J Vet Sci. 2020 Nov;21(6):e83
https://doi.org/10.4142/jvs.2020.21.e83
pISSN 1229-845X·eISSN 1976-555X

Journal of Veterinary Science

Original Article

Anatomy/Histology/Embryology

Check for updates

Comparative scanning electron microscope analysis of the enamel of permanent human, bovine and porcine teeth

Aneta Olek [1,*], **Leszek Klimek** [2], **Elżbieta Bołtacz-Rzepkowska** [1]

[1]Department of Conservative Dentistry, Medical University of Lodz, 92-217 Łódź, Poland
[2]Institute of Materials Science and Engineering, University of Technology in Lodz, 90-924 Łódź, Poland

OPEN ACCESS

Received: Mar 31, 2020
Revised: Sep 9, 2020
Accepted: Sep 14, 2020

***Corresponding author:**
Aneta Olek
Department of Conservative Dentistry, Medical University of Lodz, ul. Pomorska 251, 92-217 Łódź, Poland.
E-mail: aneta.olek@umed.lodz.pl

ORCID iDs
Aneta Olek
https://orcid.org/0000-0001-6941-6459
Leszek Klimek
https://orcid.org/0000-0003-3617-8225
Elżbieta Bołtacz-Rzepkowska
https://orcid.org/0000-0002-1696-0618

Conflict of Interest
The authors declare no conflicts of interest.

Author Contributions
Conceptualization: Olek A, Bołtacz-Rzepkowska E, Klimek L; Data curation: Olek A; Formal analysis: Bołtacz-Rzepkowska E; Funding acquisition: Olek A, Bołtacz-Rzepkowska E, Klimek L; Investigation: Olek A; Klimek L; Methodology: Olek A,

ABSTRACT

Background: Bovine and porcine teeth are often used in *in vitro* experiments as substitutes of human teeth.
Objectives: The aim of the present study was to perform a comparative analysis of enamel morphology of permanent human, bovine and porcine teeth under the scanning electron microscope.
Methods: As many as 10 human, 10 bovine, and 10 porcine teeth were studied. All the teeth were sectioned and the halves were randomly divided into 2 groups according to the examined tissue (vestibular enamel at the mid-height of the dental crown and in the cervical area). Human and bovine enamel was etched for 15 sec and porcine enamel for 30 sec. The scanning electron microscope analysis was performed. The length and width of enamel prisms were determined with the "Met-Ilo" 1.1 computer program.
Results: All enamel samples revealed the same etching pattern—Silverstone's type 2. Bovine enamel showed a similar porosity and the amount of interprismatic enamel compared to human enamel while the amount and width of interprismatic enamel bands in porcine enamel were evidently greater. The shape of the porcine prisms was visually similar to human prisms, although dimensions were significantly different. However, bovine prisms differed in form and appeared to be distinctly elongated.
Conclusions: Reported findings indicate that the results of experimental studies carried out on bovine and porcine enamel should not be compared with the results obtained on human enamel.

Keywords: Cattle; human; swine; enamel; teeth

INTRODUCTION

Enamel covering the anatomical crowns of the teeth consists of crystals built from calcium hydroxyapatite. The structural unit of enamel is the enamel prism (or enamel rod). In cross section, the broadest part of a rod is called the head and the elongated thinner portion the tail. The surface of each rod is known as the rod sheath, and the center is the core. The outer part of each enamel prism is surrounded by the interrod enamel, creating an interprismatic region (**Fig. 1**) [1].

Bołtacz-Rzepkowska E, Klimek L; Project administration: Olek A, Bołtacz-Rzepkowska E, Klimek L; Resources: Klimek L; Software: Klimek L; Supervision: Bołtacz-Rzepkowska E; Validation: Bołtacz-Rzepkowska E; Visualization: Olek A; Writing - original draft: Olek A; Writing - review & editing: Bołtacz-Rzepkowska E.

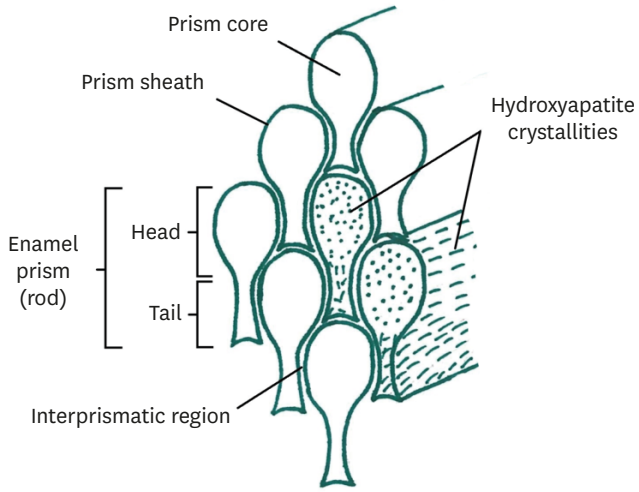

Fig. 1. The diagram illustrating shape and organization of enamel prisms with surrounding interprismatic regions. Rods are arragned regularly and are tightly packed. Note the varying crystallities orientations depending on the exposed surfaces of the rod.

