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Original Article

Ultrastructural and Chemical Composition of Dentin and Enamel in Lab Animals

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ABSTRACT

Statement of the Problem: Human tooth is clinically the most appropriate material that can be used for *in-vitro* dental research. However, there are limitations and drawbacks for using human teeth. Therefore, alternatives to samples of human teeth for dental studies are necessary.

Purpose: This study purposed to evaluate and compare the microstructure and chemical composition of enamel and dentin of teeth in some lab animals.

Materials and Method: In this experimental study, teeth of mouse, rabbit, guinea pig, dog, cat, and sheep were used. Scanning electron microscope observations and X-ray diffraction analysis were performed on samples.

Results: This study revealed resemblance in general structure of dentin and enamel between mentioned animals and human. The minimum mean of dentinal tubules diameter is found in guinea pig (0.5μ), while the highest is in cat (1.5μ). Also the lowest and the highest mean intertubular distance was measured respectively in guinea pig (3μ) and sheep (4.8μ) and the maximum and minimum mean diameter of rods was measured in rabbit (6.6μ) and guinea pig (1.5μ), respectively.

Conclusion: The recorded details and the measured values indicate great resemblance between dog and human dentin and enamel. Cat is in the second place for dentinal studies; sheep and guinea pig have the least resemblance to human within the scope of the reviewed criteria.

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Introduction

Human tooth regarding clinical view is the most appropriate material that can be used for *in-vitro* dental research. However, there are limitations and drawbacks to using human teeth. Providing human teeth in sufficient number and with venerable quality are hardly possible due to extensive caries, lesions, and other defects. Human tooth structure and composition have many variations by age and individual differences that can induce unnecessary impacts on the outcome of studies. Another problem for the application of human teeth in dental research is risk of infection and moral constraints [1].

Thus, variety of non-human teeth has been proposed as alternative and has been used in dental research. Among the various examples of non-human, bovine teeth under the age of 20 months, is one of the most popular alternative substrates [2]. In recent decades, numerous reports have been published regarding the use of bovine teeth [3-5]. Bovine teeth have been frequently used in dental research due to ease of availability, inexpensiveness, uniform composition, and comparability to human teeth with respect to crystalline structure of the calcium content. In both species, calcium contents in teeth decrease from the enamel surface to the enamel-dentin interface. In addition, dental matrix proteins in human and bovine teeth are composed of the same amino acids [6]. However, in terms of enamel, bovine and human teeth differ in the arrangement of prisms; the crystals of bovine enamel are 1.7 times thicker than human enamel, while human teeth are more resistant to wear. The bovine root dentin has been detected to have considerably more dentinal tubules per volume, despite being less hard than human root dentin. Although cow's teeth are used in dental studies, its structure does not match the human samples entirely [6].

In addition to bovine samples, pig, horse, and shark are the most common animals that their teeth are used for dental studies. Lopes and Popowics found similarities in the structure of human and pig enamel and dentin after acid etching [7-8] while Edmunds *et al.* [9] reported differences in teeth between human and horse using scanning electron microscopy (SEM) analysis on the carious lesions. In a study enrolled by Takagi *et al.* [10], they found that tooth resistance to demineralization in shark is higher than human.

Thus, alternatives to samples of human teeth for dental studies are necessary. Consequently, this study purposed to evaluate and compare microstructure and chemical composition of enamel and dentine of teeth in some lab animals and human through SEM and X-ray diffraction (XRD) analysis.

Materials and Method

In this experimental study, teeth of mouse, rabbit, guinea pig, dog, cat, and sheep were used. Five teeth without caries or other lesions such as fractures or discolorations were collected from cadaver of each species, supplied by Shiraz School of Veterinary Sciences, Shiraz, Iran.

In dog, cat and sheep first molars were extracted and in rodents, incisors were pulled out, as they were biggest with more test surface.

The surfaces of the teeth were cleaned by scaling and pumicing so that the soft and hard tissue remnants would be eliminated. To prevent microbial growth, the samples were stored in normal saline solution containing 0.4% thymol For 1 month at 4°C.

Sections of tooth containing both enamel and dentin were prepared using a saw microtome (Leitz 1600, Germany). The samples were placed in distilled water until the exact evaluation.

