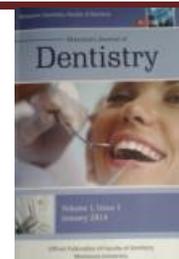




Honey Bee Propolis as a Storage Medium on Teeth Replantation in Dogs (Histological, Histochemical and Radiographic study)



Fawzy A. Darweesh¹, Ahmed R. Zaher², Adel E. Zaghloof³, Mohamed E. Helal⁴, Heba M. El-Sabaa⁵

¹Senior teaching Assistant, Oral Biology, Faculty of Dentistry, Mansoura University, Egypt.

²Professor of Oral Biology, Faculty of Dentistry, Mansoura University, Egypt.

³Professor of surgery, Anesthesiology, Faculty of Veterinary, Mansoura University, Egypt.

⁴Professor of Oral Biology, Faculty of Dentistry, Mansoura University, Egypt.

⁵Associate Professor of Oral Biology, Faculty of Dentistry, Mansoura University, Egypt.

Abstract:

Objectives: Management of both avulsions and intrusions is controversial: avulsions present the dilemma of whether or not to replant. Replantation of an avulsed tooth is an invasive procedure that requires informed consent of the patient or parent and the cooperation of the patient. Propolis may be a better storage medium in terms of maintaining PDL cell viability after avulsion, storage and re-implantation.

Methods: Thirty nine healthy mixed-breed female dogs aged from six to 11 months and had fully erupted incisors. All dogs divided into 3 dogs as Negative Control group kept without any surgical interference sacrificed at 21, 30 and 45 days from day zero of the experiment and Positive Control group: 12 dogs were subjected to surgical extraction to the upper left second incisor followed by immediate replantation. Group I (dry air group) Extracted left second incisors of this group were assigned to be left in dry air then replanted 60 minutes after extraction, Group II (propolis group), The same protocol of group I but with storage in propolis

Results: Histological examination by using H&E stain in group I dry air storage showing areas of destructed fibers of periodontal ligament with marked dissociation of PDL fibers in relation with cementum and alveolar bone, Large areas of root and alveolar bone resorption were mainly observed while in control groups or in group II propolis storage, minimal histological changes were observed. The histochemical examination by using Masson's Trichrome Stain and Mercuric-bromophenol blue stain in group I dry air storage showing weak to moderate positive reaction of PDL fibers while in control groups or in group II propolis storage, strong positive reactions were observed. The radiographic examination of group I dry air storage showed increased periodontal ligament space with loss of lamina Dura and extreme decrease of the level of the alveolar bone crest, in control groups or in group II propolis storage illustrated minimal radiological changes with decrease of the level of the alveolar bone crest.

Conclusions: Propolis is a good storage medium for avulsed teeth before replantation. Storage of the avulsed tooth in dry air decrease markedly success rate of tooth replantation.

Keywords: Propolis, periodontal ligament, storage medium.

Introduction

Tooth avulsion is defined as total displacement of the tooth out of its alveolar socket. Replantation of an avulsed tooth is an invasive procedure that requires informed consent of the patient or parent and the cooperation of the patient. The primary aim of any treatment must be to replant an avulsed adult tooth as soon as possible. The chances of success for a replanted tooth depend on the time it has been out of the mouth, so the important advice when a tooth is avulsed is to encourage an adult at the scene of the accident to replant it [1-3].

Propolis is a natural brownish-green resinous material made by the honeybees from exudates and buds of the plants and mixed with wax and bees enzymes. It is a potent antimicrobial and anti-inflammatory agent. Propolis may be a better alternative to hanks balance salt solution (HBSS), milk, or saline in terms of maintaining periodontal ligament cell viability after avulsion and storage. Exposure of PDL cells or pulp fibroblasts to propolis resulted in more viability of cells. Propolis has the ability to serve as a temporary storage medium for the maintenance of periodontal ligament (PDL) cell viability of avulsed teeth [4-5].

