A novel methodology for quantifying the removal of a biofilm-mimicking hydrogel from lateral morphological features of the root canal*

Abstract

**Aim** To introduce and characterise a reproducible hydrogel as a suitable biofilm-mimic in endodontic research. To monitor and visualise the removal of hydrogel from a simulated lateral canal and isthmus for: i) Ultrasonic Activated Irrigation (UAI) with water, ii) UAI with NaOCl, iii) NaOCl without UAI. **Methodology** A rheometer was used to characterise the viscoelastic properties and cohesive strength of the hydrogel for suitability as a biofilm mimic. The removal rate of the hydrogel from a simulated lateral canal or isthmus was measured by high speed imaging operating at frame rates from 50 to 30,000 fps. **Results** The hydrogel demonstrated viscoelastic behaviour with mechanical properties comparable to real biofilms. UAI enhanced the cleaning effect of NaOCl in isthmi (P<0.001) and both NaOCl and water in lateral canals (P<0.001). A greater depth of cleaning was achieved from an isthmus (P=0.009) than from a lateral canal with UAI, and also at a faster rate for the first 20 seconds. NaOCl without UAI resulted in a greater depth of hydrogel removal from a lateral canal than an isthmus (P<0.001). The effect of UAI was reduced when stable bubbles were formed and trapped in the lateral canal. Different removal characteristics were observed in the isthmus and the lateral canal, with initial highly unstable behaviour followed by slower viscous removal inside the isthmus. **Conclusions** The biofilm-mimic hydrogel is reproducible, homogenous and can be easily applied and modified. Visualisation of its removal from lateral canal anatomy provides insights into the cleaning mechanisms of UAI for a biofilm-like material. Initial results showed that UAI improves hydrogel removal from the accessory canal anatomy, but the creation of stable bubbles on the hydrogel-liquid interface may reduce the cleaning rate.

Introduction

Bacteria adhere to surfaces and rapidly form a biofilm, in which they are protected against chemical and mechanical stresses (Flemming et al. 2007). In infected root canals, these biofilms adhere to the root canal wall (Ricucci et al. 2009) and are particularly problematic in accessory root canal morphologies such as lateral canals, fins and isthmi because these areas are unreachable by instrumentation (Peters et al. 2001; Ricucci et al. 2013). Few studies have thus far focused on how bacteria in a biofilm cope with mechanical stresses and in endodontics this topic has yet to be addressed.

Biofilms are structured by a matrix of extracellular polymeric substance (EPS), which encompasses up to 90% of the biofilm. The EPS matrix provides the biofilm with viscoelastic properties, facilities nutrition and acts as protection from chemical and mechanical attacks imposed by dental cleaning procedures and disinfectants (de Paz 2007; Stewart & Franklin 2008). Indeed the viscoelastic properties facilitate the biofilm's ability to deform and adapt under mechanical stresses.

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Under minor stresses, a biofilm can deform elastically, whereas under more intense stresses it can flow viscously (Körstgens et al. 2001; Flemming et al. 2007). Understanding this behaviour in relation to fluid flow and chemical action of irrigants is fundamental for optimising biofilm removal strategies during root canal irrigation. Therefore, there is a need for an endodontic research model which can study the viscoelastic behaviour of biofilm during irrigation procedures.

Until now, there has been no consensus with regards to a representative endodontic biofilm model which would simulate the clinical situation in vitro, as neither the properties or behaviour of a real biofilm are easy to replicate in vitro (Stewart & Franklin 2008). A potential way forward would be the introduction of model viscoelastic materials such as hydrogels in endodontic models, which could mimic the viscoelastic properties of biofilm reported in the literature.

Ultrasonic Activated Irrigation (UAI) has been shown to be more effective than conventional syringe irrigation in mechanically cleaning confined areas of the root canal system (Burleson et al. 2007; van der Sluis et al. 2010). The results were explained by an increase of irrigant flow towards these confined areas (Jiang et al. 2010). However, the cleaning mechanism and enhancement of UAI for removing a viscoelastic material like biofilm is yet to be demonstrated. The use of optical methods, including low- and high-speed imaging, inside optically transparent root canal models can potentially provide qualitative and quantitative information on the cleaning mechanisms associated with UAI (de Groot et al. 2009).

