A new method for extraction of mandibular first molars in rats

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Abstract
The aim of the present study is to describe a new technique for the mandibular first molar extraction based on tooth section in rats. One hundred and forty Wistar rats, from three different researches underwent general anaesthesia. Each animal was then positioned on a specific board and hollemback 3ss was used to make the syndesmotomy. The mandibular first molars were extracted after tooth section with carbide ¼ drills in a high-speed hand piece under constant irrigation with sterile saline solution. The mesial portion of the first molar was removed with a modified curved mosquito forceps. The distal portion was removed and the socket was closed with 5-0 nylon thread sutures using non-traumatic needles. During the first week after tooth extraction, animals were fed with regular pressed food to avoid post-operatory trauma. This technique is careful and avoid unnecessary trauma, with minimal numbers of fractures (9.3%) and accidents such as haemorrhage (2%). All the reminiscent roots could be removed by the described method. Our technique proved to be an efficient model for future researches on alveolar wound healing with minimal fractures and accidents and provided better post-extraction outcomes for the rats.

Key Words:
tooth extraction, mandibular molar, wound healing, rats

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Introduction

It is reasonable the reduced number of studies that make morphological analysis of alveolar bone healing in humans. For such works, remove of the tissue is necessary[1-3], which goes against ethical principles of human experimentation. Alternatives such as clinical evaluation[4] and image analysis[5-6] have been used for the study of alveolar bone healing after tooth extractions in humans, with some limitations regarding histological and cellular information.

The morphological study of alveolar bone healing allows a better comprehension of this process including epithelial coverage over the extraction wound, local vascularization and newly bone formation[7-9]. It is also possible to analyze the effects of several drugs[10-12] or diseases such as osteoporosis[13-15] and diabetes mellitus[16] on bone, helping the development and improvement of drugs and biomaterials that promote alveolar bone healing[3,7].

Alveolar bone healing has been studied using several animal models like monkey[17], dog[18], ferret[19], rabbit[20] and, mainly, rats[9,21]. Superior incisors are preferentially used in rats[7,22-24], as well as maxillar molars[9,13,21,25-26], while mandibular molars are rarely used[12,14-15,27-30]. The higher bone density and resistance of mandible, mainly of the cortical bone, make the mandibular molar extraction prone to accidents and fractures. However, there are some biological advantages in studying molar sockets, like absence of continuous eruption and the presence of roots and periodontal ligament very similar to the human ones. In addition, extraction of the molar instead of incisive provided a better post-extraction outcome for extended periods for the rats[31].

In the context of its clinical relevance, molar extraction in rats seems to be an interesting model for the study of bone repair because of the absence of cartilage and the short period necessary for healing[32-33]. To our knowledge, in the indexed English-language literature, there is only three works using tooth section for mandibular molars in rats, made by our research group[12,14-15]. Considering these aspects, the main aim of this article is to describe a new method for the complete tooth extraction of first mandibular molars in rats.

Material and Methods

The technique described below has been used in our laboratory during the last few years in three different research projects and it involved 140 rats (*Ratus norvegicus albinus*,

