–839.
- Andreasen JO, Farik B, Munksgaard EC. Long-term calcium hydroxide as a root canal dressing may increase risk of root fracture. *Dent Traumatol* 2002: 18: 134– 137
- Angeloni V, Mazzoni A, Marchesi G, Cadenaro M, Comba A, Maravi T, Scotti N, Pashley DH, Tay FR, Breschi L. Role of Chlorhexidine on Long-term Bond Strength of Self-adhesive Composite Cements to Intraradicular Dentin. J Adhes Dent. 2017;19(4):341-348.
- Arends J, Ruben J, and Jongebloed WL: Dentine caries in vivo: Combined scanning electron microscopic and microradiographic investigation. Caries Res. 23, 36 (1989).
- Arends J, ten Bosch JJ. Demineralization and remineralization evaluation tech- niques. J Dent Res 1992;71:924–8.
- Ari H, Erdemir A, Belli S. Evaluation of the effect of endodontic irrigation solu- tion on the microhardness and the roughness of root canal dentin. J Endod 2004;30:792–5.
- Ari H, Erdemir A. Effects of endodontic irrigation solutions on mineral content of root canal dentin using ICP-AES technique. J Endod 2005;31:187–9.
- Ari H, Yasar E, Belli S. Effects of NaOCl on bond strengths of resin cements to root canal dentin. J Endod 2003; 29:248 –51.
- Arola D, Ivancil J, Majid H et al. Microstructure and mechanical behaviour of radicular and coronal dentin. Endod Topics. 2012; 20:30.51.
- Arola D, Reprogel RK. Effects of aging on the mechanical behavior of human dentin. Biomaterials 2005: 26: 4051-4061
- Arola D. Fracture and aging in dentin. In: Curtis RV, Watson TF, eds. Dental Biomaterials: Imaging, Testing and Modeling. Cambridge, UK: Woodhead Publishing, 2007: 314-342.

- Aslantas EE, Buzoglu HD, Altundasar E, Serper A. Effect of EDTA, sodium hypochlorite, and chlorhexidine gluconate with or without surface modifiers on dentin microhardness. J Endod. 2014 Jun;40(6):876-9.
- B. Van Meerbeek, G. Willems, J.P. Celis, J.R. Roos, M. Braem, P. Lambrechts, and G. Vanherle: Assessment by nano-indentation of the hardness and elasticity of the resin-dentin bonding area. J. Dent. Res. 72, 1434 (1993).
- Balooch G, Marshall GW, Marshall SJ, Warrel OL, Asif SAS, Balooch M. Evaluation of new modulus mapping technique to investigate microstructural features of human teeth. Journal of Biomechanics 37 (2004) 1223-1232.
- Balooch M, Demos SG, Kinney JH, Marshall GW, Balooch G, Marshall SJ. Local mechanical and optical properties of normal and transparent root dentin. J Mater Sci Mater Med. 2001 Jun;12(6):507-14.
- Bandekar J, ‘‘Amide modes and protein conformation,’’ Biochim. Biophys. Acta 1120, 123–143 (1992).
- Barreto MS, Rosa RA, Seballos VG, Machado E, Valandro LF, Kaizer OB, Só M, Bier C. Effect of Intracanal Irrigants on Bond Strength of Fiber Posts Cemented with a Self-adhesive Resin Cement. Oper Dent. 2016 Nov/Dec;41(6): 159-167.
- Barth HD, Launey ME, MacDowell AA, Ager JW, and Ritchie RO, Bone 46, 1475–1485 (2010).
- Baumgartner JC, Cuenin PR (1992) Efficacy of several concentrations of sodium hypochlorite for root canal irrigation. Journal of Endodontics 18, 605–12.
- Bella J, Brodsky B, Berman HM 1995 Hydration structure of a collagen peptide. Structure 3:893–906.
- Bergmans, L.; Moisiadis, P.; Van Meebeek, B.; Quirynen, M.; Lambrechts, P. Microscopic observation of bacteria: Review highlighting the use of environmental SEM. Int. Endod. J. 2004, 38, 775–788.
- Bertassoni LE, Swain MV. Influence of hydration on nanoindentation induced energy expenditure of dentin. J Biomech. 2012 Jun 1;45(9):1679-83. doi: 10.1016/j.jbiomech.2012.03.021.
- Bet MR, Goissis G, Lacerda CA. Characterization of polyanionic collagen prepared by selective hydrolysis of asparagine and glutamine carboxyamide side chains. Biomacromolecules. 2001;2:1074-1079.
- Bhaskar SN (1980) In: Orban's oral histology and embryology. CV Mosby, St Louis, pp 107-140
- Bitter K, Gläser C, Neumann K, Blunck U, Frankenberger R. Analysis of resin-dentin interface morphology and bond strength evaluation of core materials for one stage post-endodontic restorations. PLoS One. 2014 Feb 28;9(2): e86294.
- Bitter K, Hambarayan A, Neumann K, Blunck U, Sterzenbach G. Various irrigation protocols for final rinse to improve bond strengths of fiber posts inside the root canal. Eur J Oral Sci 2013;121:349-54.
- Bitter K, Maletic A, Neumann K, Breschi L, Sterzenbach G, Taschner M. Adhesive Durability Inside the Root Canal Using Self-adhesive Resin Cements for Luting Fiber Posts. Oper Dent. 2017 Nov/Dec;42(6):E167-E176.

- Bitter K, Neumann K, Kielbassa AM. Effects of pretreatment and thermocycling on bond strength of resin core materials to various fiber-reinforced composite posts. J Adhes Dent 2008;10:481-489.
- Bitter K, Paris S, Mueller J, Neumann K, Kielbassa AM. Correlation of scanning electron and confocal laser scanning microscopic analyses for visualization of dentin-adhesive interfaces of the root canal. J Adhes Dent 2009; 11: 7–14.
- Bitter K, Paris S, Pfuertner C, Neumann K, Kielbassa AM. Morphological and bond strength evaluation of different resin cements to root dentin. Eur J Oral Sci. 2009 Jun;117(3):326-33.
- Brackett WW, Tay FR, Looney SW, Ito S, Haisch LD, Pashley DH. The effect of subject age on the microtensile bond strengths of a resin and a resin-modified glass ionomer adhesive to tooth structure. Oper Dent 2008;33:2825.
- Breschi L, Gobbi P, Mazzotti G, Ellis TH, Sacher E, Stangel I. Field emission in-lens SEM study of enamel and dentin. J Biomed Mater Res 1999;46:315–23.
- Breschi L, Perdigão J, Gobbi P, Mazzotti G, Falconi M, Lopes M. Immunocytochemical identification of type I collagen in acid-etched dentin. J Biomed Mater Res A 2003;66:764–9.
- Breschi L, Prati C, Gobbi P, Pashley D, Mazzotti G, Teti G, et al. Immunohistochemical analysis of collagen fibrils within the hybrid layer: a FEISEM study. Oper Dent 2004;29:538-46.
- Buonocore M, Quigley M. Bonding of a synthetic resin material to human dentin: preliminary study of the bond area. J Am Dent Assoc 1958;57:807-812
- Buonocore MG. A simple method of increasing the adhesion of acrylic filling materials to enamel surfaces. J Dent Res. 1955;34: 849-853
- Burrow MF, Takakura H, Nakajima M, Inai N, Tagami J, Takatsu T. The influence of age and depth of dentin on bonding. Dent Mater 1994;10:241-246.
- Butt N, Talwar S. In-vitro evaluation of various solvents for retrieval of mineral trioxide aggregate and their effect on microhardness of dentin. J Conserv Dent. 2013 May;16(3):199-202.
- Camargo CH, Siviero M. Camargo SE, de Oliveira SH, Carvalho CA, Valera MC. Topographical, diametral, and quantitative analysis of dentin tubules in the root canals of human and bovine teeth. J Endod 2007: 33: 422-426.
- Campi LB, Lopes FC, Soares LES, de Queiroz AM, de Oliveira HF, Saquy PC, de Sousa-Neto MD. Effect of radiotherapy on the chemical composition of root dentin. Head Neck. 2019 Jan;41(1):162-169.
- Carter JM, Sorensen SE, Johnson RR, et al. Punch shear testing of extracted vital and endodontically treated teeth. J Biomech 1983;16:841–8.
- Carvalho RM, Pereira JC, Yoshiyama M, Pashley DH. A review of polymerization contraction: the influence of stress development versus stress relief. Oper Dent 1996;21:17–24.
- Carvalho RM, Tjäderhane L, Manso AP, Carrilho MR, Carvalho CAR. Dentin as a bonding substrate. Endod Top 2012;2: 62–88.

