

Histologic Assessment of Extraction Sockets Following Tooth Extraction: Suitability of a Rabbit Model

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ABSTRACT

Objective: This study was aimed at determining the suitability of rabbits for assessment of alveolar bone healing following tooth extraction.

Methods: Six (6) adult female New Zealand rabbits were used for this study. They were acclimatized under standard laboratory conditions for two weeks before the commencement of the study. Tooth extraction was carried out in all the rabbits and this was followed by histologic assessment of alveolar bone healing and osteocyte count at week 2 and week 4 post-extraction.

Results: All the rabbits tolerated the extraction procedure and no complication was recorded. Histo-architecture of alveolar bone was characterized by marked osteoblastic activity at week 2 post extraction and increased osteocyte presence at week 4 post extraction. Average value of osteocyte count (cells/ μm^2) was 20 ± 4.58 at week 2 post extraction and 32.33 ± 2.08 at week 4 post extraction.

Conclusion: The result obtained from this study shows that the rabbit could well serve as an experimental animal for assessment of alveolar bone healing following tooth extraction.

Keywords: Tooth extraction, alveolar bone healing, rabbits, osteocyte count

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INTRODUCTION

Tooth extraction is one of the oldest dental procedures and one of the most frequently performed procedures in an oral and maxillofacial surgery office.¹ The extraction socket created after tooth removal is one of the most common examples of an oral wound² and its healing follows similar

principles as soft tissue healing except that it also involves healing of the alveolar bone.³

In recent times, research involving evaluation of osteogenic potentials of alloplastic materials on sites of extraction sockets (with or without implant placement) both under normal and pathologic conditions have been done with the use of experimental animal models⁴⁻⁸. While several of such

experiments have employed the use of rats as small animal models,⁵⁻⁷ some others resorted to the use of experimental beagle dogs.^{4,8} Very limited work has utilized the alveolar bone in rabbits for periodontal regeneration or dental implant studies.⁹ Rabbits however represent a relevant model in which the physiology and the pathology of periodontal tissues resemble those of humans with respect to pro- and anti-inflammatory mechanisms⁹.

This study was aimed at determining the suitability of using rabbits as an experimental animal model for the study of alveolar bone healing following tooth extraction.

MATERIALS AND METHODS

Animal Care

Six (6) female adult New Zealand rabbits with an average weight of 1.4 kg were used for the study. The rabbits were procured from a rabbit farm located in Ilesha, Osun state in Nigeria and then housed in wooden cages in the Animal Holding of the Department of Anatomy and Cell Biology, Obafemi Awolowo University, Ile-Ife. They were acclimatized for a period of 2 weeks prior to the commencement of the study under standard laboratory conditions of light and temperature. They were fed on standard laboratory rabbit chow and allowed access to water *ad libitum*. Ethical approval for the study was obtained from the Human Research and Ethics Committee of the Institute of Public Health, Obafemi Awolowo University, Ile-Ife Nigeria.

Surgical procedure

Prior to the tooth extraction procedure, all the rabbits were deeply sedated by intramuscular administration of ketamine at a dose of 50mg/kg body weight¹⁰. Once the rabbits were confirmed to be deeply sedated, the mouth of each one was propped open using a Molt retractor (Simrix SSC-S2005, Pakistan) and the right maxillary first premolar tooth was luxated using straight Coupland elevators (OEM, BC10005) and thereafter non-toothed tissue forceps was used to complete the extraction process. Hemostasis was achieved using gauze pressure packs.

Post-operative care

Post-operative pain was controlled by a single intramuscular administration of tramadol at a dose of 2mg/kg body weight.¹¹ The rabbits were closely monitored in the immediate post-operative period. Monitoring included assessment of feeding patterns

as well as visual examination of the post extraction wound.

Sacrifice

Three of the rabbits were sacrificed 14 days' post extraction while the remaining three were sacrificed at 28 days' post extraction. Sacrifice was carried out by intravenous administration of ketamine at a dose of 50mg/kg body weight. The maxillary segments containing the extraction socket were dissected out and placed in a fixative solution (10% formal saline) for tissue processing.

Tissue Processing and Hematoxylin and Eosin Staining

After fixation, maxillary segments containing extraction socket were decalcified with freshly prepared 10% EDTA for five days with the decalcification fluid changed daily. Following decalcification, excess EDTA was washed off the bone by immersion in 10% formal saline. After decalcification, the maxillary segment tissues were processed for paraffin wax embedding. Thereafter, the paraffin blocked tissues were trimmed and mounted on blocks for sectioning. Sections of 5µm thickness were produced from the tissue blocks using a rotary microtome (Leica RM 2235, Germany). The sections were spread in warm water bath, and collected on clean grease free glass slides. The slides were then dried on a drying plate overnight to enhance adherence and thereafter subjected to Hematoxylin and Eosin staining procedure.

Histology

Stained sections were examined using Leica DM 750 research microscope connected to a computer and photomicrographs of the sections were taken. Areas showing the highest amount of bone formation/density as observed under low power magnification were selected for further assessment under at high power magnification. Photomicrographs depicting alveolar bone histo-architecture both at week 2 and week 4 were subjected to Image J software analysis to determine osteocyte count per µm².

