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Standardisation of sheep model for endodontic regeneration/revitalisation research

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ABSTRACT

Objective: Different endodontic regeneration/revitalisation protocols have been suggested for the treatment of immature permanent teeth with pulp necrosis. Many aspects of these protocols require further investigating necessitating a suitable standardised animal model for research purposes. The focus of this study was to examine the anatomy and histology of sheep teeth at different stages of development to find an appropriate dental age for endodontic regeneration/revitalisation research.
Design: Sheep teeth at mature and immature dental ages were investigated. Standardized radiography, computed tomography, and histology were used to measure root length, apical-third dentine thickness and apex diameter, and to evaluate tissue development stages.
Results: A mature sheep tooth has an apical area which consists of a major foramen, intermediate dilatation and minor foramen. From the time of eruption to maturation no major changes occur in the incisor root lengths, but the apical foramen width decreases and the dentinal wall thickness increases. The two-tooth age exhibited the most similar features to that of an immature permanent human tooth.
Conclusion: Sheep appears to be an appropriate animal model for endodontic regeneration/revitalization research with similar dimension and characteristics to human anterior teeth. Each dental age has its advantages and disadvantages. The two-tooth age showed the most favourable criteria making this age the most suitable for *in vivo* regeneration/revitalisation research.

1. Introduction

The possibility of endodontic regeneration/revitalisation treatment of immature infected teeth is a recent development offering considerable biological advantages; but a more complete understanding of the treatment requires *in vivo* research in a suitable animal model.

Primates have been used in many endodontic regeneration studies (Das, Das, & Murphy, 1997; Myers and Fountain, 1974; Nevins, Finkelstein, Borden, & Laporta, 1976; Nevins, Finkelstein, Laporta, & Borden, 1978) because of their anatomical similarity to human, but these animals are expensive, not readily available and can be difficult to manage (Torabinejad and Bakland, 1978). Dogs are seen as pets in many cultures, and have substantially different tooth anatomy to humans (Masson, Hennet, & Calas, 1992). Rodent incisor teeth are small with wide-open apices and have a continuous growth (Scarparo, Dondoni, Böttcher, Grecca, & Rockenbach, 2011; Hirschfeld, Weinreb, & Michaeli, 1973; Schumacher, 2011). Larger animal models such as pigs offer an alternative, but they can grow to an unmanageable size and can be very unpleasant and uncooperative.

Sheep, on the other hand have been used in many medical and dental studies (Danesh-Meyer, Pack, & McMillan, 1997; Mackenzie and Flake, 2001; Langhoff et al., 2008) due to their teeth being similar to humans in many anatomical and histological aspects (Thurley, 1985; Mageed et al., 2013; Ravaglioli et al., 1996; Den Boer, Patka, & Bakker, 1999). Sheep are widely available, easy to handle and are comparatively cheap to keep and maintain as they can be released to fields.

Many studies have evaluated sheep incisors from the early stages of development until eruption (Thurley, 1985; Weinreb and Sharav, 1964; GWS, 1979) but there is lack of information relating to incisor teeth root diameter, histology and development from the stage of eruption until maturation. To use sheep incisor teeth for regeneration/revitalisation research, this information is required.

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This study aimed to systematically investigate incisor roots of sheep teeth at different developmental ages to identify the most suitable dental age for endodontic regeneration/revitalisation research.

2. Material and methods

2.1. Animals

Dental development of the sheep is traditionally described in terms of the pattern of eruption of the permanent incisors (Cocquyt, Driessen, & Simoens, 2005). In this study animals were divided into mature and immature ages:

- Mature dental age has the four permanent incisors erupted (35–55 months of age).
- Immature dental ages:
  - "Two-tooth" has first permanent incisors erupted (12–18 months of age).
  - "Four-tooth" has first and second permanent incisors erupted (18–26 months of age).
  - "Six-tooth" has first, second and third permanent incisors erupted (24–36 months of age).

Sheep mandibles were scavenged from animals sacrificed at the completion of other studies following approved guidelines set by South Australia Pathology/Animal Ethics Committee (#ST25/12). The collected specimens included fourteen mandibles at two-tooth age, ten mandibles each at the four-tooth and six-tooth age, and seventeen mandibles at the mature age. The teeth at each developmental stage were examined using radiographs; CT scans, direct measurements, and histology.