For SEM, specimens were fixed in 2.5% glutaraldehyde for 12 hours (4°C). All samples were dehydrated in ascending grades of ethanol (25%, 50%, 75%, 90%, and 100%). Afterwards, the specimens were dried, gold sputter-coated and were assessed under SEM microscope (Cambridge, S360, UK).

For XRD, teeth samples (dentin and enamel together) were crushed and powdered to less than 45-μm. The obtained powders were pressed into a plastic mold under a load of 245 kN for 30 seconds resulting in disk-shaped blocks. The blocks were analyzed by XRD system (Siemens D5000, Germany). The data obtained from XRD analysis and SEM observations were recorded for each sample.

Results

SEM findings about the hard tissue of the studied animal teeth were as following:

Mouse

The dentin has cylindrical dentinal tubules (mean tubule diameter 1.3μ) which are aligned parallel (Figure 1a). In the cross section, the dentinal tubules are found to be ordered and uniformly distributed with the mean intertubular dentine diameter of 4μ (Figure 1b). In addition, the enamel rods are cylindrically positioned beside each other, showing a slight distortion (mean rod diameter

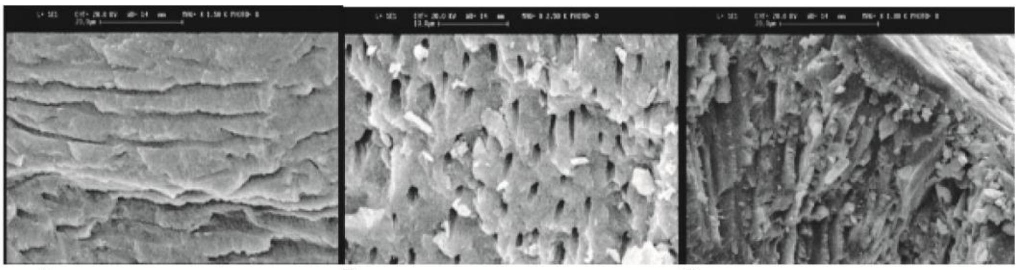

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Figure 1: Mouse: cylindrical dentinal tubules are aligned parallel (**a:** SEM, 1500X); cross section of uniformly distributed dentinal tubules (**b:** SEM, 2500X); enamel rods with slight distortion and uniform layer of nonprismatic mineral at top (**c:** SEM, 1800X)

2.8μ). A uniform layer of nonprismatic mineral with 3.6μ mean thickness covers them, towards the outer surface (Figure 1c).

Rabbit

In longitudinal section, the dentin includes parallel cylindrical dentinal tubules with the same diameter of 1μ (Figure 2a). In cross section, the dentinal tubules were observed to be non-uniformly distributed. Their intertubular distance is quite variable and random with the mean of 4μm (Figure 2b). The enamel is also a thin layer over the dentine surface and the rods are placed over the dentino enamel junction (DEJ) parallel with dentinal tubules (mean rod diameter 6.6μ) (Figure 2c).

Guinea pig

The dentin has quite thin cylindrical tubules (mean tubular diameter 0.5μ) which are distributed uniformly in the cross section with the mean intertubular dentine diameter of 3μ (Figure 3a). The enamel consists cylindrical rods that are densely intertwined (mean rod diameter 1.5μ) (Figure 3b). Their direction is almost parallel to the dentinal tubules, but the enamel thickness is too excessive, and on the outer surface, a layer of 5.6μ aprismatic nonorganic material covers the rods (Figure 3c and d).

Dog

The dentin has cylindrical dentinal tubules (mean tubular diameter 1.2μ) that are distributed uniformly and the mean diameter of intertubular dentine is measured to be 3.4μ (Figure 4a and b). The enamel has cylindrical rods aligned side by side, and reaches the surface with a bit of tortuosity. The interrods are perpendicular to the rods, creating a wicker pattern (mean rods diameter 4.2μ) (Figure 4c and d).

Cat

The dentin consists of thin cylindrical dentinal tubules that are quite parallel (Figure 5a); and in the cross section they seem to be uniform and ordered (mean tubular diameter 1.5μ, mean diameter of intertubular dentin 4.5μ) (Figure 5b). The enamel rods are thin and cylindrical and are very systematic (mean rods diameter 3.5μ) (Figure 5c).

