Research Article

Cavity preparation model in rat maxillary first molars: A pilot study

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Abstract: Objective: To conduct a standardized method for cavity preparation on the palatal surface of rat maxillary molars and to introduce a standardized method for tooth correct alignment within the specimen during the wax embedding procedure to better detect cavity position within the examined slides. Materials and methods: Six male Wistar rats, aged 4-6 weeks, were used. The maxillary molars of three animals were sectioned in the frontal plane to identify the thickness of hard tissue on the palatal surface of the first molar which was (250-300μm). The end-cutting bur (with a cutting head diameter of 0.2mm) was suitable for preparing a dentinal cavity (70-80μm) depth. Cavity preparation was then performed using the same bur on the tooth surface in the other three animals. Rats are then euthanized before dissecting, fixing, and demineralizing the teeth. For better alignment of teeth samples during the waxing procedure, K-file endodontic instrument size #8 was dipped in Indian ink. The file tip was inserted on the jaw bone at the buccal side of the tooth in a region opposed to the prepared cavity on the palatal side. Moreover, a small Dycal applicator instrument was used to mark the jaw bone on the mesial side of teeth samples as an orientation for the cutting surface. Results: Well-defined sections were obtained with a clear cavity extension within dentin and without any signs of pulp exposure in all samples. Conclusion: This pilot was conducted to perform an easy procedure for cavity preparation in rat molar teeth to obtain a clear histopathological section.

Keywords: cavity preparation, dentinal cavity, rat tooth, wax embedding.

Introduction

For many years, animal models have been used in dental research [1, 2]. Mainly to identify the effect of different stimuli on pulp tissue and host defense mechanism [3, 4]. These models can provide an environment that mimics aspects of biological processes without the risk of harming a human. Among those models, rodent animals are of particular interest due to their well-defined physiological characteristics. Rodent models, especially rats, account for most animal investigations in pulp biology [5, 6]. They are simple to handle and keep for extended periods, inexpensive, allow for larger sample numbers, are adapted to the lab setting, and have lower social and ethical problems than primates, making them excellent candidates [7].

Rat teeth can be used to produce two separate study designs. The incisors reflect the continuous growing paradigm, while the molars represent the restricted growth (human-like) type. The rat incisor's continuous development makes it a useful model for studying tissue's different forms and functions within the same tooth since it can reflect the entire life span of cell function from creation through maturity and wound healing [8, 9]. Rat molars have been regarded as an excellent tooth model because of their devel-

opmental similarities to human teeth. They have been utilized extensively as an *in-vivo* or *ex-vivo* tooth model [10]. It's also frequently utilized in cavity preparation research to mimic various human dental treatments [5, 11]. This is due to the rapid healing capacity of juvenile rat molar pulp and the subsequent prevailing results of this interaction. Thus, the results can be obtained within a relatively short period [12, 13]. However, the tiny size of rat molars and the limited accessibility to this region, especially the mandibular molars region, restricted the choice of the maxillary 1st molar, particularly its palatal surface, to be the most appropriate surface for the experimental work of cavity preparation. On the other hand, other studies employed rodents' physiological occlusal attrition as a model for dental trauma [14, 15].

There are many techniques to investigate the dentin pulpal interactions with cavity preparation, including histological and pathological tissue sections. To obtain correctly aligned tissue on a slide to be examined, it should be preceded by many oriented laboratory procedures. Because of the continuous development in dental bioactive materials, it has become necessary for studies that use animals, especially rats, to have technical and laboratory standardizations. However, there is little information in the literature regarding the exact technical procedure for specimen alignment within specific plans in the wax blocks during the procedure of paraffin wax embedding.

Therefore, this study aimed to conduct a standardized method for controlling dentinal cavity depth on the palatal surface of rat maxillary molars. In addition, to introduce a standardized method for tooth correct alignment within the specimen during the wax embedding procedure to better detect the cavity within the slides. This pilot study is supposed to facilitate these technical procedures by having a method to be easily followed by researchers in the same field.

Materials and Methods

The manuscript of this animal study has been written according to Preferred Reporting Items for Animal Studies in Endodontology (PRIASE) 2021 guidelines [16]. The following study was performed with the approval of the Research Ethics Committee of the College of Dentistry, University of Baghdad, project number 468522. Six male Wistar rats with ages 4-6w and weight of 75-125 g were used in this study and housed in animal cages as the experimental unit, for identifying the type and size of the drilling burs suitable for preparing a dentinal cavity on the palatal surface of the rat maxillary first molar without exposing the pulp. Three animals were euthanized using 0.7 ml/kg of pentobarbital injected intraperitoneally (200mg/ml). The maxillary jaw was carefully dissected before sectioning the area of the maxillary three molars from the right and left sides of the jaw. Then each maxillary first molar tooth was sectioned into two halves buccopallatally parallel to its long axis. The sections had been made with a diamond-coated disc mounted in a straight low-speed surgical handpiece under constant water cooling.