- Chai J, Takahashi Y, Hisama K, Shimizu H. Water sorption and dimensional stability of three glass fiber-reinforced composites. Int J Prosthodont 2004;17:195–199.
- Charriere E, Terrazzoni S, Pittet C, Mordasini PH, Dutoit M, Lemaitre J, et al. Mechanical characterization of brushite and hydroxyapatitecements.Biomaterials. 2001 Nov;22(21):2937- 2945.
- Cheron RA, Marshall SJ, Goodis HE, Peters OA. Nanomechanical properties of endodontically treated teeth. J Endod 2011;37:1562–5.
- Chuenarrom, C., Benjakul, P., & Daosodsai, P. (2009). Effect of indentation load and time on Knoop and Vickers microhardness tests for enamel and dentin. Materials Research, 12(4), 473–476.
- Cohen SR, Apter N, Jesse S et al. AFM investigation of mechanical properties of dentin. Isr J Chem 2008; 48(2): 65–72.
- Comba A, Scotti N, Mazzoni A, Maravic T, Ribeiro Cunha S, Michelotto Tempesta R, Carossa M, Pashley DH, Tay FR, Breschi L. Carbodiimide inactivation of matrix metalloproteinases in radicular dentine. J Dent. 2019 Mar;82:56-62.
- Cruz-Filho AM, Sousa-Neto MD, Savioli RN, et al. Effect of chelating solu- tions on the microhardness of root canal lumen dentin. J Endod 2011; 37:358–62.
- D'alpino PH, Pereira JC, Svizero NR, Rueggeberg FA, Pashley DH. Use of fluorescent compounds in assessing bonded resin-based restorations: a literature review. J Dent 2006; 34: 623–634.
- Davies JM, Horwitz DA, Davies KJ (1993) Potential roles of hypochlorous acid and N-chloroamines in collagen break- down by phagocytic cells in synovitis. Free Radical Biology & Medicine 15, 637–43.
- De Munck J, Van Meerbeck B, Wevers M, Lambrechts P, Braem M. Micro-rotary fatigue of tooth-biomaterial interfaces. Biomaterials 2005;26:1145-1153.
- De Munck J, Vargas M, Van Landuyt K, Hikita K, Lambrechts P, Van Meerbeek B. Bonding of an auto-adhesive lutingmaterial to enamel and dentin. Dent Mater 2004;20:963–971.
- De Munck J,Van Landuyt K, Peumans M, et al.A critical review of the durability of adhesion to tooth tissue: methods and results. J Dent Res 2005;84:118 –32.
- De-Deus G, Paciornic S, Mauricio MH. Evaluation of the effect of EDTA, EDTAC and citric acid on the microhardness of root dentine. Int Endod J 2006;39:401–7.
- Di Renzo M, Ellis TH, Sacher E, Stangel I (2001) A photoacoustic FTIRS study of the chemical modifications of human dentin surfaces: II. Deproteination. Biomaterials 22, 793–7.
- Dogan H, Qalt S. Effects of chelating agents and sodium hypochlorite on mineral content of root dentin. J Endod 2001;27:578–80.
- Dorozhkin SV. Chapter 7: Nanodimensional and nanocrystalline calcium orthophosphates. In: Frontiers in nanobiomedical research. Tissue regeneration. World Scientific. 2014: 219-341.
- Dotto L, Sarkis Onofre R, Bacchi A, Rocha Pereira GK. Effect of Root Canal Irrigants on the Mechanical Properties of Endodontically Treated Teeth: A Scoping Review. J Endod. 2020 May;46(5):596-604.e3.

- Doyon GE, Dumsha T, von Fraunhofer JA. Fracture resistance of human root dentin exposed to intracanal calcium hydroxide. Journal of Endodontics 2005;31:895–7.
- Driscoll CO, Dowker SE, Anderson P, Wilson RM, Gulabivala K (2002) Effects of sodium hypochlorite solution on root dentine composition. Journal of Materials Science: Materials in Medicine 13, 219–23.
- Dychdala GR (1991) Chlorine and chlorine compounds. In: Block SS, ed. Chlorine and Chlorine Compounds. Philadelphia: Lea & Febiger, pp. 131–51.
- Erdemir A, Adanir N, Belli S. In vitro evaluation of the dissolving effect of solvents on root canal sealers. J Oral Sci. 2003;45(3):123-6. doi: 10.2334/josnusd.45.123.
- Erdemir A, Ari H, Gungunes H, Belli S. Effect of medications for root canal treatment on bonding to root canal dentin. J Endod 2004; 30:113–6.
- Erdemir A, Eldeniz AU, Belli S, Pashley DH. Effect of solvents on bonding to root canal dentin. J Endod 2004;30:589 –92.
- Erİk CE, Kaya BÜ, Maden M, Orhan EO. Influence of sodium hypochlorite/etidronic acid combination and SmearOFF on push-out bond strength of fiber posts to root dentin. Dent Mater J. 2020 Aug 2;39(4):554-562.
- Eyre DR, Grynpas MD, Shapiro FD. Creasman CM (1981) Mature crosslink formation and molecular packing in ar- ticular cartilage collagen. Semin Arthritis Rheum 11:46
- Eyre DR(1980) Collagen molecular diversity in the body's protein scaffold. Science 207:1315-1322
- Fedorowicz Z, Nasser M, Sequeira P, et al. Irrigants for non-surgical root canal treatment in mature permanent teeth. Cochrane Database Syst Rev 2012:CD008948.
- Fehrenbach J, Isolan CP, Münchow EA. Is the presence of 10-MDP associated to higher bonding performance for self-etch- ing adhesive systems? A meta-analysis of in vitro studies. Dental Materials. 2021;37:1463-85.
- Feilzer AJ, de Gee AJ, Davidson CL. Curing contraction of composites and glass ionomer cements. J Prosthet Dent 1988;59:297–300.
- Feilzer AJ, de Gee AJ, Davidson CL. Increased wall-to-wall curing contraction in thin bonded resin layers. J Dent Res 1989;68:48 –50.
- Feitosa VP, Ogliari FA, Van Meerbeek B, Watson TF, Yoshihara K, Ogliari AO, Sinhoreti MA, Correr AB, Cama G, Sauro S. Can the hydrophilicity of functional monomers affect chemical interaction? J Dent Res 2014;93: 201–206.
- Ferrari M, Vichi A, Grandini S. Efficacy of different adhesive techniques on bonding to root canal walls an SEM investigation. Dent Mater, 2001; 5: 422-29.
- Ferreira I, Braga AC, Lopes MA, Pina-Vaz I. Adjunctive procedure with solvent mixtures in non-surgical endodontic retreatment: does it affect root dentin hardness? Odontology. 2021 Oct;109(4):812-818.
- Ferreira I, Soares S, Sousa J, Barros J, Braga AC, Lopes MA, et al. New insight into the dissolution of epoxy resin–based sealers. J Endod. 2017;43(9):1505-10. doi: 10.1016/j. joen.2017.03.015.