The aims of this study therefore were a) to develop and characterise a biofilm-mimicking hydrogel, b) to characterise how this hydrogel is removed from simulated lateral canals and isthmi by UAI and the role, origin and growth of large bubbles therein, and c) to measure the hydrogel removal rate from the simulated lateral canals and isthmi with and without UAI, and with water or NaOCl as irrigant.

Materials & Methods

A) Characterisation of the hydrogel model

Composition

A hydrogel was created by dissolving 3 g of gelatin (Merck, Whitehouse Station, NJ, USA) and 0.06 g of hyaluronan (sodium hyaluronate 95%, Fisher, Waltham, MA, USA) in 45 mL of filtered water at 50°C (Popa et al. 2011). Red food dye (0.25 g; KTC, Wednesbury, UK) and hollow glass spheres (0.2 g; average diameter of 10 μm, density 1100 kg/m³; Sphericel, Potters Industries, South Yorkshire, UK) were added to aid visualisation. A pilot study using a thermocouple had demonstrated that the gel would solidify after 1 minute in a model at room temperature of 21°C. Therefore, the hydrogel was kept in liquid phase in a water bath at 30°C prior to use.

Rheological measurements

A commercial rheometer (MCR501, Anton Paar, Graz, Austria) was used to measure the viscoelastic properties and cohesive strength of the hydrogel. A volume of 150 μL of hydrogel was placed between the conical top plate (diameter 40 mm) and the bottom plate (Figure 1). The storage and loss moduli were measured in the frequency range 0.1 – 100 Hz and temperatures in the range 21-31°C. These variables were controlled through the bottom plate. A
delay of 10 minutes between experiments ensured that the hydrogel reached an equilibrium temperature. These measurements were repeated three times.

The cohesive strength was determined by varying the strain from 0.5 to 500% (with 100% being the gap between the two plates) and by measuring the resulting storage and loss moduli, at a fixed frequency of 0.5 Hz and a temperature of 21°C. These measurements were repeated three times.

B) Visualisation and characterisation of the hydrogel removal process

Root canal models with simulated lateral canal and isthmus

Transparent root canal models (Figure 2) were produced by solidifying Poly-DiMethyl-Siloxane (PolyDiMethylSiloxane; Sylgard 184, Dow-Corning, Midland, MI) around a D-size finger spreader (Dentsply Maillefer, Bellaigues, Switzerland). For a model containing an isthmus, a thin metal strip (width 3 mm, thickness 0.15 mm, length 4 mm, total volume 1.8 µL) was glued to the spreader at 2-5 mm from the spreader tip. For a model containing a lateral canal, a metal wire (diameter 250 µm, length 5 mm, total volume 0.25 µL) was glued to the spreader at 3 mm from the spreader tip. The main root canal in these models had a length of 18 mm, apical diameter of 0.35 mm and a taper of 6%. The hydrogel was injected into the isthmus or lateral canal using a 30G NaviTip (Ultradent, South Jordan, UT, USA) needle whilst care was taken to avoid any air entrapment. The hydrogel was allowed to solidify for at least 1 minute at room temperature whilst the root canal models were positioned in front of a microscope (BX-FM, Olympus, Tokyo, Japan) under 2.5× magnification.

Imaging methods

A high-speed camera (SA2, Photron, Tokyo, Japan) was attached to the microscope and operated at frame rates from 50 up to 30,000 frames/second, for analysis of full lateral canal/isthmus cleaning and details of the hydrogel removal process, respectively. Illumination was provided under bright-field mode using a continuous cold-light source (ILP-1, Olympus).

C) Quantitative measurements on the hydrogel removal

The removal of hydrogel from a lateral canal (a) or an isthmus (b) was investigated by using ultrasonic activation with water (i) or NaOCl (ii) as irrigant and compared with iii) NaOCl without ultrasonic activation. Filtered water without activation was used as negative control (iv). This formed 6 experimental groups and 2 negative control groups, which were repeated 6 times.