Three small dental burs were examined to identify the suitable size for preparing the dentinal cavity (Fig.1 a): a small round carbide bur (bits FG1/4) (Fig.1 a1), a diamond depth cutter (NTI, MADC003) (Fig.1 a2), and a diamond end cutting bur (NTI, 840-010F-FG) (Fig.1 a3). The cutting part of each bur was measured parallel to the bur shank long axis by a Vernier scale (0.8 mm, 0.5 mm, 0.2 mm), respectively. Tooth halves were examined by using a digital industry microscope camera (16MP HD Digital Industry Microscope Camera C-mount Lens, Digital magnification: 5x digital zoom, Nanyang City Srate Optical

Instrument Manufactory Srate). Images were then processed using ImageJ software to identify the thickness of the palatal hard tissues of the teeth. The range was (250-300μm) for both enamel and dentine, measured from the pulp horn to the outer enamel surface, and (70-100μm) for enamel only (Fig.1 b). As a result, the end-cutting bur was chosen for cavity preparation to obtain a cavity (70-80μm) within the dentine.

Three animals were deeply anesthetized by intraperitoneal injection with a mixture of ketamine vet. (40-75 mg/kg body weight), and xylazine (5-12 mg/kg body weight) [17]. The animal was placed on a heated pad lying on its back to access the maxillary molars, and its heart rate and temperature were monitored throughout the surgical procedure. The animal was elevated to ensure water drainage, and its mouth was opened with the aid of an alms retractor (Alms-Retractor, LAWTON-210150), and a small aspirator tip was placed near the palatal surface of the operating tooth. For magnification, a dental loupe was used (NEITS Binocular Surgical Loupe, BLP-6/5.5-6.0x) during the operative procedure. A dentinal cavity was prepared on the palatal surface of the right and left maxillary first molars by the previously determined dental bur mounted on a slow-speed handpiece with constant saline irrigation.

There was difficulty in directing the long axis of the bur parallel to the palatal surface due to the limited mouth opening of the animal (Fig.1 c). Therefore, the bur shank was aligned perpendicular to the palatal surface of the tooth for better accessibility (Fig.1 d). After that, the animals were kept in cages for 24 hours before being euthanized, and the maxillary jaw was dissected and sectioned by the same method mentioned above. The left maxillary first molars were sectioned into two halves through the cavity area in a bucco-palatal direction to be examined under the digital microscope camera to identify the cavity shape and location (Fig.1 c,d). The right maxillary molars were fixed by immersing in freshly prepared 4% paraformaldehyde (PFA) in phosphate buffer saline (PBS) for 24 hours at 4°C [18]. Then the specimens were washed twice in PBS for about 10 minutes, each time with continuous agitation to eliminate any excess of the fixative solution. Then samples were demineralized using 17% ethylenediaminetetraacetic acid (EDTA) PH 7.4 at 37^{0}C [18], with continuous agitation. The solution was renewed daily, and every week each specimen was radiographically evaluated. And a surgical blade was also used to test the hard tissue cutting resistance to evaluate the demineralization process, which required between 4-6w [14].

Samples were washed twice in PBS for 10 minutes with continuous agitation to remove any remaining demineralizing substance. Then the molars were sectioned from the demineralized jaw bone piece and placed on a glass slab for examination under the digital microscope camera. For the correct alignment of the tooth specimen within wax blocks, and because the cavity was prepared on the palatal surface, the direction of sectioning would be in the frontal plane, i.e., in the bucco-palatal direction. To do so, blue Indian ink (Rotring, Germany) was placed by using a small dycal applicator instrument to mark the jaw bone on the mesial side of the tooth sample as an orientation for the cutting surface. This ink was utilized as it resists further tissue processing required for the paraffin wax embedment procedure [19]. For locating the cavity region, the tip of K-file endodontic instrument size #8 was dipped in the Indian ink; then the file tip was inserted on the jaw bone at the buccal side of the tooth in a region opposed to the prepared cavity on the palatal side (Fig.1 e). This file facilitates the handling, stabilizing, and alignment of the tooth sample during wax embedment. Then the specimen was dehydrated in ethanol and embedded in paraffin wax according to the paraffin-embedded tissue sections protocol[20].