Materials and methods

Thirty nine healthy mixed-breed female dogs were obtained from animal house of faculty of veterinary, Mansoura University. The animals aged from six to 11 months and had fully erupted incisors⁽⁴⁾. The animals were fed on a standard diet and free access to water. All dogs subjected to the same conditions then randomly divided into: Negative Control group: 3 dogs kept without any surgical interference sacrificed at 21, 30 and 45 days from day zero of the experiment. Experimental groups, 36 dogs were subjected to surgical extraction to the upper left second incisor according to the following: Positive Control group: 12 dogs were subjected to surgical extraction to the upper left second incisor followed by immediate replantation of the extracted tooth then dogs were subdivided into three subgroups A, B and C according to the time at which animals were sacrificed which were 21, 30 and 45 day starting from day zero of the experiment.

Group I (dry air group): Extracted left second incisors of this group were assigned to be left in dry air then replanted 60 minutes after extraction. Animals of this group subdivided into 3 subgroups four dogs each according to time of scarifications parallel with the negative and positive control group as: Subgroup D: sacrificed after 21 day. Subgroup E: sacrificed after 30

day. Subgroup F: sacrificed after 45 day. Group II (propolis group).

The same protocol of group I while the extracted left side teeth in this group were preserved in propolis for 60 minutes before replantation. Animals of this group follow the same scarification protocol as group I. The animals of each group were killed by overdose of thiopental and then their anterior portion of maxilla were dissected and fixed in 10% neutral buffered formalin. After radiographic examination the specimens were de-mineralized by Ethylene Di-amine Tetra Acetic acid (EDTA.). After complete demineralization, anterior region of maxilla of each dog were taken, processed for routine histological examination with Haematoxylin and Eosin Stain (H & E) and histo-chemical examination by Masson's Trichrome Stain and Mercuric-bromophenol blue.

Results

1. The macroscopic finding revealed that the teeth in study group I dry air storage especially after 45 days (subgroup F) revealed some degree of mobility to the extent of complete tooth loss, migration or exfoliation, while either in control groups or in study group II were nearly without any degree of mobility especially in negative control group.

2. The routine histological examination by using H&E stain in group I dry air storage showing areas of destructed fibers of periodontal ligament with marked dissociation of PDL fibers in relation with cementum and alveolar bone, Large areas of root and alveolar bone resorption were mainly observed while in positive control group or in group II propolis storage, minimal histological changes were observed (Fig. 1).

3. The histochemical examination by using Masson's Trichrome Stain and Mercuric-bromophenol blue stain in group I dry air storage showing showing weak to moderate positive reaction of PDL fibers while in positive control group or in group II propolis storage, strong positive reactions were observed (Fig. 2, 3).

4. The radiographic examination of group I dry air storage especially after 45 days (subgroup F) showed increased periodontal ligament space with loss of lamina Dura and extreme decrease of the level of the alveolar bone crest. In some animals, there was a complete resorption of bone surrounding the tooth to the extent that complete loss or exfoliation of the dry air stored tooth. in positive control group or in group II propolis storage illustrated minimal radiological changes with decrease of the level of the alveolar bone crest (Fig. 4).

Discussion

Blomlof et al. [6] had reported that the main reason why avulsed teeth do not reattach is that the periodontal cells on the root dry out and then die. The tooth must be kept moist. Research has shown that milk is an ideal storage medium. Provided that the tooth is kept moist in an appropriate medium it can be replanted up to 24 hours after the accident with some chance of success. Nevertheless, the watchword should be action, since the sooner the tooth is put back into its socket the better its chances of surviving for many years. Mori et al. [7] reported that both gallium nitrate and calcium hydroxide were able to limit root resorption in teeth submitted to replantation, yet they did not prevent its occurrence. Concerning the periodontal space, they demonstrate discrete presence of periodontal ligament-like

connective tissue at 30 and 60 days, which was considerably higher at 15 days. This may be explained by the presence of Periodontal tissue remnants from the alveolar wall reaching the root surface of the replanted tooth, and not by formation of new tissue. Such periodontal tissue remnants were replaced over time by a newly formed connective tissue arranged in parallel direction to the root surface.