Irrigation methods

An IrriSafe 25/25 (Acteon Satelec, Merignac, France) ultrasonic file was positioned into the centre of the main root canal as indicated in Figure 2. The files were positioned using a precision translation stage (9067M, New Focus, San Jose, CA, USA). The placement reproducibility was tested by co-registering together the first frame of 35 random movies using normalised cross-correlation (Lewis 1995). The average error in the placement differences in the two directions perpendicular to the optical axis was 10 µm and 13 µm, respectively.
The main root canal was filled with a syringe and a 30G NaviTip needle with filtered water (MilliPore, Billerica, MA, USA) or NaOCl (Sigma-Aldrich, St. Louis, MO, USA). Using a standard titration method (Vogel 1962), the concentration of NaOCl was determined to be 8.7%.

For the ultrasonic activated groups the IrriSafe file was activated for 20 sec with a power setting ‘Yellow 5’ on an ultrasonic device (Suprasson P-Max, Acteon Satelec), which corresponds approximately to 40% of its ultrasonic power range as recommended by the manufacturer. In the non-activated groups, the irrigant was left to rest in the main root canal for the same time period without any activation procedure.

Data analysis
The recorded movies of hydrogel removal were analysed in MatLab (The Mathworks, Natick, MA, USA). For each frame, the area in the lateral canal/isthmus that was occupied with hydrogel was calculated and converted into either volume or depth into the lateral canal/isthmus. The mean and standard error of the 6 measurements were plotted as a function of time. Due to the skewed distribution of the data and the null variance in some cases, nonparametric tests were employed. Kruskal–Wallis with Mann Whitney U as post hoc tests were performed to compare the volume of hydrogel removed, in each root canal model, by the 4 different irrigation protocols. Mann Whitney U test were used to compare the hydrogel removal depth from lateral canals and isthmi, by similar irrigation methods.

The null hypothesis is that there is no significant difference between the 4 irrigation protocols in removing hydrogel from lateral canals or isthmi. For all tests, P-values < 0.05 were considered statistically significant. Bonferroni correction for multiple comparisons was applied where appropriate.

D) Origin and grow of bubbles during UAI

A root canal model was created as described above but without accessory anatomy. The model was used to study the origin and growth of bubbles formed during UAI; alignment, settings and activation time of the UAI were as described above. Bubble volume growth was analysed in MatLab, by measuring the radius of a bubble in each frame of the recording.

Results

A) Characterisation of the hydrogel model:
Rheological properties

The storage ($G'$) and loss ($G''$) modulus as a function of frequency and for different temperatures are plotted in Figure 3, together with the damping factor $\delta = G''/G'$. At room temperature, the storage modulus was of the order of 102 Pa and the loss modulus was one order of magnitude lower, indicating that the hydrogel is a solid gel. At higher temperatures, the storage modulus decreased faster than the loss modulus did, resulting in a liquid gel ($G'' > G'$; $\delta > 1$) at 31°C, with a storage modulus of 10-2 Pa and a loss modulus one order of magnitude higher. With increasing frequency the (solid) hydrogel exhibited more fluid-like behaviour, as $G''$ approaches $G'$ (viz. $\delta$ approaches 1). The crossover from solid- to liquid-like behaviour was at
approximately 60 Hz (at 21°C), which indicates that the relaxation time of the cross-linking hydrogel components is 1/60 = 16.7 ms (Wloka et al. 2004). At higher frequencies, the hydrogel behaved like a liquid (G" > G', or δ > 1).

In Figure 4 the storage and loss moduli are plotted as a function of temperature at a fixed frequency of 1.33 Hz. The solid-to-liquid transition temperature was at 26.5±0.5°C. Increasing the shear in the range of 0.5 to 500% indicated that the hydrogel exhibits linear behaviour up to a strain of 50% (Figure 5). At higher strain the hydrogel started to stiffen and above 200% cohesive failure occurred.

