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Cut off values of laser fluorescence for different storage methods at different time intervals in comparison to frozen condition: A 1 year in vitro study

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Abstract

Aims: The aim of the following study is to evaluate the change in laser fluorescence (LF) values for extracted teeth stored in different solutions over a 1 year period, to give cut-off values for different storage media at different time intervals to get them at par with the in vivo conditions and to see which medium gives best results with the least change in LF values and while enhancing the validity of DIAGNOdent in research.

Materials and Methods: Ninety extracted teeth selected, from a pool of frozen teeth, were divided into nine groups of 10 each. Specimens in Groups 1-8 were stored in 1% chloramine, 10% formalin, 10% buffered formalin, 0.02% thymol, 0.12% chlorhexidine, 3% sodium hypochlorite, a commercially available saliva substitute-Wet Mouth (ICPA Pharmaceuticals) and normal saline respectively at 4°C. The last group was stored under frozen condition at −20°C without contact with any storage solution. DIAGNOdent was used to measure the change the LF values at day 30, 45, 60, 160 and 365.

Statistical Analysis Used: The mean change in LF values in different storage mediums at different time intervals were compared using two-way ANOVA.

Results: At the end of 1 year, significant decrease in fluorescence (P < 0.05) was observed in Groups 1-8. Maximum drop in LF values occurred between day 1 and 30. Group 9 (frozen specimens) did not significantly change their fluorescence response.

Conclusions: An inevitable change in LF takes place due to various storage media commonly used in dental research at different time intervals. The values obtained from our study can remove the bias caused by the storage media and the values of LF thus obtained can hence be conveniently extrapolated to the in vivo condition.

Keywords: DIAGNOdent; laser fluorescence; storage methods

INTRODUCTION

The origin of the fluorescence is not fully clear,[1,2] though it seems unlikely that apatite is responsible for the basal values associated with healthy enamel.[3] The explanation may be the result of the combination of the inorganic matrix with the absorption of organic molecules.[4] Carious lesions fluoresce more strongly than healthy tissues at an excitation wavelength in the red and infrared part of the spectrum.[1,3] This increase in observed fluorescence is related to two processes: Demineralization of the tooth and bacteria with their metabolic products (porphyrins).[1,2] Most of the fluorescence is induced by the organic components,[2,3,6] rather than by crystal disintegration and transmission through scantly homogeneous enamel.[7] This hypothesis is based on the fact that laser fluorescence (LF) does not detect lesions caused in the laboratory with acids instead of bacterial activity.[1] Bacteria responsible for caries would produce certain endogenous porphyrins (fluorophores) that fluoresce when excited by red laser light.[8]

The LF-based device for caries diagnosis is equipped with a laser diode providing the excitation light source, wavelength 655 nm, necessary for detection of these fluorophores.

Most studies evaluating LF have been carried out in vitro,[2,3,9-11] though there are also a number of in vivo studies. [12-17] The results of in vitro studies cannot be extrapolated to in vivo conditions and the limitations of these studies are well-known.
In order to get the maximal sensitivities and specificities from the LF device and to obtain an appropriate guideline for the practitioner to interpret the results, several cut-off values were presented, thus making the diagnostic decision even more difficult. In many cases, the cut-off values determined in vitro proved to be lesser than the cut-off values obtained from clinical studies. This may be due to the way in which the teeth are preserved, or changes in their organic content after extraction, inducing alterations in dental tissue fluorescence. The mentioned organic matrix begins to degrade with time. These could promote variations in the optical properties of the teeth and consequently in their LF response.

Teeth being the most essential part of the dental research methodologies invariably need to be stored under proper conditions in order for various in vitro studies to be carried out authentically. Tooth storage conditions in studies are yet to be standardized. There is a need to clarify the effect of different storage media on the fluorescence readings during a specified span of time. Hence, the prime objective of this investigation was to evaluate the change in LF for teeth stored in different storage solutions as a function of time and to give cut-off values for different storage media at different time intervals to get them at par with the clinical/in vitro conditions and to see which medium gives best results with the least change in LF values and while enhancing the validity of DIAGNOdent in research.

