An Animal Model to Study Regenerative Endodontics

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Abstract

Introduction: A growing body of evidence is demonstrating the possibility for regeneration of tissues within the pulp space and continued root development in teeth with necrotic pulps and open apices. There are areas of research related to regenerative endodontics that need to be investigated in an animal model. The purpose of this study was to investigate ferret cuspid teeth as a model to investigate factors involved in regenerative endodontics. **Methods:** Six young male ferrets between the ages of 36–133 days were used in this investigation. Each animal was anesthetized and perfused with 10% buffered formalin. Block sections including the mandibular and maxillary cuspid teeth and their surrounding periapical tissues were obtained, radiographed, decalcified, sectioned, and stained with hematoxylin-eosin to determine various stages of apical closure in these teeth. **Results:** The permanent mandibular and maxillary cuspid teeth with open apices erupted approximately 50 days after birth. Initial signs of closure of the apical foramen in these teeth were observed between 90–110 days. Complete apical closure was observed in the cuspid teeth when the animals were 133 days old. **Conclusions:** Based on the experiment, ferret cuspid teeth can be used to investigate various factors involved in regenerative endodontics that cannot be tested in human subjects. The most appropriate time to conduct the experiments would be when the ferrets are between the ages of 50 and 90 days. *(J Endod 2011;37:197–202)*

Key Words

Endodontics, ferret, histologic, radiographic, regenerative, tooth development

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When the pulp undergoes necrosis before complete root development, dentin formation ceases, and root growth is arrested. Teeth with necrotic pulps and immature apices present special challenges to clinicians during obturation. Immature teeth have open and often divergent apices that are not suitable for complete debridement and obturation with traditional materials. In addition, these teeth are susceptible to fracture after treatment because of their thin walls (1). Traditionally, an apexification procedure consists of multiple and long-term applications of calcium hydroxide to create an apical barrier before obturation of the root canals (2). Because long-term applications of calcium hydroxide might alter the mechanical properties of dentin and render these teeth more susceptible to root fracture, a 1- or 2-step artificial apical barrier with mineral trioxide aggregate (MTA) has been suggested before obturation of the coronal portion of the root canal with endodontic filling materials (2). Very high success rates have been reported for this procedure (3); however, this procedure might not result in complete root formation and might not completely reduce the chances for root fracture (4).

The ideal outcome for a tooth with an immature root and necrotic pulp would be the regeneration of pulp tissue into a canal capable of promoting the continuation of normal root development. The advantages of pulp regeneration lie in the potential for reinforcement of dentinal walls by deposition of hard tissue and for the development of an apical morphology more appropriate for routine root canal treatment procedures if future treatment becomes necessary.

There is a growing body of evidence suggesting that regeneration of the pulp space, along with continued growth of the root, might in fact be possible after pulpal necrosis and development of apical pathosis in teeth with immature apices. Several single case reports and case series have been published demonstrating radiographic signs of continued thickening of the dentinal walls and subsequent apical closure of roots in teeth with periapical lesions (2, 5–9).

These clinical cases illustrate the ability of teeth with necrotic pulps and open apices to respond to a new treatment modality, resulting in continued hard tissue deposition under certain conditions. In a comprehensive review, Murray et al (10) presented the potential of regenerative endodontics related to root canal revascularization, postnatal stem cell therapy, pulp implantation, scaffolds, and gene therapy. These authors identified many areas of research related to disinfection of root canals for regenerative endodontics, creation of replacement pulp-dentin tissues, delivery of replacement pulp-dentin tissues, dental restorative materials, and measurement of appropriate clinical outcomes. Because certain experiments are difficult or unethical to conduct in human subjects, extensive *in vivo* laboratory research with experimental animals is required to determine the efficacy and safety of regenerative endodontics (11).

The purpose of this study was to examine the developmental patterns of ferret cuspid teeth and to propose a new *in vivo* model to investigate factors involved in regenerative endodontics.

Materials and Methods

On the basis of available information (12, 13) and a preliminary experiment, we had determined that the cuspids of male ferrets erupt approximately 50 days after birth, and their apices close when these animals are approximately 110 days old. Six male ferrets (*Mustela putorius furo*) between the ages 36–133 days (Marshall BioResources, North Rose, NY; infous@marshallbio.com) were used in this

investigation. All experimental procedures were carried out in accordance with protocols approved by the Loma Linda University Animal Research Committee. After anesthetizing each animal by using intramuscular injections of 25 mg/kg of ketamine HCl (Ketaject; Phoenix Pharmaceutical, Inc, St Joseph, MO) and 2 mg/kg of xylazine (AnaSed; Lloyd Laboratories Inc, Shenandoah, IA), each animal was perfused with 10% buffered formalin (J. T. Baker Inc, Phillipsburg, NJ). Block sections including the mandibular and maxillary cuspid teeth and their surrounding periapical tissues were obtained. The specimens were radiographed and then decalcified in 10% ethylenediaminetetraacetic acid (Sigma Chemical Co, St Louis, MO) buffered to pH 7.4 at room temperature under magnetic stir bar agitation for 14–21 days. After complete decalcification and embedding each specimen in tissue prep paraffin (Fischer Scientific, Fair Lawn, NJ) by using standard paraffin processing on a Tissue-Tek VIP 5 (Sakura Finetek USA Inc, Torrence, CA), 5-μm-thick serial sections were prepared and stained with hematoxylin-eosin. The specimens were examined radiographically and histologically to evaluate stages of tooth eruption and apical closure in the maxillary and mandibular cuspid teeth.