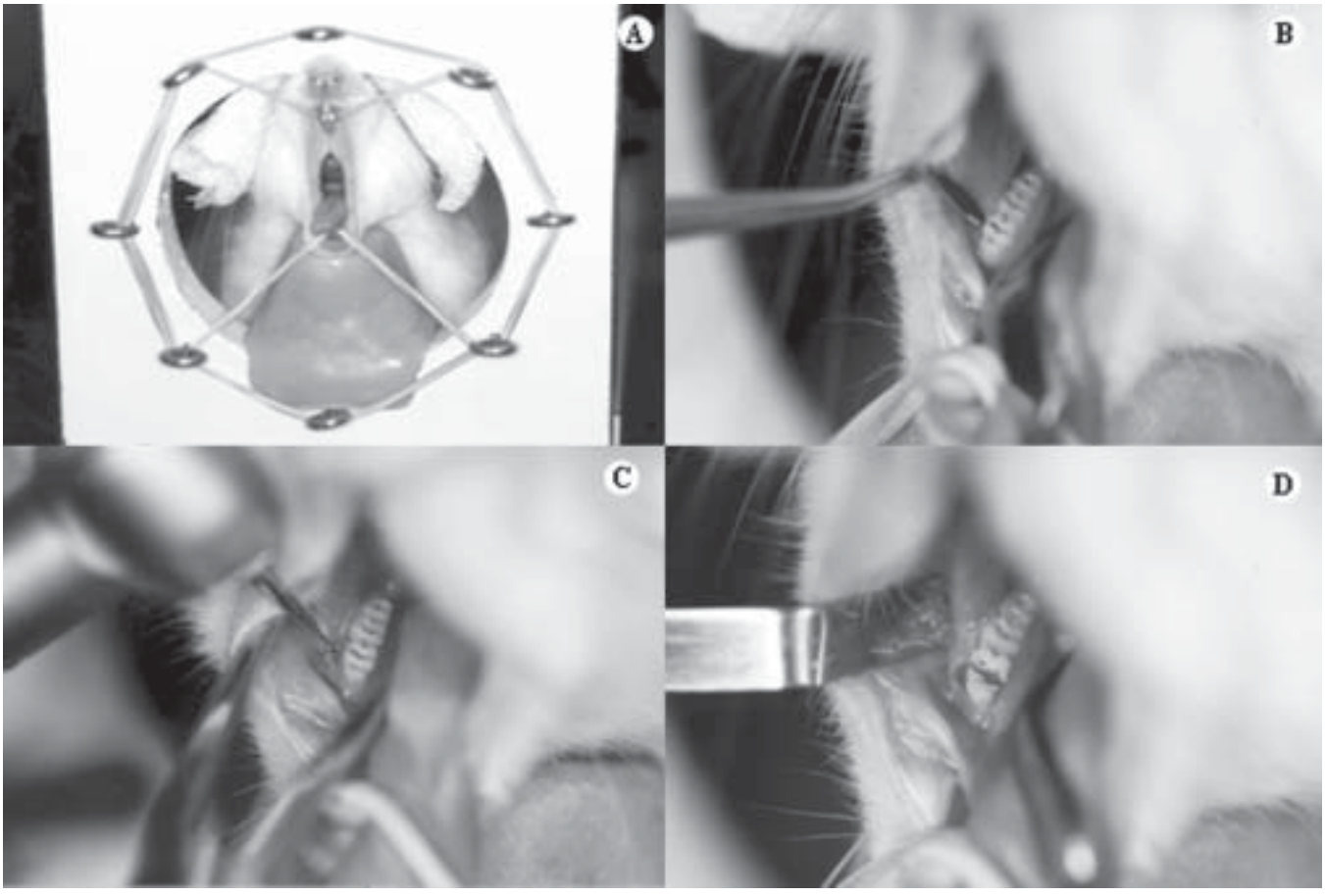

Fig 1 - *An animal positioned on a board for the extraction of the mandibular first molar and with two rubber bands maintain the mouth opened and making immovable the tong (**A**). Syndesmotomy of the mandibular first molar with a hollemback 3ss (**B**). Tooth section with carbide ¼ drills in a high-speed hand piece (**C** and **D**).*

Wistar). Morphological and molecular analysis were done about the influence of cyclosporin A (CSA) over alveolar bone healing after extraction of mandibular first molar in rats[12], as well as the influence of oestrogen's absence after ovariectomy[14-15].

