OBJECTIVE: This study evaluated the hydrophobicity of dentin surfaces that were modified through chemical silanization with octadecyltrichlorosilane (OTS). Material and Methods: An in vitro experimental study was performed using 40 human permanent incisors that were divided into the following two groups: non-silanized and silanized. The specimens were pretreated and chemically modified with OTS. After the chemical modification, the dentin hydrophobicity was examined using a water contact angle measurement (WCA). The effectiveness of the modification of hydrophobicity was verified by the fluid permeability test (FPT). Results and Conclusions: Statistically significant differences were found in the values of WCA and FPT between the two groups. After silanization, the hydrophobic intra-radicular dentin surface exhibited in vitro properties that limit fluid penetration into the sealed root canal. This chemical treatment is a new approach for improving the sealing of the root canal system.

Key words: Dentin. Surface properties. Octadecyltrichlorosilane.

INTRODUCTION

Micro-leakage directly affects the results of the root-canal treatment. Complete obturation of the root-canal with an inert filling material to create an apical seal has been proposed as the goal for successful endodontic treatment. However, micro-leakage can occur and is the primary cause of endodontic treatment failure. In cases of endodontic treatment failure, the probabilities of retreatment or surgical success range from 84% to 92%, depending on the material used (Super eBA or MTA) and the technique used (conventional or microsurgery)\(^\text{10}\). Nevertheless, the literature suggests that persistent intra-radicular infection and further leakage are the major causes of endodontic treatment failure\(^\text{21}\).

Root canal obturation involves the three-dimensional filling of the entire root canal system; this procedure is a critical step in endodontic therapy. Root canal obturation serves the following two purposes: the elimination of all sources of leakage from the oral cavity or peri-radicular tissues into the root canal system, and the sealing of any remaining irritants within the root canal system after appropriate shaping and cleaning of the canals\(^\text{8}\). The importance of three-dimensional obturation of...
Chemical treatment of the intra-canal dentin surface: A new approach to modify dentin hydrophobicity

Sample preparation

For the surface characterization, 40 human incisors that were recently extracted for surgical or periodontal reasons were collected and then stored in a 0.1% sodium azide (NaN₃) solution (Sigma-Aldrich, Paris, France). The dental crowns were sectioned and 3-mm-thick, radicular dentin slices were obtained using a low-speed cutting machine (Isomet, Buehler, Lake Bluff, IL, USA) under copious water cooling. These slices were randomly divided into the following two experimental groups (10 samples each): non-silanized and silanized. Both groups were evaluated based on the WCA measurements and fluid permeability tests (FPT).

Pretreatment protocol

All of the specimens were pretreated as previously reported to eliminate the smear layer and expose the collagen fibers. Briefly, the specimens were immersed for 3 minutes in 17% ethylene-diamine-tetraacetic acid (EDTA) (J.T. Baker, Mexico City, Mexico) solution, washed with distilled water, immersed for 5 minutes in a 2.5% NaOCl (J.T. Baker) solution, again washed with distilled water, again immersed for 3 minutes in the EDTA solution, and washed with distilled water. The specimens were examined at ×3,100 and ×5,500 magnifications under a scanning electronic microscope (SEM) (JEOL LUV 5900, Tokyo, Japan) to assess the elimination of the smear layer and the exposure of collagen fibers, respectively.

Table 1 - The mean values of contact angles (Mean±SD)

| Group       | Mean±Standard Deviation |
|-------------|-------------------------|
| Silanized   | 116°±5°                 |
| Non-silanized | 48°±16°*               |

(*Statistically significant p<0.05).
Silanization protocol
After pretreatment, the specimens underwent silanization by the immersion of each specimen for 1 minute each in 20 mL distilled water, then 20 mL ethanol (Fermont Chemicals, Monterrey, Mexico) and finally 20 mL ethyl acetate (J.T. Baker). Afterwards, the specimens were immersed for 1 minute each in an 86% octadecytrichlorosilane (OTS) (100 µL) (Fluka, Sigma Aldrich, Switzerland) solution in ethyl acetate (10 mL), then in ethyl acetate alone (20 mL) and finally in ethanol (20 mL).