- Frankenberger R, Pashley DH, Reich SM, Lohbauer U, Petschelt A, Tay FR. Characterisation of resin-dentine interfaces by compressive cyclic loading. Biomaterials 2005;26:2043-52.
- Fujimoto D, Akiba K, Nakamura N (1977) Isolation and characterization of a fluorescent material in bovine Achilles tendon. Biochem Biophys Res Commun 76:1124-1129.
- G.W. Marshall, N. Yucel, M. Balooch, and J.H. Kinney, S. Habelitz, S.J. Marshall: Sodium hypochlorite alterations on dentin and den- tin collagen. Surf. Sci. 491, 444 (2001).
- Gamsjaeger S, Robins SP, Tatakis DN, Klaushofer K, Paschalis EP. Identification of Pyridinoline Trivalent Collagen Cross-Links by Raman Microspectroscopy. Calcif Tissue Int. 2017 Jun;100(6):565-574.
- Gandolfi MG, Taddei P, Pondrelli A, Zamparini F, Prati C, Spagnuolo G. Demineralization, Collagen Modification and Remineralization Degree of Human Dentin after EDTA and Citric Acid Treatments. Materials (Basel). 2018 Dec 21;12(1):25.
- Garberoglio R, Brannstrom M. Scanning electron microscopic investigation of human dentinal tubules. Arch Oral Biol 1976: 21: 355-362.
- Geraldes, C.F.G.C. Introduction to Infrared and Raman-Based Biomedical Molecular Imaging and Comparison with Other Modalities. *Molecules* 2020, *25*, 5547.
- Giachetti L, Ercolani E, Bambi C, Landi D. Sclerotic dentin: aetio-pathogenetic hypotheses. Minerva Stomatol 2002;51:285–92.
- Giannini M, Chaves P, Oliveira MT. Effect of tooth age on bond strength to dentin. J Appl Oral Sci. 2003 Dec;11(4):342-7.
- Goldberg M, Takagi M. Dentine proteoglycans: composition, ultrastructure and functions. Histochem J 1993;25:781–806.
- Goracci C, Fabianelli A, Sadek FT, Papacchini F, Tay FR, Ferrari M. The contribution of friction to the dislocation resistance of bonded fiber posts. J Endod 2005; 31:608 –12.
- Goracci C, Sadek FT, Fabianelli A, Tay FR, Ferrari M. Evaluation of the adhesion of fiber post to intraradicular dentin. Oper Dent 2005;30: 627-635.
- Goracci C, Tavares AU, Fabianelli A, Monticelli F, Raffaelli O, Cardoso PC, Tay F, Ferrari M. The adhesion between fiber posts and root canal walls: comparison between microtensile and push-out bond strength measurements. Eur J Oral Sci 2004; 112: 353–361.
- Grigoratos D, Knowles J, Ng YL, Gulabivala K (2001) Effect of exposing dentine to sodium hypochlorite and calcium hydroxide on its flexural strength and elastic modulus.International Endodontic Journal 34, 113–9.
- Gu LS, Huang XQ, Griffin B, Bergeron BR, Pashley DH, Niu LN, Tay FR. Primum non nocere - The effects of sodium hypochlorite on dentin as used in endodontics. Acta Biomater. 2017 Oct 1;61:144-156.
- Guedes OA, Chaves GS, Alencar AH, Borges AH, Estrela CR, Soares CJ, Estrela C. Effect of gutta-percha solvents on fiberglass post bond strength to root canal dentin. J Oral Sci. 2014 Jun;56(2):105-12.
- Guidoni G, Denkmayr T, Scho¨berl T et al. Nanoindentation in teeth: influence of experimental conditions on local mechanical properties. Philos Mag 2006; 86(33/34/35): 5705–5714.

- Gwinnett AJ. Quantitative contribution of resin infiltration/hybridization to dentin bonding. Am J Dent 1993; 6:7–9.
- Habelitz S, Marshall SJ, Marshall GW Jr et al. Mechanical properties of human dental enamel on the nanometre scale. Arch Oral Biol 2001; 46(2): 173–183.
- Habelitz, S., Marshall, S.J., Marshall Jr., G.W., Balooch, M., 2001. Mechanical properties of human dental enamel on the nanometer scale. Archives of Oral Biology 46, 173–183.
- Hagger O. Swiss patent 278,946; British patent 687,299. 1951
- Hanabusa M, Mine A, Kuboki T, Momoi Y, Van Ende A, Van Meerbeek B, et al. Bonding effectiveness of a new "multi-mode" adhesive to enamel and dentin. J Dent 2012;40:475-84.
- Hannas AR, Pereira JC, Granjeiro JM, Tjäderhane L, Hannas AR, Pereira JC, et al. The role of matrix metalloproteinases in the oral environment. Acta Odontol Scand 2007;65:1–13.
- Hashimoto M, Ohno H, Endo K, Kaga M, Sano H, Oguchi H. The effect of hybrid layer thickness on bond strength: demineralized dentin zone of the hybrid layer. Dent Mater 2000;16:406 –11.
- Hashimoto M, Ohno H, Sano H, Tay FR, Kaga M, Kudou Y, Oguchi H, Araki Y, & Kubota M (2002) Micromorpho- logical changes in resin-dentin bonds after 1 year of water storage Journal of Biomedical Materials Research 63(3) 306-311.
- Hatibovic´-Kofman S, Raimundo L, Zheng L, Chong L, Friedman M, Andreasen JO. Fracture resistance and histological findings of immature teeth treated with mineral trioxide aggregate. *Dent Traumatol* 2008: 24: 272–276
- Hayashi M, Takahashi Y, Hirai M, Iwami Y, Imazato S, Ebisu S. Effect of endodontic irrigation on bonding of resin cement to radicular dentin. Eur J Oral Sci. 2005 Feb;113(1):70-6.
- Hedbom E, Heinegård D. Binding of fibromodulin and decorin to separate sites on fibrillar collagens. J Biol Chem 1993;268:27307–12.
- Helfer AR, Melnick S, Schilder H. Determination of the moisture content of vital and pulpless teeth. Oral Surg Oral Med Oral Pathol 1972: 34: 661-670.
- Hidalgo E, Bartolome R, Dominguez C (2002) Cytotoxicity mechanisms of sodium hypochlorite in cultured human dermal fibroblasts and its bactericidal effectiveness. Chemico- Biological Interactions 139, 265–82.
- Horvath SD, Altenburger MJ, Naumann M, Wolkewitz M, Schirrmeister JF (2009) Cleanliness of dentinal tubules following gutta-percha removal with and without solvents: a scanning electron microscopic study. Int Endod J 42, 1032- 1038.
- Hoshi K, Ejiri S, Probst W et al. (2001) Observation of human dentine by focused ion beam and energy-filtering transmis- sion electron microscopy. Journal of Microscopy 201, 44–9.
- Hu X, Peng Y, Sum CP, Ling J. Effects of concentrations and exposure times of sodium hypochlorite on dentin deproteination: attenuated total reflection Fourier transform infrared spectroscopy study. J Endod. 2010 Dec;36(12):2008-11.

- Huang TJ, Schilder H, Nathanson D. Effects of moisture content and endodontic treatment on some mechanical properties of human dentin. J Endod. 1992 May;18(5):209-15.
- Hulsmann M, Heckendorff M, Lennon A. Chelating agents in root canal treatment: mode of action and indications for their use. Int Endod J 2003;36:810–30.
- Ikemura K, Tay FR, Hironaka T, Endo T, Pashley DH. Bonding mechanism and ultrastructural interfacial analysis of a single step adhesive to dentin. Dent Mater 2003;19:693-804.
- Johnson AJW, Herschler BA. A review of the mechanical behavior of CaP and CaP/polymer composites for applications in bone replacement and repair. Acta Biomater. 2011 Jan;7(1): 16-30.
- Johnson GS, Mucalo MR, Lorier MA (2000) The processing and characterization of animal-derived bone to yield materials with biomedical applications: Part 1. Modifiable porous implants from bovine condyle cancellous bone and characterization of bone materials as a function of processing. Journal of Materials Science: Materials in Medicine 11, 427–41.
- Josic U, Mazzitelli C, Maravic T, et al. Evaluation of Fiber Post Adhesion to Root Dentin Achieved with Different Composite Cements: 1-year In Vitro Results. J Adhes Dent. 2022;24(1).
- Karan, K., Yao, X., Xu, C., Wang, Y., 2009. Chemical profile of the dentin substrate in non-carious cervical lesions. Dent. Mater. 25, 1205–1212.
- Karteva E, Manchorova N, Karteva T, Damyanov Z. Raman spectroscopy analysis of coronary dentin of vital and endodontically treated teeth. J of IMAB. 2022 Oct-Dec;28(4):4711-4714.
- Kaufman D, Mor C, Stabholz A, Rotstein I. Effect of gutta-percha solvents on calcium and phosphorus levels of cut human dentin. J Endod. 1997 Oct;23(10):614-5.
- Kekkonen, J.; Finnilä, M.A.J.; Heikkilä, J.; Anttonen, V.; Nissinen, I. Chemical Imaging of Human Teeth by a Time-Resolved Raman Spectrometer Based on a CMOS Single-Photon Avalanche Diode Line Sensor. Analyst 2019, 144, 6089–6097.
- Khedmat S, Hashemi A, Dibaji F, Kharrazifard MJ. Effect of chloroform, eucalyptol and orange oil solvents on the microhardness of human root dentin. J Dent (Tehran). 2015 Jan;12(1):25-30.
- Kim SK, Kim YO. Influence of calcium hydroxide intracanal medication on apical seal. Int Endod J 2002;35:623– 8.
- Kinney JH, Balooch M, Marshall SJ, Marshall GW, Jr., and Weihs TP: Atomic force microscope measurements of the hard- ness and elasticity of peritubular and intertubular human dentin. J. Biomech. Eng. 118, 133 (1996).
- Kinney JH, Gladden J, Marshall GW et al. Resonant ultrasound spectroscopy measurements of the elastic constants of human dentin. J Biomech 2004; 37(4): 437–441.
- Kinney JH, Habelitz S, Marshall SJ, Marshall GW. The importance of intrafibrillar mineralization of collagen on the mechanicalpropertiesofdentin. JdentRes 2003;82:957–61.