Sheep

The dentin has cylindrical tubules with delicate curvatures that are aligned orderly (mean tubular diameter 0.8μm) and the mean diameter of intertubular dentin is 4.8μ (Figure 6a). The enamel consists of cylindrical rods parallel to DEJ that are intertwined like a net (mean diameter of rods 2.1μ) (Figure 6b) (Table 1). The XRD findings on the hard tissue of the studied animals' teeth revealed that they were all made up of molecules of hydroxyapatite (HA), fluoroapatite (FA) and carbonate hydroxyapatite (CHA) (Table 2). The statistical analysis demonstrated no significant difference between the items in the table (*p*> 0.05).

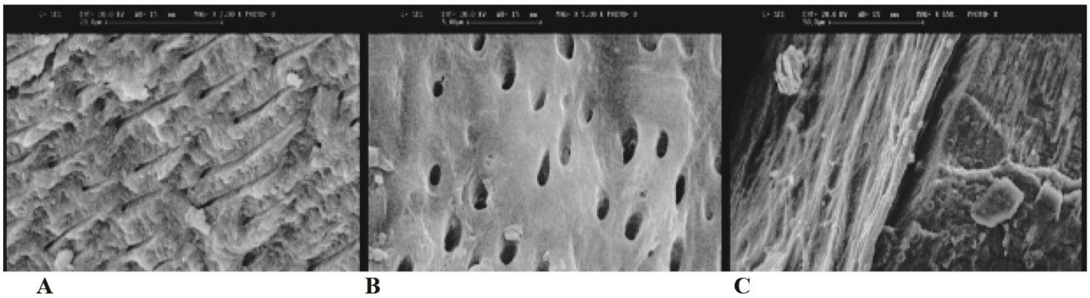

Figure 2: Rabbit: parallel cylindrical dentinal tubules with similar diameters (**a:** SEM, 2000X); cross section of non-uniformly distributed dentinal tubules (**b:** SEM, 5000X); thin layer of enamel with rods parallel to dentinal tubules (**c:** SEM, 650X)

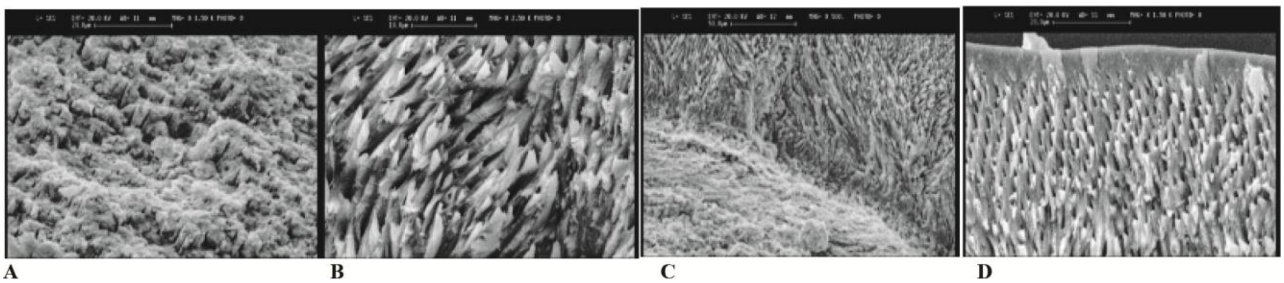

Figure 3: Guinea pig: thin cylindrical uniformly distributed dentinal tubules (**a:** SEM, 1500X); cylindrical densely intertwined enamel rods (**b:** SEM, 2500X); thick enamel and an aprismatic nonorganic material on the outer surface (**c:** SEM, 500X and **d:** SEM, 1500X)

