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Methods

A new technique to make transparent teeth without decalcifying: Description of the methodology and micro-hardness assessment

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Chemical compounds studied in this article:
Xylene C_8H_{10} (Merck F.R. Germany)
Methyl benzoate $C_8H_8O_2$ (Merck F.R. Germany)
Ethanol 30%, 50%, 80%, 96% and absolute alcohol C_2H_3OH (Merck F.R. Germany)
Glycerol 87% (Merck F.R. Germany)
Formalin (HCHO formaldehyde solution 37%) (Merck F.R. Germany)

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ABSTRACT

Diaphanisation and other in vitro endodontic models (i.e., plastic blocks, micro-CT reconstruction, computerised models) do not recreate real root canal working conditions: a more realistic endodontic model is essential for testing endodontic devices and teaching purposes. The aim of this study was to describe a new technique to construct transparent teeth without decalcifying and evaluate the micro-hardness of so treated teeth.

Thirty freshly extracted teeth were randomly divided into three groups as follows: 10 non-treated teeth (4 molars, 3 premolars, 3 incisors; control group – G1), 10 teeth were diaphanised (4 molars, 4 premolars, 2 incisors – G2) and 10 teeth were treated with the new proposed technique (2 molars, 6 premolars, 2 incisors – G3). Vickers hardness tester (MHT-4 and AxioVision microscope, Carl Zeiss, 37030 Gottingen, Germany – load = 50 g, dwell time = 20 s, slope = 5, 50× magnification) was used to determine microhardness (Vickers Hardness Number – VHN). Statistical analysis was performed using the Intercooled Stata 8.0 software (Stata Corporation, College Station, TX, USA).

Only groups 1 and 3 could be tested for hardness because diaphanised teeth were too tender and elastic. Differences in enamel VHN were observed between G1 (mean 304.29; DS = 10.44; range 283–321) and G3 (mean 318.51; DS = 14.36; range 295.5–339.2) – ($p < 0.05$); differences in dentine VHN were observed between G1 (mean 74.73; DS = 6.62; range 63.9–88.1) and G3 (mean 64.54; DS = 5.55; range 51.2–72.3) – ($p < 0.05$).

G3 teeth presented a slightly lower VHN compared to G1, probably due to some little structural differences among groups, and were dramatically harder than the diaphanised teeth. The described technique, thus, can be considered ideal for testing endodontic instruments and for teaching purposes.

1. Introduction

New endodontic devices (i.e., ultrasonic instruments, irrigation systems, and Ni-Ti rotary instruments) need to be tested on experimental models, which should have three major characteristics: (1) the models should be transparent to observe the devices while working; (2) the models should be of the same hardness of a natural tooth to test the real device efficacy and safety; and (3) the models should resemble the complex nature of the endodontic system as much as possible to validate the device efficacy in a complex environment. Such a model would be useful for both research and teaching purposes (Dummer et al., 1991; Nassri et al., 2008).

For many years, diaphanisation procedures have been employed to make teeth transparent. Diaphanised teeth are useful to evaluate root canal anatomy or the filling of root canal systems; however, these methods require massive decalcification of the tooth, which considerably alter the chemical and physical characteristics of hard tissues, softening them and giving the tooth a rubber-like consistency. Such a technique makes the tooth transparent, which is soft/elastic and cannot be used to test new endodontic devices while working within the root canal (Sidow et al., 2000; Shivapathasundharam and Berti, 2000; Yamamoto et al., 2001; Venturi et al., 2003).

Many alternative techniques and models have been developed and used to evaluate endodontic instruments, some of which attempt to recreate tooth anatomy (models consisting of transparent plastic) (Dummer et al., 1991; Bedford-Roberts et al., 1997), while others evaluate the effect of endodontic instruments via complex and expensive techniques (micro-CT reconstruction or

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Table 1
Sample age and teeth characteristics.

Group	No of teeth	Age		Tooth type			Reason for extraction	
		Mean	SD	Molars	Premolars	Incisors	Periodontal	Orthodontic
1	10	46.5	21.9	4	3	3	6	4
2	10	45.4	25.8	4	4	2	5	5
3	10	38.7	23.9	2	6	2	3	7

computerised models) (Necchi et al., 2008; Cheung and Cheung, 2008; Lee et al., 2014); however, plastic models, virtual models, and micro-CT reconstructions do not recreate the working conditions within a real root canal.

The aim of the present study was to describe a new histological procedure to make the dentine transparent without decalcifying it and without depriving it of its inorganic components and to evaluate the effect of this technique on hard tissues (enamel and dentine) micro-hardness. The study hypothesis was that mechanical characteristics (i.e., micro-hardness) of teeth treated with the new histological procedure should be comparable with that of non-treated teeth, since the new produce does not affect the tooth inorganic component.