B and C) Analysis on the hydrogel removal process

i) Ultrasonic activation with water
When the file started to oscillate, the hydrogel near the lateral canal entrance exhibited highly unstable motion. In addition, parts of the hydrogel were torn off in the lateral canal. After the first 100 µm had been removed from the lateral canal, pieces of hydrogel with an average volume of 5.1±0.9 nL were removed every 3.5±2.4 seconds (~105 oscillation cycles at a frequency of 30 kHz). Deeper into the lateral canal, the time between detaching of these pieces became larger and the fragments themselves became larger. This behaviour is illustrated by the repeated sudden increases in Figure 6, which shows the removed volume of hydrogel as a function of time. These are illustrated further with representative still images (Fig. 7).

The tracer particles embedded in the remaining hydrogel indicated that there was high-frequency oscillatory movement of these particles together with the oscillating file. However, the particles oscillation amplitude decreased rapidly with increasing distance from the file.

ii) Ultrasonic activation with NaOCl
With NaOCl no initial rapid removal of hydrogel was detected as had been with water and a more gradual cleaning was observed. When the UAI was initiated, a large number of minute bubbles formed within 20 ms (the interframe time of the imaging camera). Additionally, the characteristic sudden increases observed with water were not present when NaOCl was used. The amount of hydrogel removed after 20 seconds (16%) was lower than with filtered water (29%) (P<0.001). After approximately 10 seconds a plateau was reached, characterised by no further removal of the hydrogel. This was associated with a large amount of stable bubbles having formed in the lateral canal, which prevented streaming and further removal (Figure 4a). These bubbles had a typical radius of 60 ± 10 µm.

iii) NaOCl without activation
Within seconds after delivery of NaOCl in the root canal, a reaction was initiated place between the NaOCl and the hydrogel. This reaction was apparent from a discoloration of approximately 50 µm of the hydrogel interface, with the interface itself receding as the hydrogel was being consumed by the NaOCl. Furthermore, bubbles were formed near the interface at a growth rate of ~1.7 µm per second, up to a size of the order of 100 µm in radius.

The chemical reaction of NaOCl with the hydrogel took place at a rate of 0.6 nL/s, with a decrease in reaction rate after approximately 1 minute.
iv) Water without activation (negative control group)
After irrigant delivery we observed no quantifiable volumetric change in the hydrogel from the lateral canal. The measurement time was 120 seconds.

b) Hydrogel removal from an isthmus

i) Ultrasonic activated irrigation with water
Similar to the lateral canal case, the hydrogel near the isthmus entrance was ruptured and parts of the hydrogel exhibited highly unstable motion. Beyond a distance of approximately 500 µm into the isthmus, the removal took place more gradually and there was a smooth curved interface of the hydrogel that advanced relatively slowly into the isthmus. The amount of hydrogel removed from the isthmus during 20 seconds is plotted in Figure 8 and representative still images from one recording are shown in Figure 9. After 20 seconds, approximately 80% of the isthmus had been cleaned and further cleaning continued to occur, but at a decreasing rate.
The particles embedded in the hydrogel indicated that the interface of the hydrogel was continuously being sheared away. Meanwhile there was high-frequency oscillatory movement of the particles embedded in the hydrogel together with the oscillating file. However, the particles oscillation amplitude decreased rapidly with increasing distance from the file. Clusters of bubbles formed inside the isthmus, with a total cluster diameter of approximately 75 µm. The bubbles that comprised the clusters had radii smaller than 20 µm.

ii) Ultrasonic activated irrigation with NaOCl
With NaOCl the initial removal near the isthmus entrance was slower than with filtered water. At the start of file oscillation, a large amount of minute bubbles were formed (radius below the optical resolution of 4.2 µm; Figure 5b). Nevertheless the removal rate increased rapidly and after 2 seconds there was more hydrogel removed than with water (P=0.022). At ~15 seconds the removal stopped, because the bubbles hindered further removal.

iii) NaOCl without activation
The chemical reaction rate of NaOCl with the hydrogel was 3 nL/s, which was faster than with ultrasonic activation (P<0.05). The reaction rate was constant within the maximum measurement time of 120 seconds.

iv) Water without activation (negative control group)
After irrigant delivery we observed no quantifiable volumetric change in the hydrogel from the isthmus. The measurement time was 120 seconds.