The file was removed after that and the wax block was fixed on the stage of the microtome machine (Leica, RM2245) which was adjusted at 4-µm section thickness. During the sectioning procedure, the first few sections were discarded until the ink started to appear on the buccal side of the specimen, which gave an indication that the cavity region was reached on its palatal side. Nearly 20-25 slides showing the cavity region were collected from each sample block. After that, slides were stained with hematoxylin and eosin (H&E) staining and observed under a light microscope (Optika microscope, Italy).

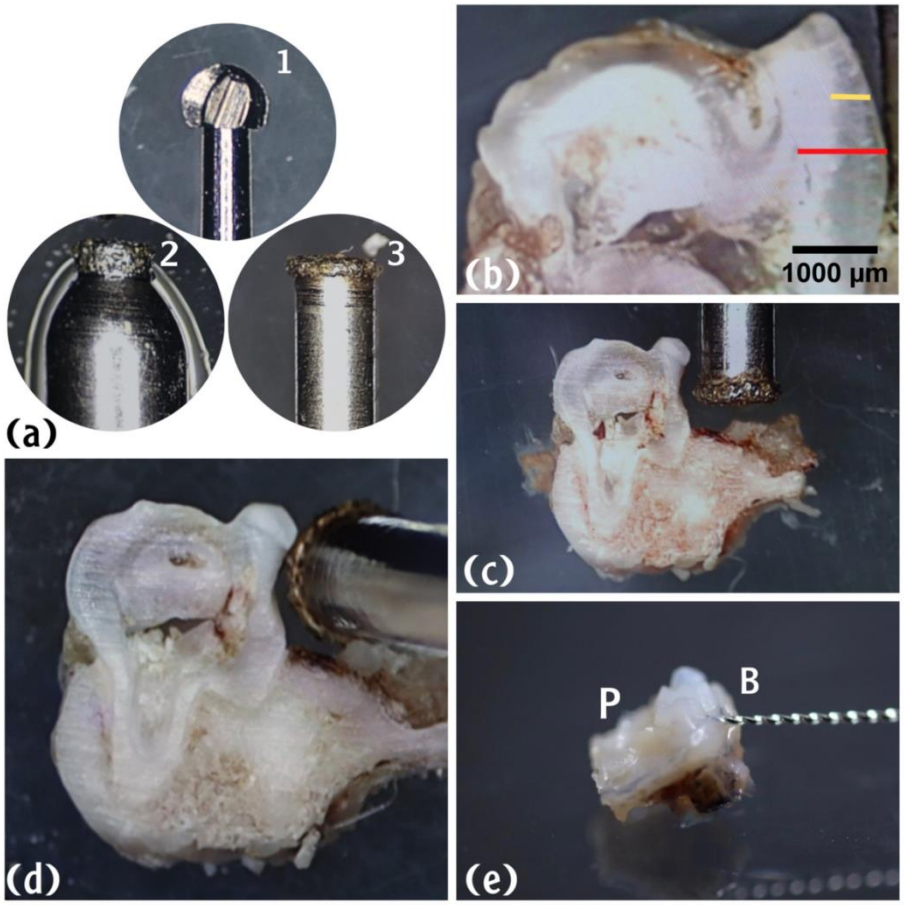

Figure 1: Cavity preparation procedure on the rat maxillary first molar. **(a)** shows the three dental burs used to identify the suitable size for preparing the dentinal cavity **(1)** the round carbide bur (bits FG1/4), **(2)** the diamond depth cutter (NTI, MADC003), and the diamond end cutting bur (NTI, 840-010F-FG) **(3)**. **(b)** shows the frontal section of the rat maxillary first molar viewed by a digital industry microscope (5x). The yellow line represents enamel thickness (about 80µm), while the red line represents whole hard tissue thickness (270µm). In images **(c)** and **(d)** show half the tooth which was frontally sectioned, and the diamond end cutting bur appears perpendicular (in c) and parallel (in d) to the tooth's long axis. **(e)** shows K-file tip was pushed slightly through the buccal bone surface **(B)** of the tooth sample opposing the palatal surface **(P)**.

Results

Images in Fig 2 show frontal sections of the tooth samples with the cavity side appearing to the right of the image (C). An overview image (a) shows the cavity extended within the dentin, leaving a

proper dentin thickness on the pulp side with no signs of pulp exposure. A higher magnification image in (b) shows a pulp-dentin region with a uniform layer of odontoblast cells (Od) lining the dentin (De).

