

## Thermographic analysis of tooth vascularization using thermal stimulation

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### ABSTRACT

**Objective:** The current pulp diagnostic techniques based on subjective patient response to electrical or thermal stimuli are unable to assess tooth vascularization, which is a true indicator of pulp vitality. The present study evaluates thermography as a pulp vitality test, assessing tooth recovery following thermal stimulation.

**Methods:** A model simulating intrapulpal circulation was developed. Superficial thermographic measurements were obtained from teeth with and without elevation of the intracoronal temperature before and after applying thermal stress with cold. The data were analyzed using analysis of variance (ANOVA), and the level of significance was set at  $p < 0.05$ .

**Results:** The model obtained could help differentiate between teeth with and without simulated pulp circulation. Recovery following application of thermal stress showed significant differences between the two types of teeth.

**Conclusion:** Thermography has the potential to be used as a diagnostic tool for the vascularity status of the dental pulp.

**Keywords:** Dental vascularization, endodontics, pulp vitality test, thermography

### HIGHLIGHTS

- The classical pulp vitality tests do not identify the presence of dental pulp vascularization.
- We present an experimental procedure to detect the status of pulp vascularization using infrared thermography.
- After a cold stimulus, recovery to the initial temperature is quicker in teeth with simulated pulp vascularization than in those without it.

### INTRODUCTION

Pulp tissue consists of richly vascularized and innervated connective tissue housed within a rigid space, with terminal blood flow and a circulatory access zone (the apical foramen) of small diameter. These characteristics imply that the defensive capacity of pulp tissue in response to a possible assault is very limited (1). Unfortunately, the amount and quality of the remnant pulp tissue can only

be determined using histological techniques (2). Pulp vitality tests are commonly used to diagnose pulp tissue disease; however, in fact, these are “pulp sensitivity tests” because they record the response of the dentin–pulp complex to external stimuli (thermal and electrical). These tests can yield false-negative and -positive results, as in the case of immature teeth or teeth that have suffered recent trauma (3, 4, 5). Thus, although the tests may yield negative results, the circulation of these teeth is usually normal (4, 6).

Other diagnostic methods have also been explored to establish pulp vascular condition, such as laser Doppler flowmetry and oximetry, although the complexity of these techniques makes their routine use questionable (4).

Thermography has been proposed for detecting pulp vitality (7) as well as for use in other fields of dentistry (8). This technique may be useful because it is noninvasive and allows a simple and precise measurement of surface temperatures (2). Technological developments in this field and use of portable systems have facilitated the use of thermography. However, in order to obtain clinically useful results, an adequate measurement protocol is needed to define the temperature difference between a healthy tooth and a tooth lacking pulp vitality, correlated to the differences in blood irrigation between the two types of teeth.

The present study was conducted to develop a thermography protocol for distinguishing between teeth with and without simulated pulp vascularization, based on the analysis of tooth temperature recovery capacity following cold stimulation. We postulated that the results ob-

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tained could be of use in developing a new pulp vitality diagnostic test.

## MATERIALS AND METHODS

### Experimental design

Forty extracted teeth were used: 10 central incisors, 10 lateral incisors, 10 canines, and 10 first premolars, which were studied using a pulp vascularization simulation system. The study was approved by the Ethics Committee of the University of Valencia (Spain). (Ref. H1448443121595).

### Procedure

The teeth were sectioned at the middle-third of the root using an 881 drill (Komet, Lemgo, Germany), and the root canal was widened to gain access to the pulp chamber. The root was perforated for placement of a needle, and the pulp tissues were removed from the chamber using a curette. (Masters, Germany). The system simulating tooth irrigation was constructed using a syringe used to inject water at 37°C, and the temperature was controlled using a thermocouple (Digital Multimeter DB 2000, Xindar, Mendaro, Spain). The syringe needle and thermocouple were fixed within the pulp chamber using a layer of photopolymerizable composite (Voco, Cuxhaven, Germany) (Fig. 1).

Thermographic video recordings were obtained in all 40 teeth in two different conditions: (a) irrigating the pulp chamber with water at 37°C and (b) without irrigation. This facilitated the comparison of thermal responses because each tooth was evaluated in both conditions.