Comparison between the hydrogel removal rates from an isthmus and a lateral canal
The dynamic removal of hydrogel from an isthmus was very different than from a lateral canal. Figures 10a and b show the borders of the hydrogel at several time intervals, displaying the different removal characteristics.
The activation with ultrasound enhanced the cleaning effect of NaOCl (P<0.001) in both lateral canals and isthmi (Figure 11). After 20 seconds, the cleaning depth in an isthmus was more than three times higher than in lateral canals (P=0.009).
The chemical reaction (in volume) between NaOCl with the hydrogel was faster inside the
isthmus than inside the lateral canal (P<0.001). However, the smaller cross-sectional area of the lateral canal meant that after 20 seconds of activation, the depth of removal was higher in a lateral canal than in an isthmus (P<0.001).

Comparison between the hydrogel removal rates with NaOCl or water
After 20 seconds of activation, lateral canals were more effectively cleaned (in volume) by UAI when using water compared with NaOCl (P=0.001). In isthmi, however, there was no difference in the total hydrogel removed from the isthmus for both irrigant types (P=0.81).

D) Origin and growth of bubbles during UAI

High-speed recordings of a bubble on a wall during ultrasonic activation in water showed bubbles oscillating together with the file. There was no measurable growth during each file oscillation. Nonetheless, on a time scale of seconds, the bubble radius was observed to grow at a rate of roughly 2 µm/s. No bubble growth was observed in the absence of ultrasonic activation.

Discussion

Anatomic complexities and high variability of the root canal system have been show as early as the work of Hess and Zurcher (1925) to the most recent microCT studies introduced by (Peters et al. 2000). It has also long been established that a root with a round tapering canal and a single foramen is exception rather than the rule, as reviewed by (Vertucci 2005). In this work, the root canal system has been simplified to a straight cone with a single lateral canal or isthmus placed in a single position in the apical third and perpendicular to the main canal. This simplification and standardisation of the accessory root canal morphology allows fair comparison between the cleaning capacity of different irrigation systems and solutions. It also provides an insight on the general mechanism of cleaning of the accessory root canal morphology, which is often neglected.

The hydrogel that was introduced in this study was intended as a controllable substitute for a natural bacteria biofilm. The measurements of the material properties showed that this hydrogel is indeed a viscoelastic material and that its storage and loss moduli are in the same range (101-104 Pa) as those reported in literature for various biofilms (Böl et al. 2013). This suggests that the hydrogel may indeed be used as a substitute for a naturally occurring biofilm for studying its interaction with flow. This could lead to insights into the removal of biofilms from lateral morphological features, in order to optimise root canal disinfection procedures. The mechanical properties of the hydrogel can be varied by adjusting the concentrations of the components. Nevertheless, a natural biofilm contains additional components from that of the hydrogel, conceivably leading to different behaviour (He et al. 2013). Furthermore, a natural biofilm may exhibit stronger adherence to the substrate, which was not controlled in the present experiment. The attachment of the hydrogel to glass is, however, difficult to control (Sagvolden et al. 1998), but is important as natural biofilms are stratified, with a stronger, well adhering layer at its base (Rochex et al. 2009). Also, natural biofilms are typically heterogeneous and contain water channels that reduce the strength of the biofilm, a property not mimicked in the hydrogel. Thus far no studies have been identified within the literature for cone-plate rheometry of natural biofilms to enable comparison with our hydrogel.
Repeated production of the hydrogel demonstrated its consistent behaviour and properties, indicating its reproducibility. The interdependence of the viscoelastic properties of the hydrogel with temperature suggests that the thermal conditions in the laboratory environment should be monitored when using this hydrogel model. In addition, in order to further reduce the variability between experiments it may be recommended to better control the placement of the file in the optical depth direction.