- Kinney JH, Marshall SJ, Marshall GW. The mechanical properties of human dentin: a critical review and re-evaluation of the dental literature. Crit Rev Oral Biol Med 2003; 14: 13-29.
- Kinney JH, Narra RK, Pople JA, Breunig TM, Ritchie RO. Age-related transparent root dentin: mineral concentration, crystallite size, and mechanical properties. Biomaterials 2005: 26: 3363-3376
- Kinney JH, Pople JA, Marshall GW, Marshall SJ. Collagen orientation and crystallite size in human dentin: a small angle X-ray scattering study. *Calcif Tissue Int* 2001:69: 31-37.
- Kishen A, Vedantam S. Hydromechanics in dentine: role of dentinal tubules and hydrostatic pressure on mechanical stress-strain distribution. Dent Mater 2007; 23:1296–306.
- Kishen A. Mechanisms and risk factors for fracture predilection in endodontically treated teeth. Endodontic Topics 2006;13:57–83.
- Komabayashi T, Nonomura G, Watanabe LG, Marshall GW Jr, Marshall SJ. Dentin tubule numerical density variations below the CEJ. J Dent 2008: 36: 953-958.
- Kurtz JS, Perdigao J, Geraldeli S, Hoges JS, Bowles WR. Bond strengths of tooth colored posts effect of sealer, dentin adhesive and root region. Am J Dent 2003;16 (special issue) No:31A-36A.
- L. Angker, M.V. Swain, and N. Kilpatrick: Characterising the micro-mechanical behaviour of the carious dentine of primary teeth using nano-indentation. J. Biomech. 38, 1535 (2005).
- L. Angker, N. Nijhof, M.V. Swain, and N.M. Kilpatrick: Influ- ence of hydration and mechanical characterization of carious pri- mary dentine using an ultra-micro indentation system (UMIS). Eur. J. Oral Sci. 112, 231 (2004).
- Lai SC, Mak YF, Cheung GS, Osorio R, Toledano M, Carvalho RM, et al. (2001). Reversal of compromised bonding to oxidized etched dentin. J Dent Res 80:1919-1924.
- Lakshmi RJ, Alexander M, Kurien J, Mahato KK, Kartha VB 2003 Osteoradionecrosis (ORN) of the mandible: A laser Raman spectroscopic study. Appl Spectrosc 57:1100–1116.
- Landis WJ, Jacquet R. Association of calcium and phosphate ions with collagen in the mineralization of vertebrate tissues. Calcif Tissue Int 2013;93:329–37.
- Larkin, P.J. *Infrared and Raman Spectroscopy*, 2nd ed.; Elsevier: Amsterdam, The Netherlands, 2018; pp. 1–5. ISBN 978-0-12-804162-8.
- Lee, J.-Y.; Kim, H.-J.; Lee, E.-S.; de Josselin de Jong, E.; Jung, H.-I.; Kim, B.-I. Quantitative Light-Induced Fluorescence as a Potential Tool for Detection of Enamel Chemical Composition. Photodiagnosis Photodyn. Ther. 2020, 32, 102054.
- Leitune VC, Collares FM, & Werner Samuel SM (2010) Influence of chlorhexidine application at longitudinal push-out bond strength of fiber posts Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology 110(5) e77-e81.
- Lewinstein I, Grajower R. Root dentin hardness of endodontically treated teeth. J Endod 1981;7:421–2.
- Limeira FIR, Carvalho MFF, Nascimento VVD, Santa-Rosa CC, Yamauti M, Moreira AN, Magalhães CS. Bond Strength of Resin

Cements Fixing Fiber Posts to Human and Bovine Teeth of Different Ages. J Adhes Dent. 2019;21(5):423-431.
- Liu Y, Tjaderhane L, Breschi L, Mazzoni A, Li N, Mao J, Pashley DH, & Tay FR (2011) Limitations in bonding to dentin and experimental strategies to prevent bond degradation Journal of Dental Research 90(8) 953-968.
- Lopes GC, Vieira LC, Araújo E, Bruggmann T, Zucco J, Oliveira G. Effect of dentin age and acid etching time on dentin bonding. J Adhes Dent. 2011 Apr;13(2):139-45.
- Lorenzetti CC, Bortolatto JF, Ramos ATPR, Shinohara AL, Saad JRC, Kuga MC. The effectiveness of glass ionomer cement as a fiber post cementation system in endodontically treated teeth. Microsc Res Tech. 2019 Jul;82(7):1191-1197.
- Loubet L, Lucas BN, Oliver WC. Some measurements of viscoelastic properties with the help of nanoindentation. NIST Spec Publ 1995; 896: 31–34.
- M. Balooch, I.C. Wu-Magidi, A. Balazs, A.S. Lundkvist, S.J. Marshall, G.W. Marshall, W.J. Siekhaus, and J.H. Kinney: Viscoelastic properties of demineralized human dentin measured in water with atomic force microscope (AFM)-based indentation. J. Biomed. Mater. Res. 40, 539 (1998).
- Macchi RL, Capurro MA, Herrera CL, Cebada FR, Kohen S. Influence of endodontic materials on the bonding of composite resin to dentin. Endod Dent Traumatol 1992; 8:26-9.
- Machnick TK, Torabinejad M, Munoz CA, Shabahang S (2003) Effect of MTAD on flexural strength and modulus of elasticity of dentin. Journal of Endodontics 29, 747–50.
- Mancuso E, Comba A, Mazzitelli C, Maravic T, Josic U, Del Bianco F et al. Bonding to dentin using an experimental zirconium oxynitrate etchant. Journal of Dentistry. 2021;108:103641.
- Mannocci F, Bhuva B, Roig M, Zarow M, Bitter K. European Society of Endodontology position statement: The restoration of root filled teeth. Int Endod J. 2021 Nov;54(11):1974-1981.
- Marchesi G, Mazzoni A, Turco G, Cadenaro M, Ferrari M, Di Lenarda R, & Breschi L (2013) Aging affects the adhesive interface of posts luted with self-adhesive cements: A 1-year study Journal of Adhesive Dentistry 15(2) 173-180.
- Marchetti C, Piacentini C, Menghini P. Morphometric computerized analysis on the dentinal tubules and the collagen fibers in the dentine of the human permanent teeth. Bull Group Int Rech Sci Stomatol Odontol 1992: 35: 125-129.
- Marending M, Luder HU, Brunner TJ, Knecht S, Stark WJ, Zehnder M. Effect of sodium hypochlorite on human root dentine--mechanical, chemical and structural evaluation. Int Endod J. 2007 Oct;40(10):786-93.
- Marending M, Stark WJ, Brunner TJ, Fischer J, Zehnder M. Comparative assessment of time-related bioactive glass and calcium hydroxide effects on mechanical properties of human root dentin. *Dent Traumatol* 2009: 25: 126–129
- Marghalani HY (2012) Sorption and solubility character- istics of self-adhesive resin cements Dental Materials 28(10) e187-e198.