The videos were recorded using a thermographic camera with an infrared resolution of 320×240 pixels and a thermal sensi-

tivity of <math><0.05^\circ\text{C}</math> (FLIR E60, FLIR, Wilsonville, USA). The camera was placed on a tripod facing the tooth, which was positioned against an anti-reflective black background at a distance of 1 m from the thermographic camera. An aluminum foil was placed on the black background to measure the reflected temperature according to the method defined by ISO standard 18434-1:2008.

The temperature of the laboratory where the study was being conducted was maintained at  $20\pm 1^\circ\text{C}$ , without air currents and with working illumination positioned at a distance of about 45–55 cm. The room temperature and humidity values were fed among the parameters of the camera for correct temperature calculation.

Enamel emissivity was determined experimentally and found to be  $0.84\pm 0.04$ . The emissivity was recorded by equally warming two teeth, one of which was fitted with an adhesive tape with a known emissivity of 0.95 (tape 0554 0051, Testo, S.A., Spain). By feeding this emissivity value in the camera, we were able to determine the temperature of the tooth with the adhesive tape; the emissivity of the camera was then modified until the tooth without the tape showed the same temperature as the tooth with the tape of known emissivity. The camera was turned on 10 min before the experiment to ensure electronic stabilization.

A thermographic video was recorded at a frequency of 7.5 images per second in the two aforementioned conditions (with and without tooth irrigation). In each case, the recording was started under the baseline conditions of the tooth, and after 150 s, thermal stress using a propane/butane nebulizer at a temperature of  $-50^\circ\text{C}$  (Endo Frost, Roeko, Cuyahoga Falls, USA) was applied. The video was further recorded for 600 s to ensure return to the baseline conditions in all cases. Within the tooth, the temperature was measured using the thermocouple on a continuous basis, enabling confirmation of return to the initial temperature value prior to application of the thermal stress. The video recordings were analyzed using FLIR TOOLS+ software (FLIR, Wilsonville, USA) by selecting the region of interest (ROI) corresponding to each tooth for calculation of the mean temperature at each moment in time.

To determine whether dentine thickness and, indirectly, the biological age of the tooth, could influence the measurements, two radiological studies were conducted using a cone-beam computed tomography system (Master 3D, Vatech, Hwaseong, Korea). Each study comprised 20 teeth distributed in arch form for evaluation of the thickness of the mineralized structure of each crown using Dental3D software (Anatomage, Silicon Valley, USA).

### Statistical analysis

Regression analysis was used for adjusting the experimental data regarding each of the study variables (time and temperature), and the corresponding correlation coefficients were calculated. Two-factor analysis of variance (ANOVA) was performed to assess the differences between the temperatures of the teeth with and without irrigation according to the thickness of the tooth along with the comparison of the paired means for studying the same tooth in two different experimental situations. For ANOVA, we used Scheffe test for mul-



**Figure 1.** Experimental preparation for thermographic video recording in the simulation of a tooth with vital pulp tissue (a) Perfusion system (syringe with warm water) and thermocouple inside the tooth (b) Thermographic camera (left) connected to a computer (right) for image recording

multiple comparisons. Significance was set at 0.05. The software Statistical Package for the Social Sciences (IBM, Armonk, USA), version 22.0, was used.

**RESULTS**

Figure 2 shows the average behaviour of the 40 teeth irrigated with water at 37°C versus the same teeth without irrigation at baseline (negative times), cooling (time 0), and subsequent recovery of the baseline temperature (positive times). An analysis of 50 s following cooling showed temperature recovery to follow a logarithmic function characterized by a rapid incremental rate at the start, followed by stabilization. The results of this analysis are shown in the equations for irrigated teeth (1) and non-irrigated teeth (2):

$$T (^{\circ}\text{C})=8.4\cdot\ln(t)+4.2 \quad R^2=0.930 \quad (1)$$

$$T (^{\circ}\text{C})=5.8\cdot\ln(t)-6.4 \quad R^2=0.933 \quad (2)$$

T (°C) is the mean temperature of the considered ROI and t is the time (in seconds) elapsed from the time of cooling.