Statistical Analysis

Statistical analyses were performed using IBM SPSS Version 21. Data was expressed in Mean ± Standard Deviation (SD). The minimum and maximum values were also recorded for each parameter.

RESULTS

All the rabbits tolerated the tooth extraction procedure, anaesthesia lasted averagely for 12



Figure 1: Extraction socket immediately after tooth extraction in a rabbit (Blue arrow).

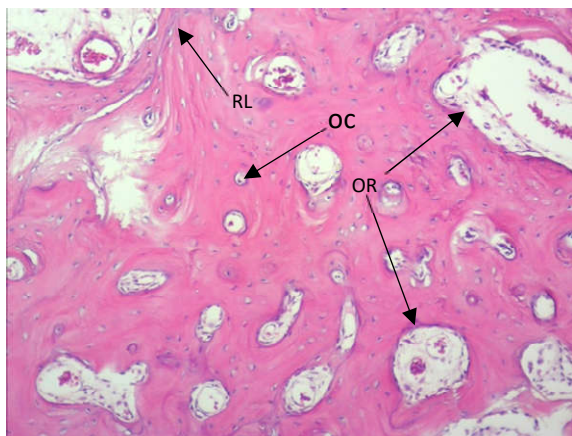


Figure 2: Photomicrograph of alveolar bone 2 weeks post-extraction (Haematoxylin and Eosin) x100. OR-Osteoblastic rimming, RL-reversal lines, OC –Osteocyte.

Table 1: Osteocyte count

Osteocyte count	mean $\pm$ sd	range
At week 2 post-extraction (cells/ μm^2)	20 $\pm$ 4.58	15-24
At week 4 post-extraction (cells/ μm^2)	32.33 $\pm$ 2.08	30-34

DISCUSSION

In Nigeria, tooth extraction in rabbits has not been previously reported to the best of our knowledge, although in western climes, it has been documented in veterinary practice for owners of pet rabbits.¹² This study demonstrated experimental tooth extraction in rabbits as well as the histologic pattern of alveolar bone healing following tooth extraction in rabbits.

minutes and recovery from anaesthesia was uneventful. Feeding pattern was not affected by tooth extraction as daily rations of feeds did not reduce. Examination of post extraction socket showed complete epithelization in all rabbits by week 1 post extraction. Also, there was no incidence of abscess formation or non-healing of extraction socket.

Histo-architecture of alveolar bone healing at week 2 post-extraction showed marked osteoblastic activity across all the groups evidenced by pronounced osteoblastic rimming and prominent reversal lines (Figure 2). Histo-architecture of alveolar bone healing at week 4 post-extraction was characterized with more of osteocytic presence and increased bone density (Figure 3). Also osteocyte count was higher at week 4 when compared to week 2 (Table 1).

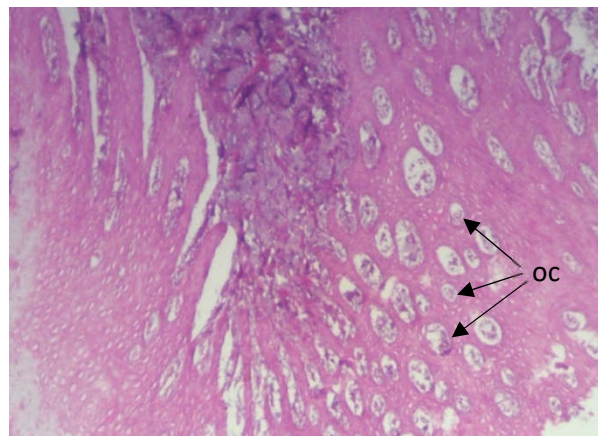


Figure 3: Photomicrograph of alveolar bone 4 weeks post-extraction (Haematoxylin and Eosin) x100. OC –Osteocyte.

The tooth extraction procedure was well tolerated in all the rabbits and there was no incidence of post-operative dentoalveolar abscess formation, despite the fact that no antibiotics were prescribed. This is in agreement with a previous report¹² which did not recommend use of antibiotics following tooth extraction in rabbits. Also, in humans, use of

antibiotics following routine extraction is not always recommended.¹³

The histo-architecture of alveolar bone healing 2 weeks following tooth extraction showed a marked osteoblastic activity which is an indicator of a high turnover rate of new bone formation. Osteoblastic activity was evidenced by pronounced osteoblastic rimming and prominent reversal lines. This finding is in agreement with findings from a previous study¹⁴ which showed that maximal new bone formation rate occurred on the 14th day post extraction.

Alveolar bone healing at week 4 was characterized with more of osteocytic presence and also osteocytes count was higher at week 4 when compared to week 2. This is similar to findings by Guglielmotti and Cabrini¹⁵ which concluded that maximal bone density was reached by 30th day post-extraction. This is further corroborated by findings from the work of Manrique *et al.*¹⁶ which also showed that bone mineral density values obtained on day 28 were higher than values obtained on days 7 and days 14 post extraction.

CONCLUSION

The result obtained from this study shows that the rabbit could well serve as an experimental animal for assessment of alveolar bone healing following tooth extraction.

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