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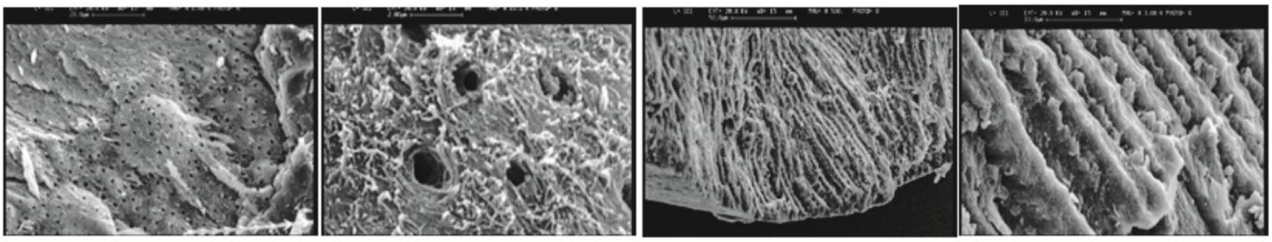

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Figure 4: Dog: cylindrical uniformly distributed dentinal tubules (**a:** SEM, 1000X and **b:** SEM, 10000X); aligned cylindrical enamel rods with a bit of tortuosity near the surface and perpendicular interrods creating a wicker pattern (**c:** SEM, 500X and **d:** SEM, 3000X)

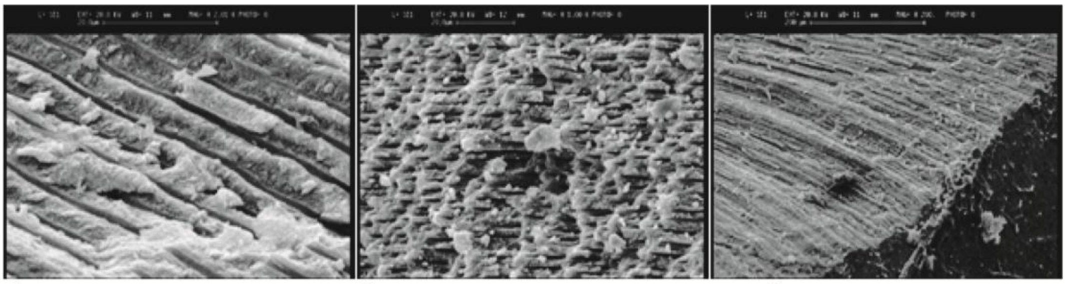

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Figure 5: Cat: thin parallel uniformly ordered cylindrical dentinal tubules (**a:** SEM, 2000X and **b:** SEM, 1000X); thin cylindrical enamel rods (**c:** SEM, 200X)

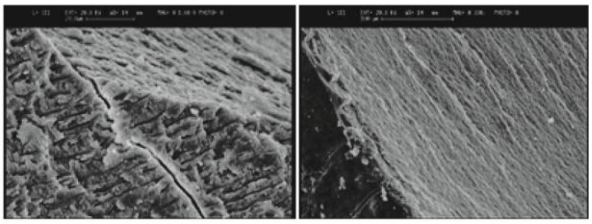

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Figure 6: Sheep: Cylindrical dentinal tubules with delicate curvatures (**a:** SEM, 1000X); cylindrical enamel rods parallel to DEJ that are intertwined like a net (**b:** SEM, 300)

Table 1: Density of dentinal tubules (DTD) per unit of area in mm2; the mean diameter of tubular dentin (MDTD), mean intertubular dentin diameter (MIDD) and the mean enamel rods diameter (MERD) in µm

Animal	MDTD	MIDD	MERD	DTD
Rat	1.3	4	2.8	45,500
Rabbit	1	4	6.6	51,000
Guinea pig	0.5	3	1.5	104,000
Dog	1.2	3.4	4..2	60,000
Cat	1.5	4.5	3.5	35,500
Sheep	0.8	4.8	2.1	40,500
Human	1.7	3.6	5.7	59,000

Table 2: The amount of minerals detected in the dental hard tissue of each studied animal

Animal	FA	HA	CHA	$ZnSo_4$	CU_2O	Not detected
Rat	1%	94%	2%	—	—	3%
Rabbit	3%	89%	2%	1%	—	5%
Guinea pig	1%	90%	5%	—	—	4%
Dog	3%	89%	2%	—	—	6%
Cat	1%	96%	—	—	—	3%
Sheep	1%	90%	5%	—	1%	3%

Discussion

Using human teeth has drawbacks and limitations. It is hardly possible to provide the proper amount of healthy teeth with a suitable quality for experiments, free of infection risk and ethical issues. Thus, researchers are looking for the proper alternative for dental research. Among all animals, the experimental and domesticated ones that are easily and widely available would be the right choice to be compared with human teeth and be adopted as the similar alternative in research.