MATERIALS AND METHODS

Sample selection

Extracted teeth were collected and subjected to an autoclave cycle for 40 min as per the guidelines of Centers for Disease Control, USA based on the study by Pantera and Schuster. Initially, all the teeth were frozen and stored at −20°C immediately after extraction and had no contact with any storage solution for 7 days. Following this, the teeth were then defrosted to room temperature over a period of 14 h placing them in plastic containers. Nearly 100% humidity was insured by placing a wet blotting paper at the bottom of each container making sure that no contact was made between the teeth and the blotting paper. All teeth were then cleaned with a toothbrush under tap water for 15 s. Only the teeth with an LF ≥30, indicative of requiring definitive restorative procedures, were used. 90 extracted teeth were selected from this pool and a specific site was selected and recorded for measurement. Each specimen had a mean fluorescence value of 92 varying from 85 to 96.

Individual storage of teeth

All 90 samples were then divided randomly into 9 groups of 10 teeth each. Measurements were made for each tooth after which the samples were stored as follows: Group 1 in 1% chloramine, Group 2 in 10% formalin, Group 3 in 10% buffered formalin, Group 4 in 0.02% thymol, Group 5 in 0.12% chlorhexidine, Group 6 in 3% sodium hypochlorite, Group 7 in a commercially available saliva substitute-Wet Mouth (ICPA Pharmaceuticals) and Group 8 in normal saline. The storage temperature was 4°C for these 8 groups. Group 9 was stored without any storage solution at −20°C.

Measurements with LF

LF measurements were performed with the same LF-based device-DIAGNOdent (Kavo, Biberach, Germany) throughout the study.

Prior to each measurement, the LF device was calibrated according to the manufacturer’s instructions. Measurements were repeated at 30, 45, 60, 160 and 365 days. In order to repeat the readings at the same site each time, an indentation was marked near the site on the teeth with a round bur [Table 1, Figure 1].

Before each measurement, all teeth were defrosted to room temperature for 14 h. Group 9 (frozen teeth) were kept at 100% humidity while the other groups were kept in their respective storage solutions following which they were rinsed under tap water for 3 min to remove residual solution before carrying out measurements. After measurement, Groups 1-8 was placed in fresh solutions while Group 9 was frozen at −20°C again. All specimens were placed in darkness.

Data analysis

In order to allow comparisons between individual LF measurements with time, the baseline was set at 0.00 and curves were plotted [Figure 2].

The mean change in LF values for the eight storage media and frozen condition was calculated. Average change at different time intervals were used as cut-off values as compared to the in vitro condition. Statistical Package for Social Sciences for Windows® version 20. Statistical significance was set at P = 0.05. The mean change in LF values in different storage mediums at different time intervals were compared using two-way ANOVA.

Table 1: Laser fluorescence values of groups at different time intervals

<table>
<thead>
<tr>
<th>Groups</th>
<th>t30</th>
<th>t45</th>
<th>t60</th>
<th>t160</th>
<th>t365</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHLX</td>
<td>−6.0018</td>
<td>−16.1478</td>
<td>−18.939</td>
<td>−17.7908</td>
<td>−15.5253</td>
</tr>
<tr>
<td>BFOR</td>
<td>−11.6185</td>
<td>−18.1938</td>
<td>−21.5608</td>
<td>−22.9719</td>
<td>−23.5339</td>
</tr>
<tr>
<td>CHLR</td>
<td>−17.8742</td>
<td>−32.0579</td>
<td>−44.0616</td>
<td>−53.4288</td>
<td>−63.0179</td>
</tr>
<tr>
<td>NS</td>
<td>−16.5447</td>
<td>−31.9059</td>
<td>−45.398</td>
<td>−57.7872</td>
<td>−68.8482</td>
</tr>
<tr>
<td>FRZ</td>
<td>−0.3101</td>
<td>−0.0915</td>
<td>−0.1044</td>
<td>0.1131</td>
<td>0.5496</td>
</tr>
<tr>
<td>THY</td>
<td>−13.6475</td>
<td>−25.7136</td>
<td>−28.8632</td>
<td>−31.9088</td>
<td>−38.0159</td>
</tr>
<tr>
<td>NaOCl</td>
<td>−13.3682</td>
<td>−30.349</td>
<td>−42.3599</td>
<td>−56.9779</td>
<td>−70.2814</td>
</tr>
<tr>
<td>WM</td>
<td>−13.6898</td>
<td>−29.0953</td>
<td>−42.6784</td>
<td>−55.1482</td>
<td>−67.7321</td>
</tr>
</tbody>
</table>
RESULTS

In all specimens the difference was statistically significant for all groups except for the frozen specimens. All curves dropped dramatically immediately after a storage in solutions in Groups 1-8. Maximum drop occurred between days 1 and 30 after which the drop was gradual. The change in LF values at 30 days as a percentage of total change was evaluated and presented in the form of a bar diagram [Figure 3].