Knowledge of the properties of the hydrogel (or a biofilm) allows the coupling of these properties to the forces exerted by a flow. However, during ultrasonic activation of the irrigant, the biofilm is exposed to forces that oscillate at 30 kHz. At such frequencies the biofilm or hydrogel may exhibit behaviour very different to those measured with the rheometer. At frequencies approaching 100 Hz, which was the maximum frequency possible with the rheometer, the hydrogel began to demonstrate different behaviour than at low (near static) frequencies. Therefore high-frequency measurements of the mechanical properties of the hydrogel and of natural biofilms should be designed, which are not straightforward.

Ultrasonic activated irrigation was capable of removing 80% of the hydrogel from an isthmus and 16-29% of the hydrogel from a lateral canal. The visualisation of the hydrogel removal allows for an interpretation of how such a viscoelastic material is removed. This is done by linking the observations to the known acoustic streaming induced by the ultrasonically oscillating file (Verhaagen et al. 2013). Near the entrance of these morphologies, there is rapid removal of hydrogel by the oscillatory component of the flow, which is known to be dominant near the file (Verhaagen et al. 2013). Further away into the isthmus, a steady jet is formed (Jiang et al. 2010; Verhaagen et al. 2013) which caused the slow, viscous removal that resembles removal by an impinging jet (Yenkel & Middleman 1987). However, in the lateral canal, there is no space for the jet to develop and the viscous removal is absent. Rather, the oscillatory pressure induced by the file is transferred into the hydrogel and causes weakening of the hydrogel, until a threshold is reached and a part of the hydrogel can be detached. This effect should also occur inside the isthmus but it is likely to be insignificant compared with the removal by the steady jet.

Optimising the removal of a viscoelastic material from lateral anatomies by UAI is not straightforward since there is no single removal mechanism for the viscoelastic hydrogel. The oscillatory flow component near the file appears to be very efficient, but its effect decreases rapidly with increasing distance from the file. The steady jet is more efficient at larger distances, but its hydrogel removal rate is lower and it is more difficult to generate inside small lateral canal anatomies. The role of cavitation in cleaning however remains unsolved.

In the absence of activation, the chemical dissolution of the hydrogel occurred at a notably lower rate. The rate of chemical dissolution depends on the concentration of NaOCl at the interface and the cross-sectional area of the lateral canal or isthmus. The slowed-down reaction inside the lateral canal could be attributed to a depletion of NaOCl availability (Baker 1947). The stable bubbles that inhibit cleaning can be introduced in the root canal in three ways. Firstly, they are generated during the reaction between NaOCl and the hydrogel. Such bubble generation has also been observed in another recent study (Sinan et al. 2013). The gas content of these bubbles is not known, however it is proposed that these are generated due to the formation of chloramines (NH2Cl) which result from the reaction between HOCl and the nitrogen compounds of the proteins present in the hydrogel (Kugler 1969; Bailarand & Dikenson 1973)
A second mechanism for introducing bubbles is by air entrainment at the irrigant-air interface at the coronal root canal opening (Macedo et al. 2014). This effect can take place when the interface becomes unstable due to ultrasonic oscillations of the file.

A third mechanism is by rectified diffusion, which is a phenomena associated with bubbles that expand and contract under ultrasound. Meanwhile there is exchange of gas between the bubble and the surrounding liquid. However, this gas exchange is bigger during the expanded phase than during the contracted phase, leading to a net diffusion of gas into the bubble, which therefore grows (Leighton 1994). The measured growth rate of ~2 µm/s is approximately 1 order of magnitude higher than that reported by Crum (1980). However that study used a frequency of 22 kHz, a larger initial radius of 45 µm and a wall was not present next to the bubble. Rectified diffusion is enhanced under higher pressure, which is the case for smaller root canal confinements (Verhaagen et al. 2013).