Mouse, rabbit, and guinea pig are laboratory animals from rodent category whose incisors are open-rooted and continue to grow permanently [11-12].

Molars of cat and dog are designed to crush and tear the flesh, and molars of the sheep to grind up the tough and cellulosic body of plants.

The results of evaluating ultrastructural hard tissues of the teeth of these animals by SEM revealed that the constructive unit of dentin in all the studied animals was cylindrical dentinal tubules that were aligned parallel and surrounded by intertubular dentin. This structure exactly resembles the dentin structure in human [13]. Except in rabbit, the cross section of dentin in all animals indicated ordered and uniform distribution of tubules. This ordered array reached its peak in cat; but in rabbit, the intertubular distance was quite variable and non-uniform. The minimum mean of dentinal tubules diameter was found in guinea pig, while the highest was in cat, which was closer to human; followed by mouse and dog, respectively. In addition, the lowest and the highest mean intertubular distance was measured respectively in guinea pig and sheep; however, the closest to human was dog.

Maximum diameter of dentinal tubules in human is on side of the pulp that is tapered toward DEJ [14]. Such a trend was not observed in the SEM graphs obtained from the teeth of the animals studied in this research. Nevertheless, such specific measurements and evaluations were not possible to be performed in this study. The highest mean diameter of dentinal tubules among these six animals in this study was that of human; it can be related to the fact that understanding the feelings and dental pains are more sophisticated in human. However, calculating the density of dentinal tubules in these animals indicated that the dog's dentin highly resembles the dentin in human. Hence, dog, and after that, cat were suggested as the best alternatives to be used in dentinal studies.

The investigations conducted by SEM on enamel showed that its constructive unit in all animals is cylindrical rods. In mouse, the rods were positioned perpendicular to the outer surface with a bit of distortion, and in rabbit, they laid on DEJ, parallel to the outer surface. In guinea pig, the rods were intertwined quite densely, i.e. they started from the DEJ, parallel to the outer surface and with a curvature of 90° ended up perpendicular to the outer surface. Dog were the only animal that resembled human, had interrods perpendicular to the rods with a wicker pattern, and at the end it became perpendicular to the outer surface with a mild curve. In cat, the rods were totally ordered and perpendicular to the outer surface; in sheep they were arrayed parallel to the surface. Only in mouse and guinea pig, a nonprismatic surface layer was observed. Skobe *et al.* [14] also reported the same phenomenon in dog and cat; however, no such result was found in the current study.

The maximum and minimum mean diameter of rods was measured in rabbit and guinea pig, respectively. However, the mean diameter of rods in human falls between the rabbit and dog [13]. According to the special array of rods and interrods, and the mean diameter of rods in dog, dog's teeth seem to be the best alternative for enamel studies.

Comparing the minerals found in hard tissues of each studied animals' teeth indicated similar chemical compound in all their teeth, the major part included HA and CHA. It was lower in rabbit and dog, so the difference was replaced with higher amount of FA; indicating diverse nutritional diet in these animals. Nutritional habits and specific behaviors would undoubtedly influence their teeth during the process of evolution. Therefore, evaluating the crystallite properties of dentin and enamel in each of these animals, as well as their organization pattern can identify the ultrastructural differences of the teeth [15]. Moreover, assessing the constituting molecules of organic matrix of dentin and enamel, in addition to specifying the amino acid compounds of their proteins can be suggested for further researches. Also, by comparing the mechanical and fatigue properties of hard dental tissues of these animals and human, like wear resistance and stress/strain ratio, it would help replacement of human teeth with animal alternatives in future dental studies.

Conclusion

The results of electronic microscopy and chemical compounds analysis revealed resemblance in general structure of dentin and enamel between studied animals and human. Nonetheless, the recorded details and the measured values indicate great resemblance between dog and human dentin and enamel. Cat is in the second place for dentinal studies; sheep and guinea pig have the least resemblance to human within the scope of the reviewed criteria.

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Conflict of Interest

Authors declare no conflict of interests.

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