It was discerned that specimens stored in Wet Mouth, sodium hypochlorite, chloramine and normal saline exhibited a slower initial decline in LF values (up to day 30) as compared to formalin, buffered formalin, thymol and chlorhexidine.

Therefore, it can be clearly discerned from Figures 2 and 3 that for short-term storage purposes-Wet Mouth, sodium hypochlorite, chloramines or normal saline should be preferred while for studies requiring long-term storage of teeth as specimens-formalin, buffered formalin, thymol or chlorhexidine should be preferred. However, the best storage condition would undoubtedly be the frozen condition which showed a statistically insignificant change ($P > 0.05$) in LF values over the entire period of the study.

DISCUSSION

Most of the research studies carried out in dentistry are first evaluated under laboratory or in vitro conditions and only after a hypothesis is tested under these conditions, is the methodology extrapolated onto the clinical or in vivo condition. Various factors such as room temperature, humidity and storage media need to be considered when working under laboratory conditions.

In the in vitro condition, the variable hypothesized to affect differences in cut-off values in different studies$^{[15,19-22]}$ has been the storage solutions. Most of the storage solutions, used in dental research for preservation of extracted teeth, are antiseptics or disinfectants which are basically a “protoplasmic poison” for the bacteria. This probably leads to decrease in the concentration of fluorophores in the specimen by dilution ultimately leading to a reduction in the LF signal. Moreover, the chemicals used might even cause a modification of the fluorophores or affect the optical properties of dental hard tissues like dispersion and scattering over time. On the other hand, freezing is known to exert a bacteriostatic effect, as this is commonly used for preservation of bacterial cultures.$^{[23]}$ Furthermore, it is highly unlikely that bacteria or their endogenous products (fluorophores) will undergo a change if no solution is in contact with them, such as in the frozen condition.

Very few studies have dealt with the issue of effect of storage on LF response. In a study$^{[23]}$ spectroscopic changes in human dentine exposed to various storage solutions were measured in the ultraviolet, visible and near-infra-red spectral ranges with an integrating
sphere spectrophotometer over a 28 day period. It was concluded that changes in surface chemistry and optical properties of dentine did occur as a function of storage solution and time, which must be considered when studying dentine. Another study[20] had a conflicting finding of increase in fluorescence after formalin fixation. However, in that experiment, the specimens had been stored in thymol saturated saline followed by subsequent storage in neutral-buffered formalin unlike our study where no prior contact was made with any other solution. Hence, the two studies cannot be compared. Few other studies[25-27] have been conducted in the recent past which concluded that a decrease in LF values takes place when teeth are stored in different storage solutions as functions of time which is in concurrence with our findings.

Our study evaluated a change in LF values for eight different storage solutions in comparison with the frozen condition which has not been previously conducted. In our study, a temporal relationship has been established in the drop in LF values wherein the maximum drop was seen during the first 30 days of storage following which the drop in the readings was found to be gradual. The observed drop in the LF values was significant for each of the eight solutions while it remained insignificant for the frozen condition, which is in agreement with the previous studies of Lussi et al.[11] Since there was little or no change in the LF values for the frozen teeth, hence it may not be wrong to assume that the frozen condition may be comparable to the in vivo condition thus enabling the practitioner to interpret results and extrapolate them with more accuracy to the clinical condition.

The study validates use of DIAGNOdent as a reliable evaluating measure in various researches being conducted globally in the field of dentistry. Recently, many researches have been conducted, utilizing DIAGNOdent as a primary tool for studying mineralization of dental specimens.[28-30]

It is our study that goes a step ahead in providing cutoff values for each case at different time intervals in an attempt to obtain appropriate guidelines for undertaking such studies in the future.

CONCLUSION

Only a few studies can be found in the literature dealing with the issue of the influence of storage on LF response. The inevitable inherent change in LF due to various storage media commonly used, at different time intervals is put forth by the study. The values thus obtained can help remove the bias caused by the storage medium and the values of LF thus obtained can hence be conveniently extrapolated to the in vivo condition.

The study enables better use of DIAGNOdent as an evaluating measure in various in vitro studies.

REFERENCES

Kaul, et al.: Establishing optimal cut-off values of laser fluorescence


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