Results

Radiographic examinations of the maxillary and mandibular teeth showed that at 36 days, the deciduous cuspid teeth were still in place, and the permanent cuspid teeth had not yet erupted into the oral cavity (Fig. 1*A*). The maxillary and mandibular cuspid teeth erupted in the oral cavity when the ferrets were 52 days old. At this stage, the root of the cuspid tooth was formed and had very thin walls, a wide canal space, and an open apex (Fig. 2*A*). At 70 days, the cuspid tooth had erupted more and had thicker root walls, a smaller root canal space, and still a very wide open apex (Fig. 3*A*). At 90 days, the cuspid tooth had completely erupted with a more complete root formation and had thicker root canal walls, smaller root canal space, and smaller apical foramen compared with 70-day specimens (Fig. 4*A*). At 113 days, the cuspid tooth had a fully formed root and a small apical foramen (Fig. 5*A*). At 133 days, the cuspid tooth had complete root formation and a closed apex (Fig. 6*A*). The root walls were thicker, and the canal space had narrowed.

Histologic examination of the block sections showed various stages of tooth eruption, root development, and apical closure. At 36 days, a developing tooth at the late bell stage of development was present (Fig. 1*B*). The inner and outer epithelia were joining to give rise to the Hertwig epithelial root sheath (HERS). Dental papilla, odontoblastic layer, developing dentin and enamel organ were visible at this stage of cuspid development. These features are very similar to those observed in a developing human tooth at the late bell stage of development. By day 52, the HERS was extending to form the root, with very thin walls, a wide canal space, and an open apex (Fig. 2*B*). The pulp-limiting membrane, periodontal ligament (PDL), and apical bone were visible at this stage of tooth formation. At 70 days, organized dental pulp, a continuous odontoblastic layer, root dentin, an open apex, PDL, and alveolar bone were observed (Fig. 3*B*). At 90 days, complete root formation and thicker dentin walls were evident, albeit with an open apex. The pulp had matured further with a distinct odontoblastic layer (Fig. 4*B*). At 113 days, the canal still had a large lumen space, thick dentinal walls, a layer of cellular cementum, closed apical region with apical ramifications, and a normal PDL (Fig. 5*B*). At 133 days, the tooth had a normal canal space, thick dentinal walls, and a mature pulp. The root apex showed presence of cellular cementum, apical ramifications (deltas), normal PDL, and periradicular bone (Fig. 6*B*).

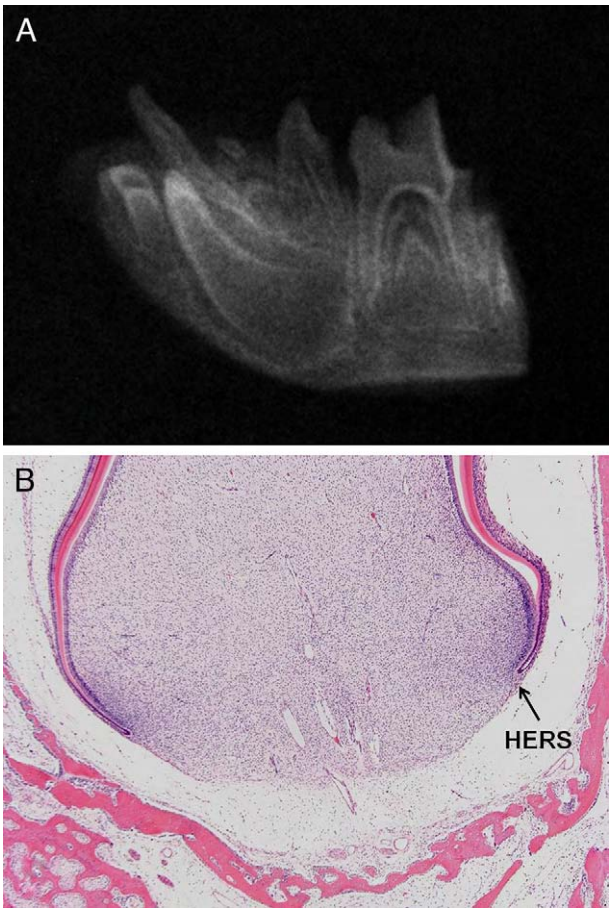

Figure 1. (*A*) Radiographic image of mandibular left quadrant of 36-day-old ferret demonstrating a retained deciduous cuspid tooth and the unerupted permanent cuspid. (*B*) Histophotographic image of mandibular left cuspid of 36-day-old ferret. Inner and outer enamel epithelia can be visualized giving rise to HERS. In addition, dental papilla, odontoblastic layer, developing dentin and enamel organ are visible at this stage of this tooth development.

Discussion

Advances in stem cell technology and the field of endodontics have created unprecedented opportunities for regenerative endodontics. Because testing some of the factors involved in regenerative endodontics in humans is impractical and sometimes unethical, it is necessary to use an animal model to study various aspects of this procedure *in vivo*. An experimental animal model should have comparable anatomical, physiologic, histologic, and pathologic characteristics to the ultimate treatment cohort (14). The animal model should provide a few reasonably large, single-rooted teeth for various endodontic procedures including vital and nonvital pulp therapies and root canal treatment. The teeth should be easy to access clinically and evaluate radiographically. In addition, the animal should be readily available, inexpensive to purchase and maintain, and easy to anesthetize.

Rats, guinea pigs, rabbits, ferrets, cats, dogs, and primates have been used in endodontic research. Rats have been used in endodontic research with respect to pulp and periapical tissue reactions to various endodontic materials as well as immunologic reactions in these tissues (14). Rats are readily available, easy to anesthetize, and inexpensive to purchase and maintain. However, these animals possess small teeth unsuitable for endodontic procedures necessary in regenerative endodontics. Rabbits and guinea pigs have also been used to test