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HISTOLOGICAL STUDIES ON EXPERIMENTALLY REPLANTED TEETH IN DOGS

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Summary

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The studies were performed on 8 dogs, 4 of each gender, weighing 12-16 kg, aged 2-4 years. They were divided into two groups – control (intact animals) and experimental (with dental replantation). The latter were submitted to a dental intervention consisting in reproduction of dental avulsion following anaesthesia, endodonthic treatment, apical dentotomy, tooth replantation and fixation with orthodontic splint, a figure-of-eight wire ligature and photopolymer. The histological studies were performed in experimental dogs at days 60, 90 and 120 following replantation. Parallel studies were performed in the control group as well. Each histological sample included a complex of the replant with the neighboring two teeth together with the adjacent solid and soft tissues (gingiva and bone alveolus with the adjacent area of the jaw bone). Total paraffin cross-sections with a thickness up to 10 μm including all elements of the bioptate were made. Permanent histological preparations were obtained following staining with hematoxylin (Erlich)-eosin and hematoxylin-hydrochloric acid.

The light microscopic study of samples from the control group as well as of samples from teeth, adjacent to the replant in experimental groups, did not reveal any changes in the dental histostructure and that of neighboring elements. The histological follow-up of replantation showed that it was similar to bone grafts and occurred as a mixed type of healing in the various parts of the tooth – periodontal–fibrous, cement-bone or both types of healing.

Key words: avulsio dentis, dogs, histostructure, tooth replantation

INTRODUCTION

The recent reports in the field of dental traumatology showed a significant increase in the percentage of traumatic injuries of teeth (luxations and avulsions) in pets (dogs and cats) (Emily, 1991; Hennet, 1998). The commonest causes according to Jacobs (1989), Harvey and Emily (1993) are fights with other animals, traffic accidents, falls, playing with hard objects etc. Spondick (1992) and Wiggs and Loprise (1997) indicate that dental traumatic injuries in dogs affect most commonly the maxillar incisors and canine teeth. The dental replantations are known from the remote past (Karchemskii, 1960). Recently, a number of authors have studied dental transplantation (Karchemskii, 1960; Gracis and Orsini, 1998; Spondik, 1992; Borisov and Lazarova, 2000). Clinical and experimental studies in human dentistry are predominating. Such studies in Bulgaria have been performed by Kadankov (1967, 1976). Reports on dental replantation in world veterinary dentistry are sporadic (Boudrieau and Kudisch, 1966; Gracis and Orsini, 1998). The number of those using histological methods for the study of dental

replants is also limited. Despite the subject of the study (man, laboratory animal, pets etc.), all observations are confined to histological analysis of separate elements of the posttransplantation process. In some studies (Romer, 1951) this is the pulp of replanted teeth while others observed the periodontium (Neuwirt, 1925; Axhausen and Hammer, 1937), the dental ligaments (Oikarinen, 1996), the gingiva (Kayalibay et al., 1996; Pitt, 1996) etc.

Taking into account the actuality of the problem and the lack of complete morphological studies in dogs, we aimed to follow up the histological status of teeth and the surrounding structures (teeth, gingiva, soft and bone alveoli together with adjacent jaw bone area) after experimental induction of traumatic dental avulsion and subsequent replantation.

MATERIALS AND METHODS

Eight dogs, 4 of each gender, aged between 2-4 years and weighing 12-16 kg were divided in two groups – control (with intact animals) and experimental (dogs subjected to experimental dental replantation). The experiments were performed from October 1998 to January 2000. For anaesthesia, xylazine (Rometar, Spofa, Prague, Czech Republic) and ketamine hydrochloride (Rhône Mérieux, France) were used (Dinev and Aminkov, 1998). The dental avulsion was performed on the second left maxillar incisor. A wick soaked with saline was put in the freed alveolus and an endodonthic treatment (pulpotomy) (Hennet, 1991; Borissov and Sivrev, 1995), neurectomy, apical dentotomy and obturation of the pulpal cavity with Forident (Spofa, Prague, Czech Republic) were performed. Thus prepared, the tooth was put back into the alveolus and the replant was further fixed with a curved splint, a figure-of-eight wire ligature and photopolymer Solidex (Shofu Dental, Germany). At the end of the 4th week, the orthodontic splint was removed and after the 6th week – the photopolymer from the two neighboring teeth was also removed. At determined intervals after the dental replantation, the dogs were euthanized (via intravenous injection of magnesium sulfate) and specimens for histological study were obtained: one dog at days 60 and 90; 2 dogs at day 120. In a similar way, parallel specimens were obtained from the control group as well.

Each specimen included the replant with the neighboring two teeth together with the adjacent solid and soft tissues (gingiva, soft and bone alveoli with the adjacent area of the jaw bone). The material was processed according to the method of Romeis (1953), modification of Volkova and Eletski (1986). Following fixation of bioptates in 10% neutral formol, the fixative was removed via a water bath for 10-12 hours. The decalcification was performed in 10% nitric acid for 15-20 days followed by washing in 96^{o} ethanol for 24 hours. The next procedures consisted in 24-hour water bath, dehydratation in ethanol and embedding in paraffin (for 72 hours). The material was cut to obtain total cross-sections beginning from the crowns and reaching tooth roots thus including the whole bioptate. The paraffin cross-sections (10 μm thick) were stained with hematoxylin (Erlich)-eosin and hematoxylin-hydrochloric acid to obtain permanent histological preparations. They were submitted to a light microscopic study with a NU-2 microscope.