- Maroulakos G, He J, Nagy WW. The Post-endodontic adhesive interface: Theoretical perspectives and potential flaws. J Endod. 2018 Mar;44(3):363-371.
- Marshall GJ, Balooch M, Kinney J, Marshall S. Atomic force microscopy of conditioning agents on dentin. Journal of Biomedical Materials Research 1995;29:1381–7.
- Marshall GW Jr, Marshall SJ, Kinney JH, Balooch M. The dentin substrate: structure and properties related to bonding. J Dent 1997: 25: 441-458.
- Marshall GW, Wu-Magidi IC, Watanabe LG, et al. Effect of citric acid concentration on dentin demineralization, dehydration, and rehydration: atomic force microscopy study. J Biomed Mater Res 1998;42:500–7.
- Matsumoto T, Nagai T, Ida K et al. (1987) Factors affecting successful prognosis of root canal treatment. Journal of Endodontics 13, 239–42.
- Mayhew L, Smith D. On the decomposition of life expectancy and limits to life. Popul Stud (Camb). 2015;69(1):73-89.
- Mayrand D, Grenier D. Detection of collagenase activity in oral bacteria. Canadian Journal of Microbiology 1985;31:134–8.
- Mazzitelli C, Monticelli F, Osorio R, Casucci A, Toledano M, Ferrari M. Water sorption and solubility of different self-adhesive cements. Dental Materials 2009;5:e37.
- Mazzitelli C, Monticelli F, Toledano M, Ferrari M, Osorio R. Dentin treat- ment effects on the bonding performance of self-adhesive resin ce- ments. Eur J Oral Sci 2010;118:80-86.
- Mazzoni A, Apolonio FM, Saboia VP, Santi S, Angeloni V, Checchi V, Curci R, Di Lenarda R, Tay FR, Pashley DH, & Breschi L (2014) Carbodiimide inactivation of MMPs and effect on dentin bonding Journal of Dental Reasearch 93(3) 263-268.
- Mazzoni A, Breschi L, Carrilho M, Nascimento FD, Orsini G, Ruggeri A, et al. A review of the nature, role, and function of dentin non-collagenous proteins. Part II: enzymes, serum proteins, and growth factors. Endod Top 2009;21: 19–40.
- Mazzoni A, Marchesi G, Cadenaro M, Mazzotti G, Di Lenarda R, Ferrari M, Breschi L. Push-out stress for fiber posts luted using differ- ent adhesive strategies. Eur J Oral Sci 2009;117:447-453.
- Mazzoni A, Tjäderhane L, Checchi V, et al. Role of dentin MMPs in caries progression and bond stability. *J Dent Res*. 2015;94(2):241-251.
- Mazzoni, V. Angeloni, F.M. Apolonio, N. Scotti, L. Tjäderhane, A. Tezvergil- Mutluay, et al., Effect of carbodiimide (EDC) on the bond stability of etch-and-rinse adhesive systems, Dent. Mater. 29 (2013) 1040–1047.
- McCreadie BR, Morris MD, Chen TC, Sudhaker Rao D, Finney WF, Widjaja E, Goldstein SA. Bone tissue compositional dif- ferences in women with and without osteoporotic fracture. Bone. 2006;39:1190–1195.
- Mencik J and Swain M: Micro-indentation tests with pointed indenters. *Mater*. *Forum* **18,** 277 (1994).
- Micheletti CM. Dental histology: study of aging processes in root dentine. Boll Soc Ital Biol Sper 1998: 74: 19-28.

- Miguez PA, Pereira PN, Atsawasuwan P, Yamauchi M. Collagen cross-linking and ultimate tensile strength in dentin. J Dent Res 2004: 83: 807-810.
- Mine A, De Munck J, Cardoso MV, Van Landuyt KL, Poitevin A, Van Ende A, Matsumoto M, Yoshida Y, Kuboki T, Yatani H, Van Meerbeek B. Den- tin-smear remains at self-etch adhesive interface. Dent Mater 2014;30:1147–1153.
- Miotti LL, Follak AC, Montagner AF, Pozzobon RT, da Silveira BL, Susin AH. Is Conventional Resin Cement Adhesive Performance to Dentin Better Than Self-adhesive? A Systematic Review and Meta-Analysis of Laboratory Studies. Oper Dent. 2020 Sep 1;45(5):484-495.
- Miura J, Nishikawa K, Kubo M, Fukushima S, Hashimoto M, Takeshige F, et al. Accumulation of advanced glycation end-products in human dentine. Arch Oral Biol 2014;59:119-24.
- Mjor IA, Nordahl I. The density and branching of dentinal tubules in human teeth. Arch Oral Biol 1996: 41: 401-412.
- Montanari M, Prati C, Piana G. Differential hydrolytic degradation of dentin bonds when luting carbon fiber posts to the root canal. Med Oral Patol Oral Cir Bucal. 2011;16:e411-7.
- Monticelli F, Osorio R, Mazzitelli C. Limited decalcification/diffusion of self-adhesive cements into dentin. J Dent Res 2008;87:974-979.
- Montoya C, Arango-Santander S, Peláez-Vargas A, Arola D, Ossa EA. Effect of aging on the microstructure, hardness and chemical composition of dentin. Arch Oral Biol. 2015 Dec;60(12):1811-20.
- Morris MD, Lee KW, Agee KA, Bouillaguet S, Pashley DH. Effects of sodium hypochlorite and RC-prep on bond strengths of resin cement to endodontic surfaces. J Endod 2001;27:753–7.
- Morris MD, Mandair GS. Raman assessment of bone quality. Clin Orthop Relat Res. 2011 Aug;469(8):2160-9. doi: 10.1007/s11999-010-1692-y. PMID: 21116756; PMCID: PMC3126952.
- Morris MD, Mandair GS. Raman assessment of bone quality. Clin Orthop Relat Res. 2011 Aug;469(8):2160-9.
- Müller, F., Shimazaki, Y., Kahabuka, F., Schimmel, M., 2017. Oral health for an ageing population: the importance of a natural dentition in older adults. Int. Dent. J. 67, 7–13.
- Muniz L, Mathias P. The influence of sodium hypochlorite and root canal sealers on post retention in different dentin regions. Oper Dent 2005;30:533–9.
- Nagarkar S, Theis-Mahon N, Perdigão J. Universal dental adhesives: Current status, laboratory testing, and clinical performance. Journal of Biomedical Materials Research Part B: Applied Biomaterials. 2019;107:2121-31.
- Nakabayashi N, Saimi Y. Bonding to intact dentin. J Dent Res 1996; 75:1706-15.
- Nakabayashi N. Resin reinforced dentin due to infilitration of monomers into the dentin at the adhesive interface. J Jpn Dent Mat Devices 1982; 1:78-81.
- Nakajima M, Kitasako Y, Okuda M, Foxton RM, Tagami J. Elemental distributions and microtensile bond strength of the adhesive interface to normal and caries-affected dentin. J Biomed Mater Res B Appl Biomater 2005;72: 268-275.