All experiments were approved by the Ethics Committee of Experimentation on Animals of the State University of Campinas (UNICAMP), Brazil. All procedures for tooth extraction were done under general anaesthesia. Once verified sedation and anaesthesia signals, humidified gauze was placed to cover the animal's eyes to avoid dryness of the corneas. The animal was then positioned on a board similar to that described by Doku[34]. Two rubber bands were used to maintain the mouth opened and to make immovable the tong (Fig. 1 A). Hollemback 3ss was used to make the syndesmotomy, disconnecting the surrounding gingiva of the mandibular molar (Fig. 1 B). After that, the tooth was sectioned with carbide ¼ drills in a high-speed hand piece (Fig. 1 C and D). Constant irrigation with sterile saline solution in a disposable Pasteur pipette was done to maintain the surgical site clean and to avoid overheating of the tissues. The mesial portion of the first molar was removed (Fig. 2 A) with the aid of small dental chisel and modified curved mosquito forceps. A chisel or a hollemback was then positioned between the first and second molars allowing avulsion of the dental reminiscent (Fig. 2 B and C). The socket was closed with 5-0 nylon thread sutures using non-traumatic needles (Mononylon®, Ethicon, Brazil) (Fig. 2 D). Similar procedures were done in the opposite site. Much care should be taken to remove all blood and debris of the rat's mouth. In order to avoid a fatal haemorrhage, the mandibular third molar should not be removed, even when luxation accidentally occurs, because a large blood vessel runs below this tooth. Animals were maintained in individual cages until the total recuperation of the anaesthesia (around 20 to 24 hours) with water *ad libitum*. During the first week after tooth extraction, animals were fed with regular pressed food to avoid post surgery trauma.

Results

The methodology for tooth extraction described in this article proved to be careful and was able to avoid unnecessary trauma, allowing an uneventful recovery for the vast majority of the rats, with minimal numbers of fractures and accidents. There were some fractures (9.3%) at the cementoenamel junction of the mesial tooth portion (Table 1). This problem