Water contact angle measurement
The WCA of the dentin surfaces was measured by the sessile drop technique using a DGD-DX model analysis system (GBX Scientific Instruments, Valence, France). One drop (4 µL) of distilled water was deposited on each dentin surface with a micro-syringe (Figure 1). Images were captured immediately after the deposition using a micro-video system (GBX). The acquired images were analyzed by the Visiodrop software (GBX). The WCA values were analyzed by the Wilcoxon test; the statistical significance was set at p<0.05.

Fluid permeability test
Recently extracted uniradicular teeth were used. The crowns were sectioned to obtain a standard tooth root length of 10 mm. Patency of the root canal was achieved with a 21-mm file, type K #10; the working length was reached at 0.5 mm from the apex. The root canal was reamed with the ProTaper Universal system until its size corresponded to that of an F3 instrument. For irrigation, 2.0 mL of 5.25% NaOCl was used at the change of each instrument; a final irrigation was performed with 5 mL of 17% EDTA. All of the specimens were obturated using gutta-percha with RoekoSeal (Coltene Whaledent, Cuyahoga Falls, Ohio, USA) as a sealer. For the FPT, the external surfaces of the root were sealed with nail varnish, except for the apical third. The FPT was performed using a closed pressure system under a constant pressure of 3 psi. This pressure was applied with nitrogen for 16 minutes. An air bubble was introduced with a syringe and it was positioned in the millimeter ruler. The FPT was performed by measuring the path of advancement of the air bubble, and the reading was converted to µL/min using the linear velocity formula, \( V = \frac{\pi r^2 L}{T} \). The FPT values were analyzed by the Wilcoxon test; the statistical significance was set at p<0.05.

RESULTS
Figure 2 shows the SEM images after pretreatment. As observed in Figure 2A, the smear layer and debris were effectively eliminated by pretreatment. The presence of collagen fibers was also confirmed by the SEM image, as shown in Figure 2B. Table 1 shows the means and standard deviations of the WCAs of the dentin surfaces in the non-silanized and silanized groups. The contact

![Figure 2](image)

**Figure 2** - The scanning electron microscopy (SEM) image of the pretreated dentin surfaces: A- shows open dentinal tubules and the absence of the smear layer at a magnification of x3,100; B- shows collagen fibers on the dentin surface at x5,500 magnification

| Table 2 - The values for liquid flow at 16 min of evaluation |
|-------------------------------------------------------------|
|                | 2 min     | 4 min     | 6 min     | 8 min     | 16 min    |
| **Group**      | **Time**  | **Mean±Standard Deviation** |
|----------------|-----------|---------------------------|
| Silanized      | 0.013±0.0013 | 0.013±0.0012 | 0.007±0.0002 | 0.005±0.0001 | 0.001±0.0001 |
| Non-silanized  | 0.039±0.0021 | 0.026±0.0012 | 0.026±0.0012 | 0.013±0.0011 | 0.005±0.0001 |

Values represent the mean of 3 samples; data are expressed in µL/min⁻¹.
angles were significantly higher after treatment with OTS (p<0.05), thereby showing a decreased wettability of the modified surfaces.

The liquid flow was assessed for 16 minutes (at 2, 4, 6, 8 and 16 min). The FPT showed a liquid flow of 0.3264±0.0123 μL/min⁻¹ through the non-silanized root canals and a liquid flow of 0.1172±0.005 μL/min⁻¹ through the silanized samples. These values represent the total liquid flow at all times in each group. The difference in the total liquid flow between the two groups was statistically significant (p<0.05). These results are shown in Table 2.

**DISCUSSION**

This study was focused on the modification of the intra-radicular dentin surface with OTS in ethyl acetate solvent after chemical pretreatment. OTS is the most effective silanizing agent that is used for the production of hydrophobic surfaces. OTS has been extensively used in semiconductors, bio-membrane models, nanolithography, infrared spectroscopy and Raman spectroscopy²⁵.