- Nalci G, Alaçam T, Altukaynak B. Microhardness evaluation of root dentin after using resin sealer solvents. J Dent Res Dent Clin Dent Prospects. 2021 Fall;15(4):256-261. doi: 10.34172/joddd.2021.042.
- Natusch I, Pilz ME, Klimm W, Buchmann G. Transparent dentinal sclerosis and its clinical significance. ZahnMund Kieferheilkd Zentralbl 1989;77:3–7.
- Nogueira CHP, Gelio MB, Besegato JF, Ramos ATPR, Fernández E, Kuga MC, Saad JRC. Effect of aging and cementation systems on the bond strength to root dentin after fiber post cementation. Braz Dent J. 2023 Jan-Feb;34(1):52-60.
- Notarstefano, V.; Belloni, A.; Sabbatini, S.; Pro, C.; Orilisi, G.; Monterubbianesi, R.; Tosco, V.; Byrne, H.J.; Vaccari, L.; Giorgini, E. Cytotoxic Effects of 5-Azacytidine on Primary Tumour Cells and Cancer Stem Cells from Oral Squamous Cell Carcinoma: An In Vitro FTIRM Analysis. *Cells* 2021, *10*, 2127.
- Notarstefano, V.; Sabbatini, S.; Conti, C.; Pisani, M.; Astolfi, P.; Pro, C.; Rubini, C.; Vaccari, L.; Giorgini, E. Investigation of Human Pancreatic Cancer Tissues by Fourier Transform Infrared Hyperspectral Imaging. *J. Biophotonics* 2020, *13*, e201960071.
- Oliveira GC, Oliveira GM, Ritter AV, Heymann HO, Swift EJ, Yamauchi M. Influence of tooth age and etching time on the microtensile bond strengths of adhesive systems to dentin. J Adhes Dent. 2012 Jun;14(3):229-34.
- Oliveira LD, Carvalho CA, Nunes W, et al. Effects of clorhexidine and sodium hypo- chlorite on the microhardness of root canal dentin. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2007;104:e125–8.
- Oliver W and Pharr G: An improved technique for determining hardness and elastic modulus using load and displacement sensing indentation experiments. J. Mater. Res. 7, 1564 (1992).
- Oliver, W.C., Pharr, G.M., 2004. Measurement of hardness and elastic modulus by instrumented indentation: advances in understanding and refinements to methodology. J. Mater. Res. 19, 3–20.
- Orsini, G.; Orilisi, G.; Notarstefano, V.; Monterubbianesi, R.; Vitiello, F.; Tosco, V.; Belloni, A.; Putignano, A.; Giorgini, E. Vibrational Imaging Techniques for the Characterization of Hard Dental Tissues: From Bench-Top to Chair-Side. *Appl. Sci.* 2021, *11*, 11953.
- Oyarzun A, Cordero AM, Whittle M (2002) Immunohisto- chemical evaluation of the effects of sodium hypochlorite on dentin collagen and glycosaminoglycans. Journal of Endo- dontics 28, 152–6.
- Oyarzún A, Rathkamp H, Dreyer E. Immunohistochemical and ultrastructural evaluation of the effects of phosphoric acid etching on dentin proteoglycans. Eur J Oral Sci 2000;108:546–54.
- Ozer F, Sengun A, Ozturk B, Say EC, Tagami J. Effect of tooth age on microtensile bond strength of two fluoride-releasing bonding agents. J Adhes Dent 2005;7:289-289.
- Padmakumar I, Hinduja D, Mujeeb A, Kachenahalli Narasimhaiah R, Kumar Saraswathi A, Mirza MB, Robaian A, Basheer SN, Karobari MI, Scardina GA. Evaluation of Effects of Various Irrigating Solutions on Chemical Structure of Root Canal Dentin Using FTIR, SEM, and EDS: An In Vitro Study. J Funct Biomater. 2022 Oct 20;13(4):197.

- Pala K, Demirbuga S, Gümüs HÖ, Arslan S, Zorba YO. Effect of different bonding techniques on the bond strength of two different fiber posts. J Restor Dent 2014;2:32.
- Papa J, Cain C, Messer HH. Moisture content of vital vs endodontically treated teeth. Endod Dent Traumatol 1994: 10: 91-93.
- Park JW, Ferracane JL. Water aging reverses residual stresses in hydrophilic dental composites. J Dent Res 2014;93:195–200.
- Paschalis EP, Gamsjaeger S, Klaushofer K. Vibrational spectroscopic techniques to assess bone quality. Osteoporos Int. 2017 Aug;28(8):2275-2291.
- Paschalis EP, Tatakis DN, Robins S, Fratzl P, Manjubala I, Zoehrer R, Gamsjaeger S, Buchinger B, Roschger A, Phipps R, Boskey AL, Dall'Ara E, Varga P, Zysset P, Klaushofer K, Roschger P (2011) Lathyrism-induced alterations in collagen cross-links influence the mechanical properties of bone material without affecting the mineral. Bone 49:1232–1241.
- Pascon FM, Kantovitz KR, Sacramento PA, et al. Effect of sodium hypochlorite on dentine mechanical properties: a review. J Dent 2009;37:903–8.
- Pashley D, Okabe A, Parham P. The relationship between dentine microhardness and tubule density. Endod Dental Traumatol 1985;1:176–9.
- Pashley DH, Tay FR, Yiu C, et al. Collagen degradation by host-derived enzymes during aging. J Dent Res 2004;83:216 –21.
- Pelegrine RA, Paulillo LA, Kato AS, Fontana CE, Pinheiro SL, De Martin AS, Bueno CE. Effect of Endodontic Retreatment on Push-out Bond Strength and Quality of Fiber Post bonding Interface of Resin Cements. J Contemp Dent Pract. 2016 Jan 1;17(1):42-8.
- Pelozo LL, Silva-Neto RD, Corona SAM, Palma-Dibb RG, Souza-Gabriel AE. Dentin pretreatment with Er:YAG laser and sodium ascorbate to improve the bond strength of glass fiber post. Lasers Med Sci. 2019 Feb;34(1):47-54.
- Penel, G.; Leroy, G.; Rey, C.; Bres, E. MicroRaman Spectral Study of the PO4 and CO3 Vibrational Modes in Synthetic and Biological Apatites. *Calcif. Tissue Int.* 1998, *63*, 475–481.
- Perdigao J, Araujo E, Ramos RQ, Gomes G, Pizzolotto L. Adhesive dentistry: current concepts and clinical considerations. Journal of esthetic and restorative dentistry. 2021;33:51-68.
- Perdigao J, Gomes G, Augusto V. The effect of dowel space on the bond strengths of fiber posts. J Prosthodont 2007; 16:154–64.
- Perdigão J, Sezinando A, Monteiro PC. Effect of substrate age and adhesive composition on dentin bonding. Oper Dent. 2013 May-Jun;38(3):267-74.
- Pereira KF, Vencão AC, Magro MG, Belizário LG, Porto TS, Andrade MF, Duarte MAH, Kuga MC. Effect of endodontic retreatment on the bond strength of resin cements to root canal dentin. Am J Dent. 2019 Jun;32(3):147-151.
- Porter AE, Nella RK, Minor A, Jinschek JR, Kisielowski C, Radmilovic V, Kinney JH, Tomsia AP, Ritchie RO. A transmission electron microscopy study of mineralization in age-induced transparent dentin. Biomaterials 2005: 26: 7650-7660.

- Prestes RC, Golunski SM, Toniazzo G, Kempka AP, Di Luccio M. Caracter- ização da fibra de colágeno, gelatina e colágeno hidrolisado. Rev Bras Prod Agroindustriais. 2013; 15:375-382.
- Price RI, Lees S, Kirschner DA 1997 X-ray diffraction analysis of tendon collagen at ambient and cryogenic temperatures: Role of hydration. Int J Biol Macromol 20:23–33.
- Pulido CA, de Oliveira Franco APG, Gomes GM, Bittencourt BF, Kalinowski HJ, Gomes JC, Gomes OMM (2016) An in situ evaluation of the polymerization shrinkage, degree of conversion, and bond strength of resin cements used for luting fiber posts. J Prosthet Dent 116:570–576.
- Radovic I, Mazzitelli C, Chieffi N, Ferrari M. Evaluation of the adhesion of fiber posts cemented using different adhesive approaches. Eur J Oral Sci 2008, 116:557-563.
- Radovic I, Monticelli F, Goracci C, Vulicevic ZR, Ferrari M. Self-adhesive resin cements: a literature review. J Adhes Dent. 2008 Aug;10(4):251-8. PMID: 18792695.
- Radovic I, Monticelli F, Papacchini F, Magni E, Cury AH, Vulice- vic ZR, et al. Accelerated aging of adhesive-mediated fiber post-resin composite bonds: A modeling approach. J Dent. 2007;35:683-9.
- Ramírez-Bommer C, Gulabivala K, Ng YL, Young A. Estimated depth of apatite and collagen degradation in human dentine by sequential exposure to sodium hypochlorite and EDTA: a quantitative FTIR study. Int Endod J. 2018 Apr;51(4):469-478.
- Reeh ES, Messer HH, Douglas WH. Reduction in tooth stiffness as a result of endodontic and restorative procedures. J Endod. 1989 Nov;15(11):512-6.
- Rey C, Combes C, Drouet C, Sfihi H, Barroug A. Physico-chemical properties of nanocrystalline apatites: implications for biominerals and bio- materials. Mater Sci Eng: C. 2007 Mar;27(2):198-205.
- Robins SP (2007) Biochemistry and functional significance of collagen cross-linking. Biochem Soc Trans 35:849–852.
- Rodig T, Muller C, Hoch M, et al. Moisture content of root canal dentine affects detection of microcracks using micro-computed tomography. Int Endod J 2018; 51:357–63.
- Rodrigues RV, Sampaio CS, Pacheco RR, Pascon FM, Puppin-Rontani RM, Giannini M. Influence of adhesive cementation systems on the bond strength of relined fiber posts to root dentin. J Prosthet Dent 2017;118: 493–499.
- Rotstein I, Cohenca N, Teperovich E, Moshonov J, Mor C, Roman I et al. (1999) Effect of chloroform, xylene, and halothane on enamel and dentin microhardness of human teeth. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 87, 366-368.
- Rotstein I, Cohenca N, Teperovich E, Moshonov J, Mor C, Roman I, et al. Effect of chloroform, xylene, and halothane on enamel and dentin microhardness of human teeth. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 1999;87(3):366-8.
- Roulet JF. Marginal integrity: clinical significance. J Dent 1994;22(Suppl 1):S9 –12.