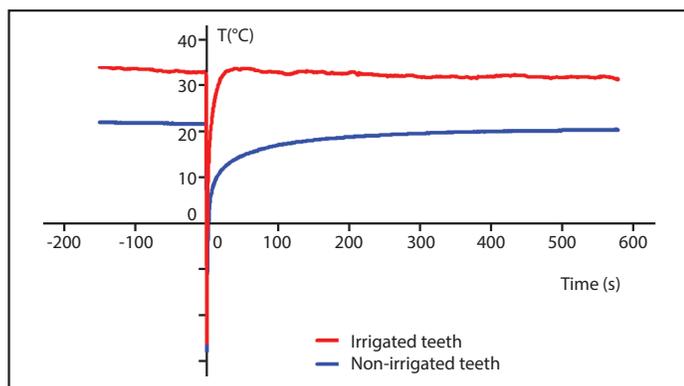
From these equations, it can be noted that a difference of over 10°C between the two types of teeth was observed as soon as 1 s after cooling and the temperature recovery rate of the irrigated teeth was 44.8% greater than that of the non-irrigated teeth.

To determine the moment after cooling at which the temperature difference was greatest between the two types of teeth, the difference between the mean temperatures at each time point for both types of teeth was calculated. Results referring to these temperature differences are shown in Figure 3.

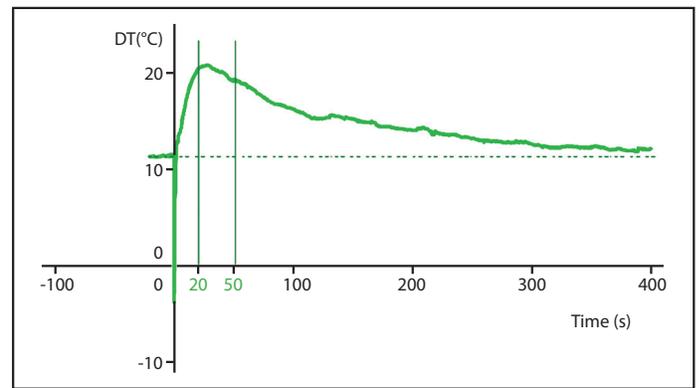
Figure 3 shows a sharp increase in the temperature difference, allowing initial polynomial adjustment (valid for the first 50 s) (eq. 3), that reached maximum after 20–30 s. This was followed by a slow decrease in the difference, characterized by a decremental exponential function [valid from 50 s after application of the thermal stress (eq. 4) till the return to baseline after approximately 300–500 s).

$$DT (^{\circ}\text{C})=0.24 \cdot 10^{-3}\cdot t^3-0.034\cdot t^2+1.124\cdot t+8.882 \quad R^2=0.960 \quad (3)$$

$$DT (^{\circ}\text{C})=41.347\cdot e^{-0.207\cdot t} \quad R^2=0.977 \quad (4)$$



**Figure 2.** Temperature (in °C) in both groups of teeth (with and without irrigation) during the experiment. Time zero corresponds to thermal stress (cooling), the negative values correspond to the baseline situation, and the positive values correspond to baseline temperature recovery



**Figure 3.** Temperature difference (DT, in °C) between the irrigated and non-irrigated teeth following thermal stress. The broken line indicates the temperature differences between the two types of teeth under baseline conditions. The maximum temperature difference between the irrigated and non-irrigated teeth was recorded approximately 20 s after thermal stress induction. The temperature difference corresponding to the baseline situation was recovered after 300 s. The vertical lines delimit the optimum time interval for recording the images in a pulp diagnosis protocol

**TABLE 1.** Maximum temperature difference, DTmax (°C) between the irrigated and non-irrigated teeth according to enamel thickness (ET) No significant differences were found (p<0.05)

ET (mm)	<1.5 n=5	1.5–2.0 n=17	2.0–2.5 n=15	>2.5 n=3
DTmax (°C)	11.5±3.1	8.0±2.2	9.1±2.3	7.2±3.3

DT (°C) is the temperature difference between the irrigated and non-irrigated teeth and t is the time elapsed after application of the thermal stress.