2. Materials and methods

2.1. Specimen preparation

Thirty teeth, freshly extracted for orthodontic or periodontal reasons (Table 1), were used for this study. After extraction, the teeth were cleaned with gauze and physiological solution and stored in a solution of 98% glycerol and 2% formalin until the start of the experiment. All patients signed an informed consent agreement. Teeth were randomly divided into the three following groups by a block randomisation sequence: Group 1 (G1), 10 natural non-treated teeth as the control group; Group 2 (G2), 10 teeth treated with diaphanisation procedures, as previously described (Venturi et al., 2003); and Group 3 (G3), 10 teeth were treated with the new proposed technique, as described below. Teeth from the three groups were cut along the longitudinal axis, embedded and stacked in a chemically transparent cured acrylic resin (Dentaurum, Bologna, Italy) on glass and ground using the Exact cutting and grinding system (Exact Technologies Inc., 7002N. Broadway Extension (Oklahoma City, OK 73116, USA).

2.2. Description of the new technique to create transparent teeth (G3)

The principal prerequisite for achieving good transparent sections is to maintain the hydration of the tooth. Care must be taken that the dentine is not dehydrated during any phase of the process because microscopic air bubbles, if they enter the dentinal tubules, can make the teeth opaque. Thus, the teeth cannot be made completely transparent. The best procedure is to work with vital teeth as soon as possible after extraction. The teeth should be immediately stored in water, and then washed with a mild (10%) solution of hydrogen peroxide. Any tissue adhering to the root surface should be removed using curettement, and rinsed under running water. The tooth should then be stored in a solution of 98% glycerol and 2% formalin until further analyses. When the teeth were removed from the solution, excess glycerol must be removed using lens cleaning paper or a microfiber cloth to ensure that no small fibres are left on the root surface. The tooth is later placed in a Petri dish and covered with a 30% alcohol solution. Using a small brush and alcohol solution, the tooth is further cleaned, followed by rinsing under running water. The pulp chamber can now be opened. The pulp is completely removed from the pulp chamber, after which the tooth is immersed in a 0.5% aniline blue solution for ten minutes. Opening the pulp chamber and removing its contents are necessary to allow the dye to thoroughly penetrate the root canal. At this point, inspection of the superficial anatomy of the root under a microscope is performed to determine which planes are to be cut to best indicate the features of interest, taking care not to allow the specimens to dry. The tooth is now ready for preparation. This preparation entails grinding the roots down to the chosen planes, following the line of the canal, and taking care to keep the canal well within the section. Canals frequently present a double curvature on two different planes, and thus very frequently, in preparing these sections, the tooth must also be curved. Using a diamond disc or bur, with a water spray, the root is thinned slightly, leaving a thick layer (2 mm) of dentine between the surface of the section and the canal. After this initial rough shaping of the roots and crown, the tooth is immersed in distilled water and subsequently stained, selecting among the various methods for staining pulp tissue and cementum. We used the modified Mallory's mixture (Spielman and Joseph, 1995). The time of staining was variable and dependent on the staining technique, the thickness of the section and the age of the patient from whom the tooth was removed. Each day, the section is rinsed in distilled water and assessed to determine adequate penetration of the stain. The teeth of young patients require between 1 and 3 days, and should not be left longer in the staining solution to avoid the stain from penetrating too deeply into the dentine. Elderly teeth may require longer staining periods.

After staining, the section is washed under running water and then shaped to its final thickness, first using a fine turbine diamond or bur with water spray to a thickness of 2–3 mm, and subsequently polished with fine abrasive discs mounted on a micro-engine using progressively finer grit and working under running water. This was the most delicate phase of the procedure. If a mistake is made and the cut touches the canal, then the section cannot be utilised. Importantly, when determining the thickness of the section, it is necessary to calculate how much dentine will be removed within the canal using instruments during the preparation phases of the specimen. In addition, sufficient dentine thickness is necessary to avoid perforation or splitting of the section. To achieve adequate control during this finishing phase, it is advisable to work under a microscope.

The section is then washed in distilled water and subjected to a process during which water is gradually replaced with alcohol by passing it through a series increasing concentrations of alcohol solutions at 30%, 50%, 80%, 96% and absolute alcohol. The specimens are left to incubate at each dilution for approximately five minutes until the dentine begins to lose its opacity and the root becomes transparent. The stained pulp tissue becomes dark red or brown (if Mallory's Mixture is used). At this point, the sections were transferred to a bath of absolute alcohol in a Petri dish to better observe the anatomy under the microscope. Next, we determined how the tooth would be oriented for mounting, which is dependent upon the purpose for which it is will be used. The sections were then transferred into methyl benzoate for at least two or three days during which time, the oil of this solution will replace any small bubbles of air in the dentinal tubules to create transparency. If the tooth has not reached the desired transparency within two or three days, then it will remain in the methyl benzoate until the dentine