Suttovia et al. [8] demonstrated that root surface treatment with sodium hypochlorite and sodium fluoride for avulsed tooth before replantation had no remarkable effect in preventing either ankylosis or root resorption. Martin et al. [9] found that Propolis may be a better alternative to Hank's balanced salt solution HBSS, milk, or saline in terms of maintaining PDL cell viability after avulsion and storage. Numerous studies have examined various media in an attempt to determine the ideal material for storage of the avulsed tooth. Martin et al. [9] conducted a study and compared various storage media and it appeared that propolis may be a better alternative to hanks balance salt solution (HBSS), milk, or saline in terms of maintaining periodontal ligament cell viability after avulsion and storage.

These results agreed with Ozan et al. [10], they suggested that propolis has the ability of maintaining the vitality of periodontal ligament cells which is important for increasing the survival rate of replanted teeth. Ozan et al. [10] determined the ability of propolis to serve as a temporary storage medium for the maintenance of periodontal ligament (PDL) cell viability of avulsed teeth. The results showed that 10% propolis was more effective storage medium than other groups. Graziela Garrido Mori et al. [11] demonstrated that root surface treatment with Zoledronic Acid for avulsed tooth before replantation had a remarkable effect in preventing root resorption.

Chappuis et al. [12] reported that the survival rate of a tooth that was completely avulsed and replanted was 95.6% one year after replantation. They suggested that the induction of replacement resorption after replantation was influenced by the time lag between avulsion and replantation during which the tooth is exposed to dry condition.

Donaldson et al. [13] reported that the time limit for successful replantation was 15 minutes while McIntyre et al. [14] reported a time limit of 20 minutes if the tooth had been exposed to dry conditions. Niikuni et al. [2] reported that complete root resorption had occurred within a very short period of 5 months after the replantation and this could be explained by the poor condition of the periodontal ligament, probably due to the exposure of the avulsed tooth to dry conditions for two hours. Cho S et al. [1] reported that the long-term prognosis for the replanted incisor in case of prolonged dry storage is not good. Teeth replanted after 60 minutes of dry storage become ankylosed and are resorbed within 7 years in young patients, whereas teeth replanted under similar conditions in patients older than 16 may remain functional for considerably longer periods.

Cho S et al. [1] reported that avulsion is a more serious assault on the gingiva, the periodontal ligament and the pulp, so teeth replanted within 5 minutes after avulsion had the best prognosis and the chance of pulpal and periodontal healing was inversely related to the stage of root

development and the period of dry storage. Cho S et al. [1] reported that the long-term prognosis for the replanted incisor in case of prolonged dry storage is not good. Teeth replanted after 60 minutes of dry storage become ankylosed and are resorbed within 7 years in young patients, whereas teeth replanted under similar conditions in patients older than 16 may remain functional for considerably longer periods [15-19].

Al-Shaher et al. [20] examined the tolerance of fibroblasts of the periodontal ligament (PDL) and dental pulp to propolis and compared it with that of calcium hydroxide in vitro. Data revealed that exposure of PDL cells or pulp fibroblasts propolis resulted in > 75% viability of cells. On the contrary, calcium hydroxide 0.4 mg/ml was cytotoxic and < 25% of the cells was found to be viable. The authors concluded that, propolis can be recommended as a suitable transport medium for avulsed teeth.

Amita Coutinho [21] found that Sub gingival irrigation with propolis extract as an adjuvant to periodontal treatment was more effective than conventional treatment both by clinical and microbiological parameters. Toket et al. [22] analyzed the morphometric and histopathologic changes associated with experimental periodontitis in rats in response to the systemic administration of propolis showing Changes in alveolar bone levels were clinically measured and tissues were histopathologically examined. Propolis was found significantly reduces the periodontitis – related bone loss [15,16]. The finding of this study provided

morphologic and histologic evidence that propolis when administered systemically, prevented alveolar bone loss in rat model.

Coutinho A [23] revealed that Sub gingival irrigation with propolis extract as an adjuvant to periodontal treatment was more effective than scaling and root planning both by clinical and microbiological parameters. De Maral. [24] concluded that when propolis was applied in certain periodontal pockets once weeks for five weeks all the periodontal pockets irrigated with propolis showed a 95% decline. It is recommended that 10% Brazilian green propolis be used in conjunction with treatment of chronic periodontitis. Dodwad and Kukreja [25] found that Propolis – containing mouth rinse has an important role in inhibition of plaque formation, and they concluded that propolis can be used as a natural mouthwash alternative to chemical mouthwash as it was effective in inhibition of plaque formation and in improvement of gingival health [17].