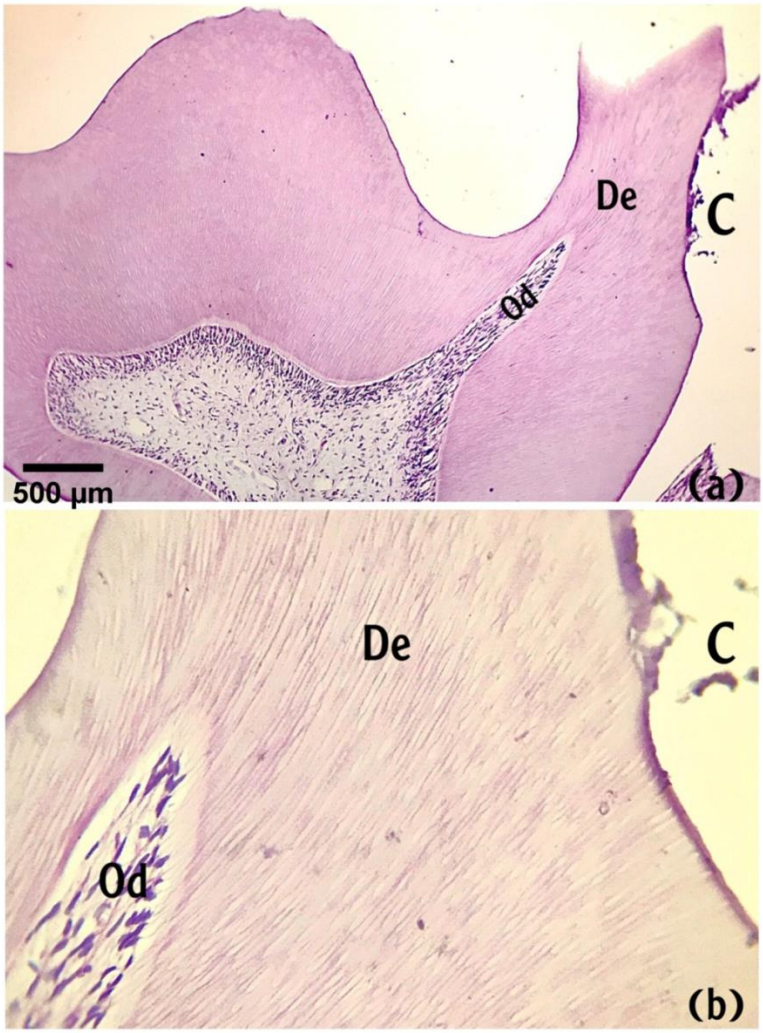

Figure 2: Demineralized frontal sections of rat maxillary first molar stained for hematoxylin and eosin (H&E). The (C) represents the cavity sides in both images. (a) shows an overview low magnification (x10) image, while (b) is a higher magnification (x40) image for the region of interest. (Od) is the odontoblast layer, and (De) is the dentin.

Discussion

The host response to an injurious agent sustains a high degree of complexity which makes it impossible to be in vitro. The associated inflammatory and reparative processes are multifactorial, and in order to study these processes from all perspectives, a suitable experimental model is required. However, it is important to address the fact that there is no universal animal model that is ideal for all research needs. Therefore, clinicians and researchers must be aware of the relative strengths and weaknesses of the diversity of the available animal models.Nevertheless, animal models assist science and scientists to obtain new knowledge and better understanding of various physiological and pathological Conditions.

Rodents models comprise the majority of animal studies in pulp biology, mainly due to the well-defined physiological parameters. They are easy to handle and house for long periods, are relatively low cost which enhances the possibility of large sample sizes, adaptable to the lab environment, have low social and ethical concerns compared to primates makes them a good choice [21]. In addition, most commercially available antibodies for cellular and molecular techniques are available for rats. In dentistry and despite the differences, rats presented a suitable model to study various fields. Two different developmental models can be obtained from rat teeth. The continuous growing model is represented by incisors and limited growth (human-like) model by molars. Continuous growth makes the rat incisor a valuable model to investigate tissue structure and function within a single organ that may represent the whole life cycle of cellular activity from formation to maturation and repair after injury [8, 22]. In addition, rat incisors provide the researcher with appreciable amounts of pulp tissue suitable for tissue cultures [23], and enough to provide a sufficient quantity of RNA even from a single pulp tissue [24].

The cavity preparation model in rat molar teeth is frequently used in dental research to mimic various human dental treatments or to investigate the biological responses of the dentin-pulp complex to cavity preparation [12, 25] or any restorative material. The rat molar teeth are of particular interest since they have the same pulp chamber, pulp tissue, and root structural traits as human teeth [26]. The majority of previous studies use the mesial surface for cavity preparation without explaining in detail the method of preparation[27, 28]. The palatal surface of the maxillary first molar is particularly interesting because it is not affected by attrition, which is already present on the occlusal surface. Moreover, the accessibility of this surface is more than the buccal surface which is covered by the strong cheek muscles, and this makes the reflection and drilling on the buccal surface is very difficult. However, cavity preparation on the palatal surface of the maxillary first molar is not an easy procedure.

