International Journal of Research in Medical Sciences
Rehan AD et al. Int J Res Med Sci. 2017 Jul;5(7):3126-3128
www.msjonline.org
pISSN 2320-6071 | eISSN 2320-6012
DOI: http://dx.doi.org/10.18203/2320-6012.ijrms20173000

Original Research Article

Comparison of microwave decalcification with conventional decalcification method by using different decalcifying agents

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Received: 01 May 2017
Accepted: 29 May 2017

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ABSTRACT

Background: In routine histopathology, decalcification of teeth is an essential and important step during tissue processing. The present study was attempted to decalcify teeth using microwave method and to compare it with conventional decalcification method. The aim of the study was to compare microwave decalcification with conventional decalcification method with respect to the speed of decalcification, preservation of tissue structure, and efficacy of staining.
Methods: In our study, the total sample size used for both routine and microwave decalcification was 30 premolar teeth. The three solutions were diluting nitric acid (5%), formic acid (5%), and EDTA (14%).
Results: The results in the present study confirmed the fact that the microwave method using nitric acid was indeed the fastest decalcifying method needing just about 4 days for premolars, compared with routine decalcification. The results also showed that the overall structural details and good staining characteristics were better in teeth decalcified by 5% nitric acid in comparison to EDTA and formic acid in both the methods used. But nitric acid showed good staining details in microwave method in comparison to conventional method.
Conclusions: 5% nitric acid by microwave method proved to be the best decalcifying agent as it was fast and gave good structural details and staining characteristics.

Keywords: Decalcification, Microwave oven, Teeth

INTRODUCTION

Pierre de Coubertin proposed the Olympic motto “citius, altius, forties” which is Latin for faster, higher, and stronger. With this basis, we too have adopted the same in our quest for a decalcifying agent.[1] Hard tissue preservation close to the viable state is indispensable for the understanding of structural and sub-structural details and functions. The cutting of thin sections by conventionally used methods is impossible in cases of tissues such as bone, teeth, odontomas, and calcified lesions. For such tissues, removal of calcium is done by a process known as "decalcification" which in turn makes the tissue soft enough to be cut by the microtome.[2]

Decalcification of hard tissue is one of the most technique-sensitive procedures in the histopathology laboratory. It is of special significance in oral pathology as decalcification of bone and teeth is a routinely required procedure.[3] Decalcification is carried out by chemical

agents, either with acids to form soluble calcium salts or chelating agents that bind to calcium ions.[4]

In the manual method of decalcification, hard tissues are placed in a decalcifying agent at room temperature with changes of the solution at regular intervals till the end point is reached. Microwave decalcification is a novel technique compared to the manual method. In this method, hard tissues are placed in the decalcifying agent in a microwave oven for intermittent periods with regular changes of the solution till the end point is reached. Microwave irradiation has been shown to speed up the process of decalcification significantly-from days to hours.[5]

The present study was aimed to study decalcification of teeth by conventional method and microwave method using three different decalcifying agents and to find the best decalcifying solution to be used with respect to the speed of decalcification, preservation of tissue structure, and staining efficacy in both the methods.

METHODS

A total of 30 extracted premolar teeth were used in the study and divided equally into two groups based on the method by which the teeth were decalcified; Group 1: Conventional decalcification method and Group 2: Microwave decalcification method. Three different acids were used as the decalcifying agents, i.e. 5% nitric acid, 5% formic acid and 14% EDTA. 5 teeth were decalcified in each acid by using both conventional and microwave decalcification method. Kitchen microwave oven of LG Company with 800 W, model 2049 was used for microwave decalcification.

All the specimens were weighed and labeled for standardization of procedure. Each sample was then suspended in a Coplin jar with the help of a thread in approximately 100 ml of decalcifying agent for decalcification. The exact time at the start of decalcification was noted. The decalcifying solutions were changed and pH and temperature of the solutions were recorded on a daily basis.

In the manual decalcification procedure, the solution was changed once in 3 days and the pH and temperature were recorded on a daily basis. In the microwave technique, the specimens were irradiated for eight cycles of 8 s each (at 1-h intervals) per day for formic and nitric acids, and eight hours cycles of 10 s each at 1-hour intervals per day for EDTA so that the temperature of all three hours decalcifying solutions was maintained at around 41-43°C. The decalcifying solution was changed every day and the end-point was ascertained on a daily basis using the calcium oxalate method by Clayden.[4] Macroscopically, yellow discoloration of specimen, tearing, and shrinkage were assessed. Specimens were then processed; sectioned and stained with hematoxylin and eosin. Microscopically, sections were assessed for the quality of staining and overall structural detail. The macroscopic parameters were recorded as present or absent, and the microscopic parameters were graded as good, fair, and poor.