2.2. Radiographs

Occlusal radiographs were obtained using a long cone X-ray machine (Siemens Heliodent, Germany). A film holder designed especially for this study was used to ensure that the X-ray tube head, film and object were at the same position throughout the study (Fig. 1A). Radiographic images were analysed using ImageJ software (ImageJ v 1.48, US National Institutes of Health, Bethesda, MD) to measure root length, apical third dentine thickness and apical the diameters of the teeth on both the left and right sides (Fig. 1B,C).

2.3. Computer tomography (CT)

High resolution CT scan images of mandible specimens were obtained using a live animal micro CT scanner (SkyScan 1076, Belgium). Scans were loaded to CTAn software (version 1.91.0, SkyScan) to measure root canal length, apical third dentine thickness and apical diameter. CT VOX software was used to create a 3D model.

2.4. Direct measurements

The crowns of the teeth were removed at the cemento-enamel junction (CEJ) level under water cooling using a low speed handpiece with a diamond disc. For mature teeth, root length was measured by introducing an endodontic file (size 10 K-file (SybronEndo, Glendora, USA)) into the root canal until the file tip was visible at the apex. Measurements were repeated twice and the average of the two measurements was taken. To replicate clinical endodontic procedures, 0.5 mm was subtracted from the measured length when determining root canal length. The apical third of each root was dissected and the mesial and distal root thicknesses of each piece were measured using digital callipers, and the average was considered as the apical third root wall thickness. The width of the apical foramen was measured by introducing K-files (size 15–70) through the apical third segment. The maximum size file that passed easily through the foramen was recorded as the apex diameter.

For immature teeth the widths of the apical foramina were larger than endodontic file sizes, necessitating the use of digital callipers to measure the root length, root wall thickness and apical diameters. Apical foramen width was calculated by subtracting dentinal wall thicknesses from root width at the apex.

2.5. Evaluation of method reliability

High resolution micro CT scans are ideal for measuring small distances and examining structures but are difficult to use on large live animals. Dental radiography is commonly used clinically and for *in vivo* research but sometimes obtaining measurements is difficult due to the superimposition of other anatomical structures on the site under investigation. To evaluate the reliability of radiographs and CT scans, measurements were collected from the same teeth using both radiographs and CT scans and compared to direct measurements.

To avoid biological hazards, sheep mandibles were fixed prior to radiography and CT scanning. After fixation, extraction of the teeth was difficult as they fractured easily which reduced the number of teeth investigated in each group.

2.6. Histology

Three mandibles from each age group were fixed in 10% neutral buffered formalin (Australian Biostain, VIC, Australia), decalcified in 4% EDTA in hydrochloric acid solution (Scharlau, Spain), and embedded in paraffin wax. Serial longitudinal sections (7 μm thick) were stained with hematoxylin and eosin. The stained sections were scanned and viewed using a slide scanner (NanoZoomer, Hamamatsu, Japan).

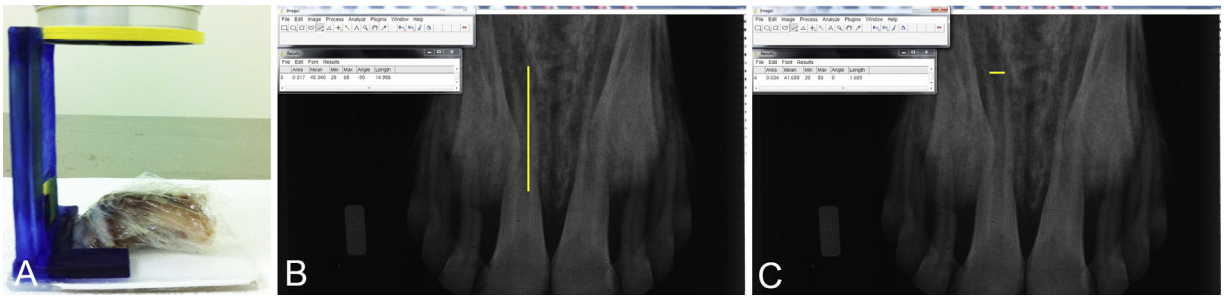

Fig. 1. Radiographic method and measurement on images using ImageJ software. Radiograph localising frame (A), measuring root length (B), measuring apex width (C).