The tiny size of these teeth makes it difficult to choose the drilling instrument that would prepare a cavity without exposing the pulp. Besides, it is challenging to reach the treatment region because of the molar teeth's anatomical placement posterior to a significant diastema that isolates them from the incisor teeth. The visual access to the operative region may also be significantly impeded by soft tissues of the mouth and cheeks. To properly complete any surgery, the rat's head must be positioned correctly with clear visual access to the molar teeth [5]. At the same time, sparse information is available in the literature regarding this issue [29, 30]. This procedure is almost left for a try and error by the researcher, who may spend extra animal samples, effort, and time before obtaining a consistent protocol. According to this study, the thickness of the palatal hard tissues of the teeth range was (250-300μm) for both enamel and dentine. Therefore, this study suggests using the diamond end cutting bur (NTI, 840-010F-FG) to prepare shallow to moderate dentinal cavities (the cutting part of this bur is 0.2 mm). The round carbide bur (bits FG1/4), and the diamond depth cutter (NTI, MADC003), can be used to prepare deeper cavities or to make pulpal exposure (with cutting parts of 0.5 mm and 0.8 mm respectively). In addition, this study provided a simple method to locate the cavity region in the wax-embedded teeth samples. This will save the time that would be probably spend during the sectioning procedure to obtain slides showing the area of interest (cavity area).

In a conclusion, this pilot was conducted to perform an easy procedure for cavity preparation in rat molar teeth to obtain a clear histopathological section. This was a preliminary investigation, however, and further study is planned using a larger sample size with different tissue markings to identify pulp-dentin complex responses for cavity preparation.

Conflict of interest

The authors have no conflicts of interest to declare**.**

Author contributions

All authors contributed to the study's conception and design. Material preparation, data collection, and analysis were performed by [Muna Sh. Ahmed]. The first draft of the manuscript was written by [Muna Sh. Ahmed] and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Informed consent

Consent to participate For this type of study, formal consent is not required.

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دراسة تجريبية لنموذج تحضير التجويف السني داخل الأضراس الأولى للفئران

منى شهاب احمد انس فلاح مهدي, سيف العرب محمد

المستخلص:

الهدف: إجراء طريقة معيارية لتحضير التجويف على السطح الحنكي للأضراس العلوية للجرذان ، وإدخال طريقة موحدة للمحاذاة الصحيحة للأسنان داخل العينة أثناء إجراء تضمين الشمع من أجل الكشف بشكل أفضل عن موضع التجويف داخل الشرائح التي تم فحصها. المواد والطرق: تم استخدام ستة ذكور من فئران ويستار تتراوح أعمارهم بين 4 و 6 اسابيع. تم قطع الأضراس العلوية لثلاثة حيوانات في المستوى الأمامي للتعرف على سمك النسيج الصلب على السطح الحنكي للرحى الأول والذي كان (250-300 ميكرومتر). كان برغي القطع النهائي (بقطر رأس القطع 0.2 مم) مناسبًا لتحضير تجويف عاجي (70-80 ميكرومتر) بعمق. ثم تم تحضير التجويف باستخدام نفس الطبق على سطح السن في ثلاثة حيوانات أخرى. ثم تم قتل الفئران بطريقة القتل الرحيم قبل تشريح الأسنان وتثبيتها ونزع المعادن منها. للحصول على محاذاة أفضل لعينات الأسنان أثناء إجراء إزالة الشعر بالشمع ، تم غمس حجم أداة اللبية K-file # 8 بالحبر الهندي وتم إدخال

طرف الملف على عظم الفك في الجانب الشدق من السن في منطقة تعارض التجويف المحضر على الجانب الحنكي. علاوة على ذلك ، تم استخدام أداة قضيب صغيرة لتمييز عظم الفك على الجانب الإنسي من عينات الأسنان كتوجيه لسطح القطع. النتائج: تم الحصول على مقاطع محددة جيدًا مع امتداد واضح للتجويف داخل العاج وبدون أي علامات لتعرض اللب في جميع العينات. الخلاصة: تم إجراء هذا الدليل لأداء إجراء سهل يجب اتباعه لإعداد التجويف في ضرس الفئران للحصول على قسم تشريح نسيجي مرضي واضح.