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.

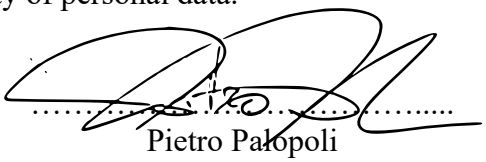
Pietro Palopoli
* * * * * *

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.