RESULTS AND DISCUSSION

The light microscopic analysis of teeth from control group and of neighboring teeth from the experimental group did not reveal any changes in their histostructure

and in that of surrounding elements, so only the histological status of the replant will be described. The free part of the tooth (the crown) as well as the initial zone of the intermediate part (the dental neck) were characterized by no deviation from the normal histostructure. The dental alveolus in the region of the gingival edge possessed its cover of multiple-layer squamous epithelium, whose basal membrane was repeatedly pleated in a wave-form manner from the underlying connective tissue basement and showed no histological deviations. In the deeper part of the neck and particularly in the root zone, the dental alveolus was structurally similar to the region of the gingival edge, but there also was a slight thickening of the connective tissue and single diffuse pleiomorphic cellular infiltrates (day 60 after the replantation, Fig. 1). We observed that the replanted tooth was firmly attached to the surrounding structures in a various manner. The predominating form was a close contact between the cement of the replant and the surface of bone alveolus. In this case, there was an alternation of multiple areas of close attachment with rare small incisure-like spaces where the cement surface was not in contact with the alveolus along the cutting surface (day 90, Fig. 2). The microscopy at bigger magnifications revealed that in areas of close attachment, the cement surface was irregular (appeared as lacunae), filled with granulation tissue that turned to newly formed bone tissue in the direction of bone alveolus. This form of attachment was characteristic especially for the deep parts of tooth root while in the superior area, capillary incisure-like spaces were hardly visible. There, the cement was with smooth surface while the periosteal layer of the bone alveolus was in a process of significant transformation with fibrillary structures resembling Sharpey's fibers, grown towards the cement.

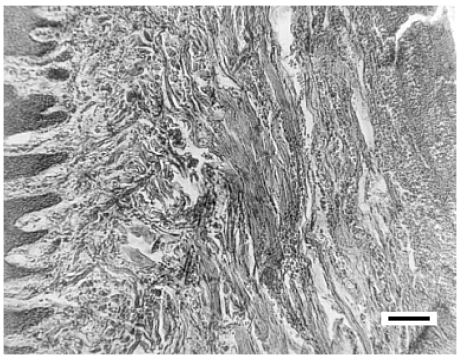

Fig. 1. Area of alveolus in the region of the tooth root with a slightly thickened connective tissue and a small diffuse pleiomorphous infiltrate (day 60 after the replantation). Bar ≈10 μm, H/E.

Fig. 2. Cross-section of the replant with a part of the alveolus in the region of tooth root. Area of close contact between the cement of the tooth and the bone alveolus (day 90 after the replantation). Bar ≈10 μm, H/E.

The interspaces among the fiber clusters were occupied by tube-shaped holes surrounded by ingrowing blood vessels, elements of differentiating connective tissue and nervous bundles (Fig. 3, day 120 after the replantation). Both parts of attachment were present between the deep and the top part of root. Hyaline cartilagi-

nous fragments undergoing transfromation were observed as single findings in the newly forming bone tissue of the dental root (day 120 after the replantation, Fig. 4). The present histological study did not show the presence of inflammatory microfoci or an uncontrolled destructuve effect of tissue around the replant.

Fig. 3. Area of close contact between the replanted tooth and the intensively transfoming periosteal layer of the alveolus (day 120 after the replantation). Bar ≈10 μm, H/E.

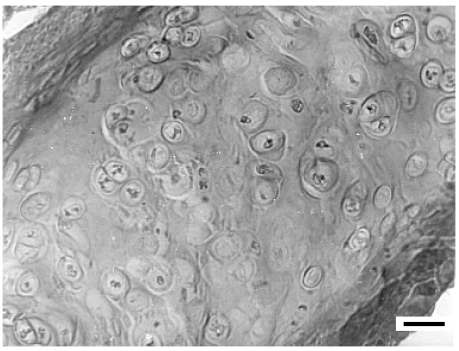

Fig. 4. Fragment of a hyaline cartilage undergoing transformation in the newly forming bone tissue (day 120 after the replantation). Bar ≈10 μm, H/E.

The areas with thickened connective tissue, single diffuse pleiomorphic cellular infiltates and lymphoid cellular clusters in the gingiva are also described by others (Kadankov, 1967; Kodukova et al., 1986; Krastev and Vitanov, 1993). They are considered as a normal finding in the actively functioning gingiva in both men and animals.

Our results evidenced that the process of reconstruction of dental replants in dogs follows the pattern described by Axhausten and Hammer (1937), Heiss (1944) and Kadankov (1976) and the known mechanisms of bone grafts, studied in detail by Paskalev (1997). According to our opinion, a granulation tissue was initially formed, that destroys the cement and forms a lacuna. Then, it indergoes a metaplasia to bone tissue that replaces the cement and dentin of tooth root and thereafter, the tooth unites immovably to the alveolus. This is similar to the description of analoguous processes, reported in the experiment of Oikarinen and Stoltze (1996) with monkeys.

Our experimental studies upon dental replantation in dogs following dental avulsion and the histological follow-up of the replantation suggest that the course of the healing is like that of bone grafts. The process of replantation is realized by a mixed type of healing in the different parts of the tooth – periodontal fibrous healing, cement-bone healing or both types.

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