In dentistry, silanes have been used to coat the inorganic filler particles on polymer matrix composites and for adhesive bonding to resin and luting porcelain cements in restorative procedures¹³,¹⁸. Silanization is also performed for post collocation in endodontics, where silanes are coated on the surface of the post for repelling water, blood and saliva²³,²⁹. We report a new approach to the modification of the intra-radicular dentin surface by converting it to a hydrophobic surface to improve root canal sealing. There are a few reports that focused on changing the properties of an intra-radicular dentin surface¹²,²².

In this study, subsequent treatments were performed to modify the intra-radicular dentin surface. First, we carried out a pretreatment with EDTA (17%) and NaOCl (2.5%) solutions. Further, the effectiveness of EDTA concentrations in the range of 15%–24% within a period of less than 8 minutes for preventing cell necrosis and maintaining the integrity of the collagen was demonstrated. The use of a 2.5% concentration of NaOCl for less than 1 hour was shown to cause minor changes in the collagen fibers. The intra-radicular dentin was not damaged at these concentrations and exposure times, and the integrity of the collagen fibers was conserved and they remained functional for chemical interaction with the OTS.

The WCA measurement determined the wettability of the surface. A surface is considered to be hydrophobic when the contact angle is over 90 degrees¹¹,²⁰. The hydrophobicity of the intra-radicular dentin surface was assessed by the WCA measurements before and after the chemical modification. The pretreatment group showed an average contact angle of 48°±16°, which indicates the original hydrophilic nature of the intra-radicular dentin. After silanization, the average contact angle was 116°±5°, thereby indicating the hydrophilic to hydrophobic transformation of the intra-radicular dentin surface.

The purpose of this procedure is to allow the OTS to react chemically with the collagen fibers and hydroxyapatite (HA). The chemical interaction between the OTS and the dentin takes place in HA and the collagen fibers. In 22% of the interactions in the collagen, the principal amino acids for that chemical interaction are glycine, proline and hydroxyproline. OTS interacts with the phosphates in HA, creating the P-O-Si bonds, and in the collagen fiber trough, the hydroxyl group (OH) creates the Si-O bonds²⁸.

The fluid filtration technique has been used to measure micro-leakage since 1988¹⁷, and various studies have used radioisotopes, dyes, fluids and bacterial growth to evaluate apical leakage as the possible cause of endodontic treatment failure²⁻¹⁹. The effectiveness of hydrophobic modification was verified by the FPT. In this test, the water flow was directed towards roots that had undergone a hydrophobic chemical treatment, and the opening was sealed with gutta-percha and RoekoSeal. After polymerization and cross-linking, the solid RoekoSeal samples exhibited an external hydrophobic surface, and they did not allow aqueous solvents to infiltrate and swell the material. Therefore, RoekoSeal can be used in combination with water without inducing material deformation, indicating that this compound is an ideal complement for use with the dentin surface treatment¹. The FPT results indicated that the modified surface effectively reduced the water flow; this result is important to avoid fluid micro-filtration. In previous reports, all of the FPTs were performed under hydrophilic conditions to evaluate the techniques and materials for endodontic obturation³,⁶,¹⁶. These studies have reported fluid permeability values under hydrophilic conditions that range from 0.50 μL/min⁻¹ to 0.26 μL/min⁻¹, depending on the obturation technique used. In our study, the fluid permeability value under hydrophilic conditions was 3.09x10⁻⁵ μL/min⁻¹. These results show that the hydrophilic-to-hydrophobic chemical transformation is effective in reducing the fluid permeability.

The silanization process has some disadvantages due to the possible cytotoxicity of the solvents used and sub-products derived from the chemical reaction. The proposed chemical modification of the intra-radicular dentinal surfaces is a new strategy in the prevention of apical leakage and could be an alternative approach for achieving a better clinical prognosis. The next step is to perform an
in vivo study to confirm the absence of toxicity in the periapical region and improved effectiveness in preventing endodontic treatment failure.

CONCLUSIONS

The intra-radicular dentinal surface was effectively modified into a hydrophobic surface by chemical silanization with OTS. The hydrophilic dentinal surface exhibited in vitro properties that limit fluid permeability into the root canal, which was sealed using a lateral gutta-percha condensation and silicon-based sealer. This chemical treatment is a new approach for improving the sealing of the root canal system.

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