- Ryou H, Romberg E, Pashley DH et al. Nanoscopic dynamic mechanical properties of intertubular and peritubular dentin. J Mech Behav Biomed Mater 2012; 7: 3–6.
- S. Poolthong, D. Low, M. Swain, T. Sumii, and T. Mori: Predic- tion of positional dependence of mechanical properties of dentine. J. Dent. Res. 77, 917 (1998).
- Saeki K, Marshall SJ, Gansky SA, et al. Etching characteristics of dentin: effect of ferric chloride in citric acid. J Oral Rehabil 2001;28:301–8.
- 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;58:59-66.
- Sakae T, Mishima H, Kozawa Y. Changes in bovine dentin mineral with sodium hypochlorite treatment. J Dent Res 1988;67:1229–34.
- Salehi H, Terrer E, Panayotov I, Levallois B, Jacquot B, Tassery H, Cuisinier F. Functional mapping of human sound and carious enamel and dentin with Raman spectroscopy. J Biophotonics. 2013 Oct;6(10):765-74.
- Santana FR, Soares CJ, Ferreira JM, Valdivi AD, Souza JB, Estrela C. Effect of root canal sealer and artificial accelerated aging on fibreglass post bond strength to intraradicular dentin. J Clin Exp Dent. 2014 Oct 1;6(4):e350-6.
- SanterreJP,ShajiiL,LeungBW.Relationofdentalcompositeformulationstotheir degradation and the release of hydrolyzed polymeric-resin-derived products. Crit Rev Oral Biol Med 2001;12:136 –51.
- Sarkis-Onofre R, Skupien JA, Cenci MS, Moraes RR, Pereira-Cenci T. The role of resin cement on bond strength of glass-fiber posts luted into root canals: a systematic review and meta-analysis of in vitro studies. Oper Dent 2014;39:E31–44.
- Sathorn C, Palamara J, Palamara D, Messer H. Effect of root canal size and external root surface morphology on fracture susceptibility and pattern: a finite element analysis. Journal of Endodontics 2005;31:288–92.
- Sayin TC, Serper A, Cehreli ZC, Otlu HG. The effect of EDTA, EGTA, EDTAC and tetracycline-HCl with and without subsequent NaOCl treatment on the microhard- ness of root canal dentin. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2007;104:418–24.
- Scheffel DLS, Hebling J, Scheffel RH, Agee K, Turco G, de Souza Costa C, Pashley DH. Inactivation of matrix-bound matrix metalloproteinases by cross-linking agents in acid-etched dentin. Operative Dentistry 2014;39:152–8.
- Schroeder HE. Age-related changes in the pulp chamber and its wall in human canine teeth. Schweiz Monatsschr Zahnmed 1993: 103: 141-149.
- Schwartz RS, Fransman R. Adhesive dentistry and endodontics: materi-als, clinical strategies and procedures for restoration of access cavi- ties: a review. J Endod 2005;31:151–165.
- Sebold M, Giannini M, André CB, Sahadi BO, Maravic T, Josic U, et al. Bonding interface and dentin enzymatic activity of two universal adhesives applied following different etching approaches. Dental Materials. 2022.

- Sedgley CM, Messer HH. Are endodontically treated teeth more brittle? J Endod. 1992 Jul;18(7):332-5.
- Senawongse P, Otsuki M, Tagami J, Mjör I. Age-related changes in hardness and modulus of elasticity of dentine. Arch Oral Biol 2006;51:457- 463.
- Serino G, Comba A, Baldi A, Carossa M, Baldissara P, Bignardi C, Audenino A, Torres CGR, Scotti N. Could light-curing time, post-space region and cyclic fatigue affect the nanomechanical behavior of a dual-curing cement for fiber post luting? J Mech Behav Biomed Mater. 2022 Jan;125:104886.
- Sevimay S, Oztan MD, Dalat D. Effects of calcium hydroxide paste medication on coronal leakage. J Oral Rehabil 2004;31:240 – 4.
- Seyedmahmoud R, McGuire JD, Wang Y, Thiagarajan G, Walker MP. The interrelationship of microstructure and hardness of human coronal dentin using reference point indentation technique and micro-Raman spectroscopy. Dent Mater. 2017 Oct;33(10):1069-1074.
- Shafiei F, Memarpour M, Vafamand N, Mohammadi M. Effect of antibacterial/adhesive approaches on bonding durability of fiber posts cemented with self-etch resin cement. J Clin Exp Dent. 2017 Sep 1;9(9):e1096-e1102.
- Shafiei F, Yousefipour B, Mohammadi-Bassir M. Effect of Carbodiimide on Bonding Durability of Adhesive-cemented Fiber Posts in Root Canals. Oper Dent. 2016 Jul-Aug;41(4):432-40.
- Shemesh H, Lindtner T, Portoles CA, Zaslansky P. Dehydration induces cracking in root dentin irrespective of instrumentation: a two-dimensional and three-dimensional study. J Endod 2018;44:120–5.
- Sim TP, Knowles JC, Ng YL, Shelton J, Gulabivala K (2001) Effect of sodium hypochlorite on mechanical properties of dentine and tooth surface strain. International Endodontic Journal 34, 120–32.
- Slutzky-Goldberg I, Maree M, Liberman R, Heling I. Effect of sodium hypochloride on dentin microhardness. J Endod 2004;30:880–2.
- Souza NO, Sousa RS, Isolan CP, Moraes RR, Lima GDS, Lomonaco D, de Paula DM, Alves AH, Sabóia VP, Feitosa VP. Intraradicular Dentin Biomodification with Natural Agents for Bonding Glass-fiber Posts. J Adhes Dent. 2021 Jun 5;23(3):223-230.
- Stape THS, Uctasli M, Cibelik HS, Tjaderhane L, Tezvergil-Mutluay A. Dry bonding to dentin: Broadening the moisture spectrum and increasing wettability of etch-and-rinse adhesives. Dental Materials. 2021;37:1676-87.
- Suchanek W, Yoshimura M. Processing and properties of hydroxya-patite-based biomaterials for use as hard tissue replacement implants. J Mater Res. 1998 Jan;13(1)94-117.
- Swift EJ, Perdigao J, Heymann HO. Bonding to enamel and dentin: a brief history and the state of the art, 1995. Quintessence Int 1995;26:95-110.
- Taddei, P.; Prati, C.; Gandolfi, M.G. A poly (2-hydroxyethyl methacrylate)-based resin improves the dentin remineralizing ability of calcium silicates. Mater. Sci. Eng. C 2017, 77, 755–764.
- Tagami J, Nakajima M, Shono T, Takatsu T, Hosoda H. Effect of aging on dentin bonding. Am J Dent 1993;6:145-147.