RESULTS

The decalcification of premolars in nitric acid, formic acid and EDTA took 35, 42 and 85 days, respectively for complete decalcification whereas the microwave oven technique took 4, 9 and 20 days, respectively. Out of the two methods which were used, microwave method of decalcification was faster than the conventional method.

Table 1: Macroscopic parameter - discoloration of teeth.

Decalcifying agent	Number of tooth	Discoloration	
		Yes	No
Conventional nitric acid	05	05	00
Conventional formic acid	05	00	05
Conventional EDTA	05	00	05
Microwave nitric acid	05	05	00
Microwave formic acid	05	00	05
Microwave EDTA	05	0	05

Table 2: Macroscopic parameter - tearing and shrinkage of teeth.

Decalcifying agent	Number of tooth	Tearing and Shrinkage	
		Yes	No
Conventional nitric acid	05	00	05
Conventional formic acid	05	04	01
Conventional EDTA	05	05	00
Microwave nitric acid	05	01	04
Microwave formic acid	05	05	00
Microwave EDTA	05	05	00

Yellow discoloration of the teeth specimens was present in 5% nitric and absent in formic acid and EDTA irrespective of the method used (Table 1). Formic acid and EDTA showed tearing and shrinkage in either of the percentages by both conventional and microwave method, which was statistically significant (Table 2).

Table 3: Microscopic parameter - overall staining.

Decalcifying agent	Number of tooth	Overall staining		
		Good	Fair	Poor
Conventional nitric acid	05	03	02	00
Conventional formic acid	05	02	02	01
Conventional EDTA	05	02	03	00
Microwave nitric acid	05	04	01	00
Microwave formic acid	05	03	02	00
Microwave EDTA	05	03	02	00

In microwave and conventional method 5% nitric acid showed good staining characteristics, 5% formic acid and EDTA showed fair staining characteristics (Table 3). Out of all the decalcified teeth, microwave method of decalcification showed significantly better staining characteristics than conventional method.

DISCUSSION

Tooth decalcification is a time-consuming process. It takes weeks and preservation of the tissue structure depends on the quality and velocity of the demineralization process. A new method using microwave oven was seen to accelerate the decalcification.[6] The choice of decalcifying agent and method is largely dictated by the urgency of the procedure.[7]

The potential application of microwave energy in histotechnology was first recognized by Mayers (1970). This form of nonionizing radiation produces alternating electromagnetic fields that result in the rotation of dipolar molecules such as water and the polar side chains of proteins through 180°C at the rate of 2.45 billion cycles/second. The molecular kinetics so induced result in the generation of energy flux which continue until radiation ceases.[8]

A study done on decalcification by Pitol et al, using microwave method has shown that there was 30-fold increase in decalcification speed with microwave method as compared with the conventional method. Another study has shown a reduction of decalcification time from 45 days to 2 days when done by microwave method.[6] Others opinion that the bone decalcification is accelerated by about 10 times using microwave method.[9] The results of the present study were consistent with the previous studies, concluding that the microwave-assisted method is faster than the conventional method.

According to a study done by Moore et al, Lynch and Culling et al, yellow staining of the tissue results when nitric acid is used as a decalcifying agent.[2,10,11] In our study, we also observed that nitric acid produced yellowish discoloration of teeth when used as a decalcifying agent irrespective of the method used. The other decalcifying agents did not produce any discoloration of teeth. Formic acid and EDTA produced tearing and shrinkage of teeth in both the microwave and conventional methods.

Nitric acid has shown good overall staining details in comparison to ethylene di-amine tetra acetic acid (EDTA) and formic acid.[12] The overall histological impression in the microwave method compared to conventional method was significantly better. Cellular structures could be well-appreciated in almost all the sections of nitric acid.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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Cite this article as: Rehan AD, Saigal S, Bhargava A, Kumar U, Thakur P, Kausar T. Comparison of microwave decalcification with conventional decalcification method by using different decalcifying agents. Int J Res Med Sci 2017;5:3126-8.