Conclusion

From this study, we can conclude that;

1. Immediate replantation of avulsed tooth increase success rate of tooth replantation.
2. Propolis is a good storage medium for avulsed teeth before replantation.
3. Storage of the avulsed tooth in dry air decrease markedly success rate of tooth replantation.

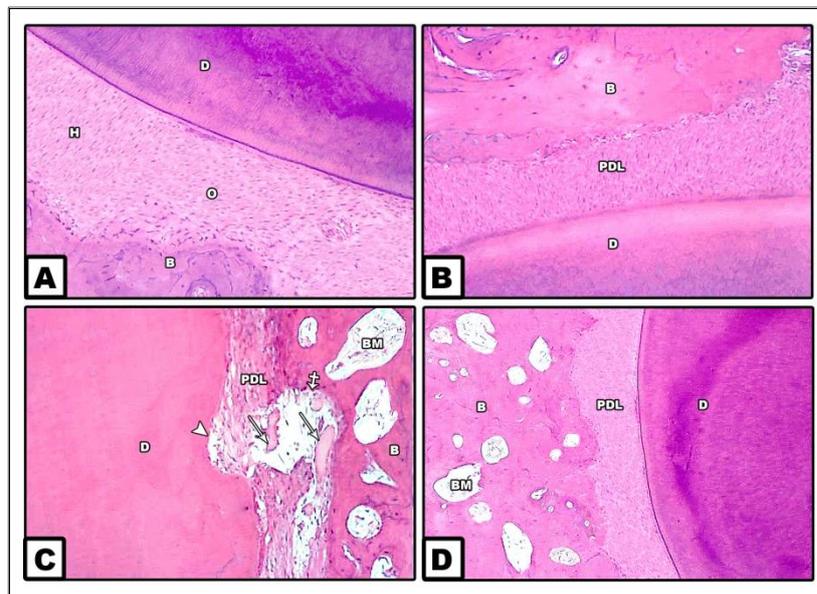


Figure.1: Photomicrograph of periodontium (H&E X100). (A) Negative control group showing normal arrangement of horizontal (H) and oblique (O) PDL fibers bundles. (B) Positive control group showing disorientation of the fiber bundles with un-uniformed thickness of PDL in relation with cementum and alveolar bone. (C) Group I showing huge areas of root resorption (arrow head) and alveolar bone resorption (crossed arrow) with widening of PDL space and dilatation of blood vessels (arrow). (D) Group II animals showing nearly normal appearance of PDL fibers in relation with cementum and alveolar bone with loss of orientation of PDL fibers.

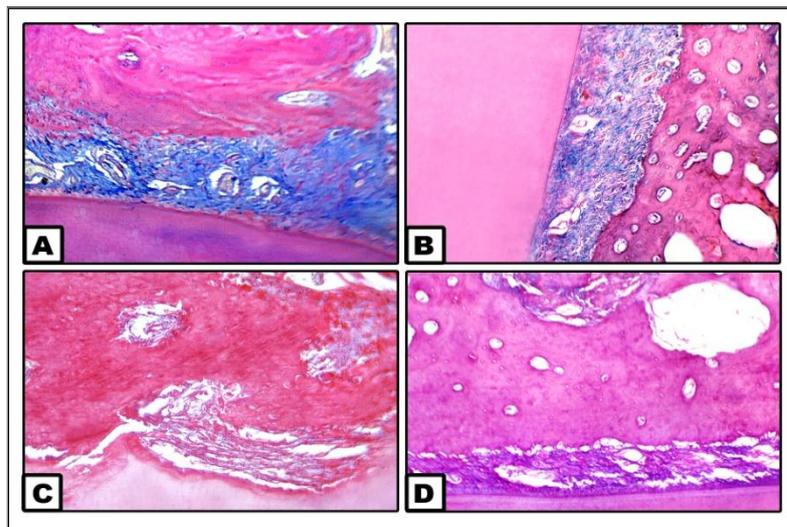


Figure 2: Photomicrograph of periodontium (Trichrome X100). **(A)** Negative control group showing high positive reaction of PDL region. **(B)** Positive control group showing high positive reaction of PDL region. **(C)** Group I showing weak positive reaction of PDL region. **(D)** Group II showing high positive reaction of PDL region.