- Tang W, Wu Y, Smales R. Identifying and reducing risks for potential fractures in endodontically treated teeth. J Endod 2010;36:609–17.
- Tay FR, Loushine RJ, Lambrechts P, Weller RN, Pashley DH. Geometric factors affecting dentin bonding in root canals: a theoretical modeling approach. J Endod 2005;31:584 –9.
- Tay FR, Pashley DH, Yoshiyama M. Two modes of nanoleakage expression in single- step adhesives. J Dent Res 2002; 81:472– 6.
- Ten Cate AR. *Oral Histology: Development, Structure and Function*, 7th edn. St. Louis: Mosby-Year Book Inc., 2008.
- Tezvergil-Mutluay A, Mutluay MM, Agee KA, Seseogul- lari-Dirihan R, Hoshika T, Cadenaro M, Breschi L, Vallittu P, Tay FR, & Pashley DH (2012) Carbodiimide cross-linking inactivates soluble and matrix-bound MMPs Journal of Dental Reasearch 91(2) 192-196.
- Thomas GJ, Whittaker DK, Embery G. A comparative study of translucent apical dentine in vital and non-vital human teeth. Arch Oral Biol 1994: 39: 29-34.
- Tjäderhane L, Carrilho MR, Breschi L, Tay FR, Pashley DH. Dentin basic structure and composition-an overview. Endod Top 2009;20: 3–29.
- Toledano M, Aguilera FS, Cabello I, Osorio R. Masticatory function induced changes, at subnanostructural level, in proteins and mineral at the resin-dentine interface. J Mech Behav Biomed Mater. 2014 Nov;39:197-209.
- Tronstad, L. (2008). Clinical endodontics: a textbook. Stuttgart: Thieme p. 5.
- Tsuda H, Ruben J, Arends J. Raman spectra of human dentin mineral. Eur J Oral Sci 1996;104:123–31.
- Ubaldini ALM, Benetti AR, Sato F, Pascotto RC, Medina Neto A, Baesso ML, Peutzfeldt A. Challenges in luting fibre posts: Adhesion to the post and to the dentine. Dent Mater 2018;34:1054–1062.
- Van Meerbeek B, De Munck J, Yoshida Y. Adhesion to enamel and dentin: current status and future challenges. Oper Dent 2003;28:215-35.
- Veis A, Schlueter RJ. The macromolecular organization of dentine matrix collagen I. Characterization of dentine collagen. Biochemistry 1964;3:1650–7.
- Veis A. Mineralization in organic matrix frameworks. Rev Miner Geochem 2003;54: 249–89.
- Vichi A, Vano M, Ferrari M. The effect of different storage conditions and duration on the fracture strength of three types of translucent fiber posts. Dent Mater 2008;24:832–838.
- Walters C, Eyre DR. Collagen crosslinks in human dentin: increasing content of hydroxypyridinium residues with age. Calcif Tissue Int. 1983 Jul;35(4-5):401-5.
- Wang L, Zhao Y, Mei L, et al. Effect of application time of maleic acid on smear layer removal and mechanical properties of root canal dentin. Acta Odontol Scand 2017;75:59–66.
- Wang RZ, Weiner S. Strain–structure relations in human teeth using Moire´ fringes. J Biomech 1997; 31(2): 135–141.
- Wang TF, Feng XW, Gao YX, Wang M, Wang YN, Sa Y, Jiang T. Effects of different concentrations and exposure time of sodium hypochlorite on the structural, compositional and mechanical properties

of human dentin. J Huazhong Univ Sci Technolog Med Sci. 2017 Aug;37(4):568-576.
- Waters NE: Some mechanical and physical properties of teeth, in Mechanical Properties of Biological Materials, edited by J.F. Vincent and J.D. Curry (Cambridge University Press, Cam- bridge, UK, 1980), p. 99.
- Weber DF. Human dentine sclerosis: a microradiographic survey. Arch Oral Biol 1974;19:163–9.
- Weerakoon AT, Cooper C, Meyers IA, Condon N, Sexton C, Thomson D, Ford PJ, Symons AL. Does dentine mineral change with anatomical location, microscopic site and patient age? J Struct Biol X. 2022 Jan 23;6:100060.
- Wopenka B, Kent A, Pasteris JD, Yoon Y, Thomopoulos S. The tendon-to-bone transition of the rotator cuff: a preliminary Raman spectroscopic study documenting the gradual minerali- zation across the insertion in rat tissue samples. Appl Spectrosc. 2008;62:1285–1294.
- Xu C, Wang Y. Chemical composition and structure of peritubular and intertubular human dentine revisited. Arch Oral Biol. 2012 Apr;57(4):383-91.
- Xu H, Zheng Q, Shao Y, Song F, Zhang L, Wang Q, Huang D. The effects of ageing on the biomechanical properties of root dentine and fracture. J Dent 2014;42:305–311.
- Xu, C.; Karan, K.; Yao, X.; Wang, Y. Molecular Structural Analysis of Noncarious Cervical Sclerotic Dentin Using Raman Spectroscopy. J. Raman Spectrosc. 2009, 40, 1780–1785.
- Yamauchi M, Shiiba M. Lysine hydroxylation and cross-linking of collagen. Methods Mol Biol 2008;446:95–108.
- Yan W, Montoya C, Øilo M, et al. Reduction in fracture resistance of the root with aging. J Endod 2017;43:1494–8.
- Yan W, Montoya C, Øilo M, Ossa A, Paranjpe A, Zhang H, Arola DD. Contribution of Root Canal Treatment to the Fracture Resistance of Dentin. J Endod. 2019 Feb;45(2):189-193.
- Yasui T, Tohno Y, Araki T. Determination of collagen fiber orientation in human tissue by use of polarization measurement of molecular second-harmonic-generation light. Appl Opt 2004: 43: 2861-2867.
- Yerramshetty JS, Akkus O. The associations between mineral crystallinity and the mechanical properties of human cortical bone. Bone. 2008 Mar;42(3):476-82.
- Yiu CK, Garcia-Godoy F, Tay FR, et al. A nanoleakage perspective on bonding to oxidized dentin. J Dent Res 2002;81:628 –32.
- Yoshida Y, Van Meerbeek B, Nakayama Y, Snauwaert J, Hellemans L, Lambrechts P, et al. Evidence of chemical bonding at biomaterial-hard tissue interfaces. J Dent Res2000;79:709-14.
- Yoshihara K, Yoshida Y, Hayakawa S, Nagaoka N, Torii Y, Osaka A, Su- zuki K, Minagi S, Van Meerbeek B, Van Landuyt KL. Self-etch monomer- calcium salt deposition on dentin. J Dent Res 2011;90:602–606.
- YoshikawaT,SanoH,BurrowMF,TagamiJ,PashleyDH.Effectsofdentindepthand cavity configuration on bond strength. J Dent Res 1999;78:898–905.

- Yuanli H, Juan W, Mengzhen J, Xuan C, Kaixin X, Xueqin Y, Xin Q, Hantao H, Yuan G, Ling Z. The effect of two endodontic sealers and interval before post-preparation and cementation on the bond strength of fiber posts. Clin Oral Investig. 2021 Nov;25(11):6211-6217.
- Zehnder M (2006) Root canal irrigants. Journal of Endodontics 32, 389–98.
- Zelic K, Milovanovic P, Rakocevic Z, Askrabic S, Potocnik J, Popovic M, Djuric M. Nano-structural and compositional basis of devitalized tooth fragility. Dent Mater. 2014 May;30(5):476-86.
- Zhang K, Kim YK, Cadenaro M, et al. Effects of different exposure times and concentrations of sodium hypochlorite/ethylenediaminetetraacetic acid on the structural integrity of mineralized dentin. J Endod 2010;36:105–9.
- Zhang TH, Yang YM. Development and application of nanohardness technique. Adv Mech 2002; 32(6): 349–364.
- Zhang YR, Du W, Zhou XD, Yu HY. Review of research on the mechanical properties of the human tooth. Int J Oral Sci. 2014 Jun;6(2):61-9. doi: 10.1038/ijos.2014.21
- Zheng, L., Nakajima, M., Higashi, T., Foxton, R. M., & Tagami, J. (2005). Hardness and Young's modulus of transparent dentin associated with aging and carious disease. Dental Materials Journal, 24(4), 648–653.
- Zhou J, Yang X, Chen L, Liu X, Ma L, & Tan J (2013) Pre-treatment of radicular dentin by self-etch primer containing chlorhexidine can improve fiber post bond durability Dental Materials Journal 32(2) 248-255.
- Ziskind D, Hasday M, Cohen SR et al. Young's modulus of peritubular and intertubular human dentin by nano-indentation tests. J Struct Biol 2011; 174(1): 23–30.