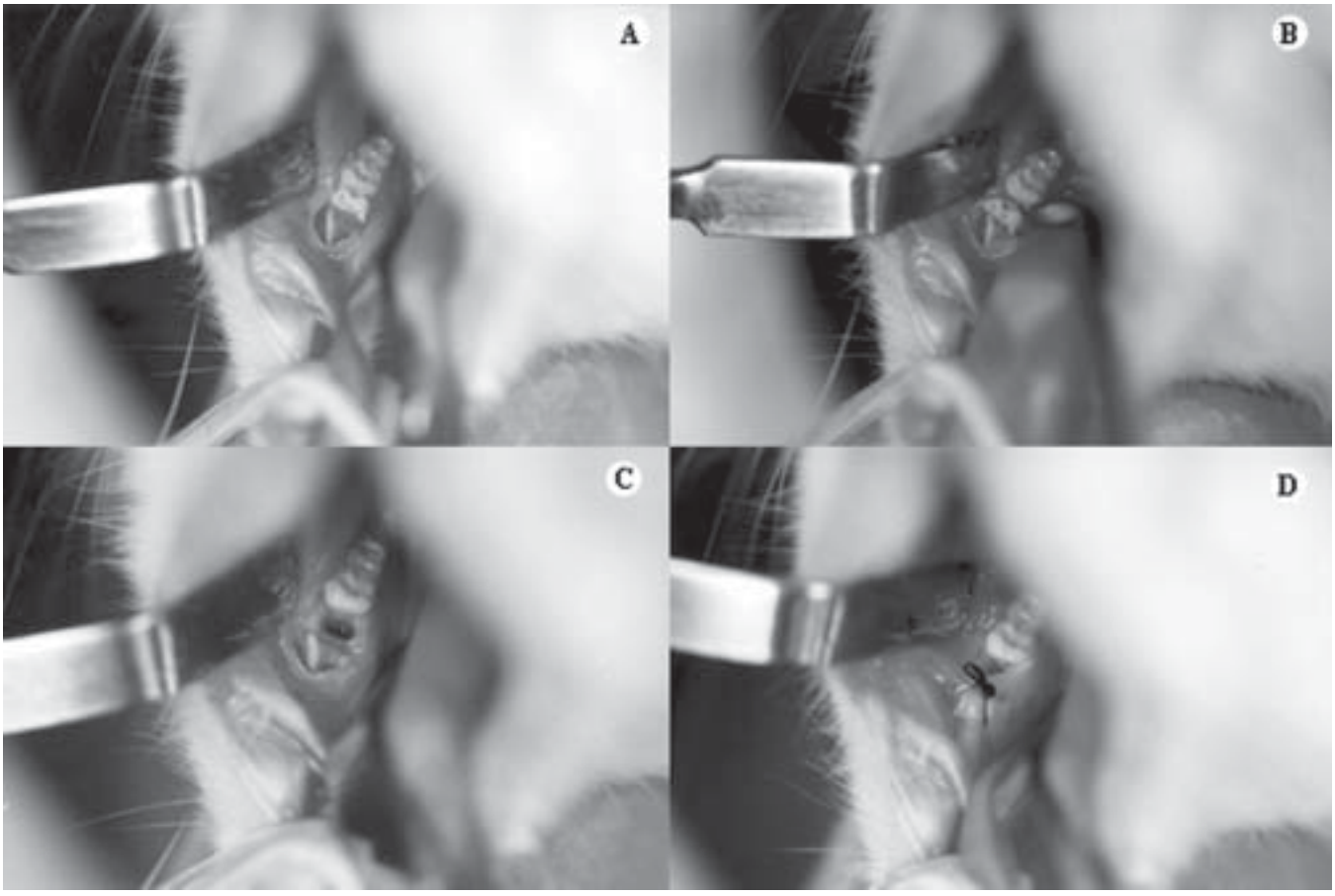

Fig 2 - *Socket of the mesial section of the first molar (**A**). Avulsion of the distal portion of the first molar with a hollemback 3ss (**B**). Socket of the mandibular first molar before (**C**) and after the suture (**D**).*

Table 1: Complications occurred during or after mandibular molar extraction in Wistar rats.

Troubles during and after tooth extraction	Number of rats
Dental fractures	13
Haemorrhage	3
Death by anaesthesia complications	6
Death by general infection	6
Total	140

was resolved with aid of the hollemback and the small dental chisel, allowing the complete avulsion of these roots. Three animals (2%) presented haemorrhage, controlled by local pressure, as consequence of accidental removal of the third molar or during the suture (Table 1). Twelve animals (8,6%) were lost, being six as consequence of anaesthesia complications and six of general infection, possibly because of immunosuppression by CSA (Table 1).

Discussion

The methodology developed for extraction of the mandibular first molar is careful, quick (about 15 to 20 minutes per animal) and allowed an adequate post surgery recovery[12,14-15].

This paper describes in details the technique and the instruments employed on tooth extraction. Especially adapted mosquito forceps[30,35-36], enamel hatchets[31,37] and modified scalers[27] have been used for mandibular molar extraction. The use of the small dental chisel as a dental elevator was very important for the complete avulsion of reminiscent roots, since residual roots retards the normal chronology of alveolar bone healing. Other authors also described the use of forceps, enamel hatchets and especially designed instruments for extraction of upper incisors[23-24,38-40], whereas maxillary molars generally are extracted with dental explorers and modified scalers used as elevators, as well as with modified curved mosquito forceps[25-26,41-42].

Animal age is a major factor to be considered, since older rats frequently present increased deposition of cementum around the root, a predisposing factor for dental fractures[9]. On the other hand, young rats present limited mouth opening, making difficult the visualization, tooth section and suture. Among the difficulties found, we emphasize the possibility of accidental extraction of the third molar which can lead to fatal haemorrhage. The technique presented in this paper allows the complete extraction of mandibular molars, opening opportunities for the development of new research models using molar sockets, without the interference of large residual roots frequently observed after the use of conventional methods for extraction. The use of this technique allows the establishment of new experimental protocol for alveolar wound healing and biocompatibility with minimal dental fractures and surgical accidents.

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