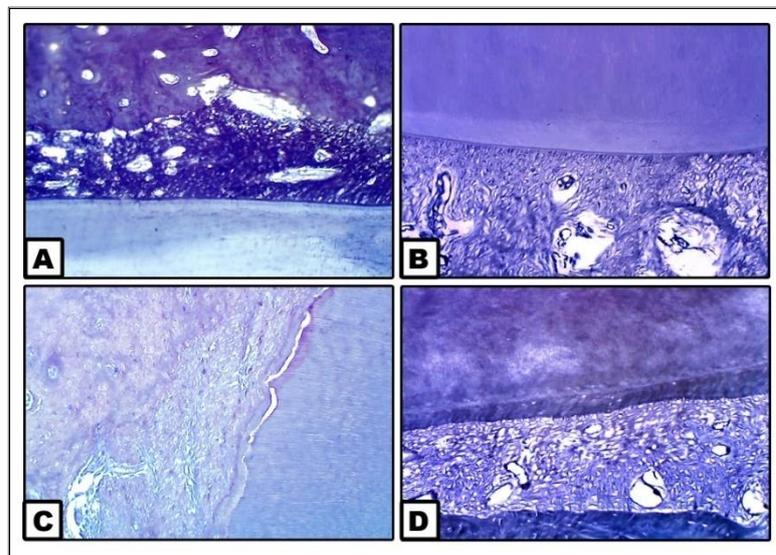


Figure 3: Photomicrograph of periodontium (Bromophenol Blue X100). **(A)** Negative control group showing high positive reaction of PDL region. **(B)** Positive control group showing high positive reaction of PDL region. **(C)** Group I showing weak positive reaction of PDL region. **(D)** Group II showing high positive reaction of PDL region.

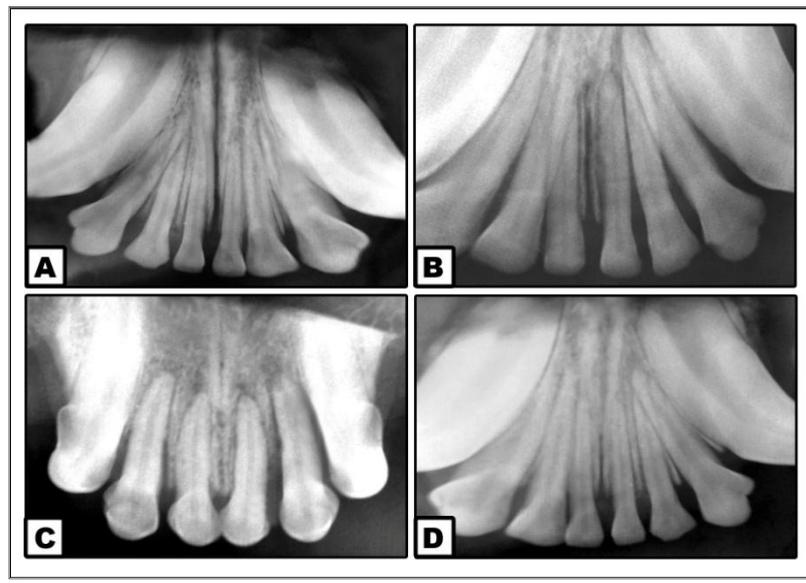


Figure 4: Photoradiograph (periapical x-ray film). **(A)** Negative control group showing normal appearance of the periodontium surrounding the teeth. **(B)** Positive control group showing minimal radiological changes with some degree of loss of continuity of lamina Dura and decrease of the level of the crest of the alveolar bone. **(C)** Group I showing increased the periodontal ligament space with loss of lamina Dura and extreme decrease of the level of the alveolar bone crest. **(D)** Group II showing minimal radiological changes with decrease of the level of the crest of the alveolar bone.

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