This behaviour allowed us to establish a time interval following application of the thermal stress in which the temperature difference between the teeth with and without irrigation was greatest, thereby providing a clear evidence of the different thermal behaviours of the two types of teeth.

The thickness of the mineralized tissue of the tooth was not relevant in this study because no significant differences were observed in the thermal response of the teeth according to enamel thickness (Table 1).

**DISCUSSION**

The few studies on this topic found in the literature stress the importance of the environmental conditions in obtaining the different measurements. In this regard, Smith et al. (9) and Kells et al. (10, 11) used a protocol similar to that of our study for obtaining the thermographic videos. The first study (9) recorded the temperature changes in the tooth for 10 min after application of the cold stimulus. The second study described a protocol for clinical recording of thermographic images. The third investigation (11) was an *in vitro* study in mandibular incisors to determine which cold application technique is best for clinical use with thermography, and it was found that the pressurized air delivered by certain dental instruments is more advisable than pieces of ice because the

stimulus must be applied in the same zone of the tooth each time.

The study samples were sectioned at the middle-third of the root and widened the root canal to allow injection of water at 37°C, as done by Smith (9). A digital thermocouple was fixed within the pulp chamber, and the same teeth were used as vital specimens (irrigating with water at 37°C to simulate the effect of intrapulpal blood circulation) and devitalized specimens (without irrigation), thereby facilitating the comparison of the effect of irrigation. The cold stimulus (-50°C) was applied using a nebulizer. In all cases, a video recording was obtained at 2.5 min prior to the application of the thermal stress, at the moment of temperature reduction (time 0), and 10 min after temperature recovery. In this way, we controlled and checked thermal stability until cold induction and were able to establish the progression of temperature recovery.

The conclusions drawn by the studies on tooth temperature variations using thermography are heterogeneous. According to some authors, temperature recovery is initially observed, followed by gradual slowing of recovery (9, 10); however, it is difficult to clinically confirm whether a tooth presents blood flow (and is therefore vital) because tooth temperature is affected by different environmental situations (9). Our study confirms observations with regard to the initially rapid temperature recovery followed by stabilization, although the effect of thermal stress manifested during a 5-min interval after cooling. However, it must be underscored that recording at ROI [ $>50\%$  of the maximum temperature difference (DT<sub>max</sub>)] where the temperature difference between the vital and devitalized teeth is analyzed should be made between 10–100 s after cooling. According to Kells et al. (10), tomographic images offering valid results regarding temperature differences can only be obtained during the first 3 min. In our study, this 3-min interval could also be used to assess the thermal stress produced, although we considered it advisable not to exceed the limit of 2 min to obtain greater differences.

On the other hand, the study on the influence of the thickness of the mineralized dental tissues revealed no significant differences among the different thicknesses. It can be concluded that at least under the conditions of our study, tooth thickness exerts no influence upon the thermographic measurements of the pulp temperature changes.

Regarding the limitations of our study, a mention must be made of its *in vitro* design in which the difference at the baseline between an irrigated and a non-irrigated tooth is already almost 11°C, with a temperature difference of almost 20°C obtained as a result of application of the thermal stress after 20 s. However, under *in vivo* conditions, the initial temperature difference can be expected to be noticeably smaller due to the influ-

ence of the surrounding tissues. Nevertheless, *in vivo* recovery process would also allow recording of a temperature difference of some degrees between the two types of teeth that would be sufficient enough to establish a diagnostic protocol. This aspect is the subject of an ongoing study by our group.

## CONCLUSION

Thermographic recordings within a few minutes (particularly within the first 20–30 s) after application of thermal stress, and the subsequent thermal recovery, are able to distinguish between a tooth with a pulp temperature equivalent to that of a vital tooth and a tooth with a pulp temperature equivalent to that of a tooth with necrotic pulp chamber.

## Disclosures

**Conflict of interest:** No conflict of interest was declared by the authors.

**Ethics Committee Approval:** University of Valencia (Spain). (Ref. H1448443121595).

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