The accumulation of bubbles at the interface between NaOCl and hydrogel inside lateral canals/isthmi can hinder irrigant penetration and convection and decrease the rate of cleaning. The coalescence of small bubbles into larger stable bubbles (with a radius ~100 µm) could potentially inhibit the local acoustic streaming activity which may further decrease the hydrogel removal rate. Clinically, persistent infections in confined areas adjacent to portals of exit of the root canal system often lead to endodontic treatment failures (Wu et al. 2005; Ricucci et al. 2013). Therefore strategies to avoid the formation of stable bubbles, its accumulation, in addition to its efficient removal, should be explored. In addition, it would be interesting to replace the hydrogel in this model by an in vitro microbial biofilm in order to study the diffusion of irrigant into more naturally derived biofilms and their disruption by means of different irrigant systems available.

**Conclusions**

The hydrogel approach developed in this study displayed viscoelastic behaviour with material properties similar to those reported for natural biofilms. The removal of the hydrogel from a lateral canal or isthmus of the root canal resulted from convection and was characterised by an initial rapid and unstable removal, followed by slower, constant viscous removal (isthmus) or the detaching of pieces of hydrogel (lateral canal). More hydrogel was removed from the lateral canal using UAI when using water as irrigant than with NaOCl, because of the formation of bubbles that hindered further removal. The chemical dissolution of the hydrogel by NaOCl took place at a much lower rate than that measured in the presence of activation. No hydrogel was removed from lateral canals or isthmi with water and with no activation.
Figures

**Figure 1:** Sketch of the functional aspects of the rheometer, with the hydrogel (red) placed between the conical top plate and the fixed flat bottom plate. The top plate is oscillating with a frequency $f$ and amplitude $A$; the temperature $T$ of the bottom plate is controlled.

**Figure 2:** Sketch of the root canal models with a lateral canal (a) and isthmus (b). The dimensions and locations of the canals are indicated, as well as the position of the ultrasonic file (black undulated shape). The red areas are occupied by hydrogel.
Figure 3: Storage (blue) and loss (red) modulus and damping factor (green) measured with the rheometer as a function of frequency and at different temperatures as shown.

Figure 4: Storage (blue) and loss (red) modulus measured as a function of temperature, for a fixed frequency of 1.33 Hz. The three individual measurements (thin lines) as well as the averages (thick lines) are shown. The solid-liquid transition is at the crossover between the two moduli, which is at 26.5±0.5°C.
Figure 5: Storage (blue) and loss (red) modulus and damping factor (green) as a function of shear strain, at a fixed frequency of 0.5 Hz and a temperature of 21°C. Three separate measurements are shown. Stiffening behavior is observed above a shear of 50%, followed by cohesive failure at 200% shear.

Figure 6: Removed hydrogel volume from a lateral canal as a function of time. The solid lines represent the average of 6 measurements; the thin dashed lines and open symbols indicate two times the standard error. The single thin blue line represents a single measurement, showing sudden increases in the removed hydrogel volume.
Figure 7: Three frames from two recordings showing hydrogel removal from a lateral canal: before ultrasonic activation, after 1 and after 4 seconds of activation, in water. In the first recording (a-c) stable bubbles are hindering further removal; in the second recording (d-f) no bubbles were observed.

Figure 8: Removed hydrogel volume from an isthmus, as a function of time. The solid lines represent the average of 6 measurements; the thin lines and open symbols indicate 2 times the standard error.
Figure 9: Three frames from the recordings of hydrogel removal from an isthmus, before ultrasonic activation and after 1 and 4 seconds of activation. In (a-c) water was used as irrigant; in (d-f) NaOCl was used, which lead to the rapid generation of many minute bubbles that darkened the image.
Figure 10: Hydrogel removal from a lateral canal (a) and isthmus (b). The color of the lines represents the border at a specific time, with fixed time intervals (0.55 s for the lateral canal, 0.25 s for the isthmus) and a total time of 38 s for the lateral canal and 10 s for the isthmus. For the lateral canal, several lines overlap because there was no removal between those time points. For the isthmus, initial unsteady removal can be observed (green lines, followed by steady removal (purple lines)).

Figure 11: Removed depth of hydrogel from an isthmus and a lateral canal, as a function of time, with and without UAI. The solid lines represent the average of 6 measurements; the thin lines and open symbols indicate 2 times the standard error.
References