Many dental procedures are performed on the enamel and various substances such as bleaching agents, tooth pastes or filling materials are applied to it. Knowledge of the enamel structure has become particularly important when adhesive materials, which are able to bond to the tooth tissues, were introduced into dentistry. To facilitate this attachment, the enamel requires etching with phosphoric acid. This procedure is used to remove some organic parts of the enamel crystals to increase porosity and enable a bonding resin to flow into the newly created gaps. Then the adhesive restorative material is placed and chemically bonded to the resin and enamel [1].

Due to the constant development of materials science and the need to evaluate new dental materials in laboratory research, numerous natural teeth are required. Only sound teeth with a lot of preserved, unchanged tissues are suitable for *in vitro* studies. Unfortunately, human teeth are usually extracted due to severe carious damage. Moreover, growing awareness of patients who are oriented towards preserving their own dentition and wide reconstruction abilities cause serious problems in obtaining a sufficient number of natural teeth [2-5]. The ethical aspect is also important and includes gaining patient's consent to use their tissues as an experimental material.

Therefore, attempts have been made to use the teeth of different species of mammals, for example rats, cats, dogs, and monkeys [6-9]. It seems, however, that the teeth of breeding animals are the best. They are easily accessible and allow researchers to precisely select the examined group in terms of age and diet. Both these factors, among many others, influence mineralization and chemical composition of the enamel [4,10]. Similar living conditions facilitate standardization and comparability of experimental studies. The use of animal teeth in laboratory tests also eliminates the problem of cross-infection, which is emphasized by the ethics committees [4,10,11]. The International Standards Organization also encourages researchers to use the teeth of breeding animals.

Based on the literature [2-5,12-26] in *in vitro* tests, human teeth have been frequently replaced by bovine incisors and porcine molars in the field of materials science and dentistry. Despite the common use of animal teeth, it remains doubtful whether the results obtained

can be uncritically related to human teeth and clinical conditions. Although the histology, microstructure and mechanical properties of human teeth have been thoroughly studied, tissues of teeth of other mammals require further research.

Therefore, the aim of the present study was to perform a comparative analysis of enamel morphology of permanent human, bovine, and porcine teeth under the scanning electron microscope (SEM).

MATERIALS AND METHODS

Ethical Approval was given by Bioethics Committee of the Medical University in Lodz, RNN/162/10/KE and the Institutional Animal Care and Use Committee of the Local Ethical Committee in Lodz (Poland) Ldz.LKE/12/2011.

Ten fully erupted permanent human lower third molars extracted from 20–35-year-old patients for medical reasons, 10 bovine permanent lower central incisors (animal age was about 36 months) and 10 porcine permanent molars (animal age was about 36 months) were used in this study. Bovine and porcine teeth were obtained from a slaughterhouse.

Immediately after the extraction and removal of periodontal ligament remnants, the teeth were stored in 0.5% chloramine T (Chloramin T; Schulke CZ, the Czech Republic) solution for maximum 3 months at room temperature, according to ISO guidelines (ISO/TS 11405/2003).

Preparation of the samples

All the teeth were sectioned in a bucco-lingual plane with a diamond bur in order to assess the enamel prism orientation. Then the tooth halves were divided into 2 groups according to the examined tissue (Group 1: vestibular enamel at the mid-height of the dental crown; and Group 2: vestibular enamel in the cervical area) (**Table 1**). The samples were ground with water-cooled carborundum discs (800, 1,000, and 1,200 grit) and polished to obtain a smooth surface perpendicular to a long axis of enamel prisms (**Fig. 2**).

Etching of the samples

Human and bovine enamel was etched for 15 sec with 35% orthophosphoric acid (Blue Etch, Poland) in order to reveal the prismatic structure, and then, rinsed with distilled water from an air-water syringe. Porcine enamel was etched for 30 sec because the pilot study showed that etching for a shorter period of time was inefficient to expose enamel prisms.

SEM investigation

Specimens were dehydrated through an ascending series of ethanol (30–100%), mounted on aluminum stabs with their treated surfaces facing upwards and sputter-coated with

Table 1. Distribution of enamel samples in each group

Type of teeth	Examined area
Human (10 teeth = 20 samples)	Mid-height of the crown (10) Cervical (10)
Bovine (10 teeth = 20 samples)	Mid-height of the crown (10) Cervical (10)
Porcine (10 teeth = 20 samples)	Mid-height of the crown (10) Cervical (10)

https://doi.org/10.4142/jvs.2020.21.e83

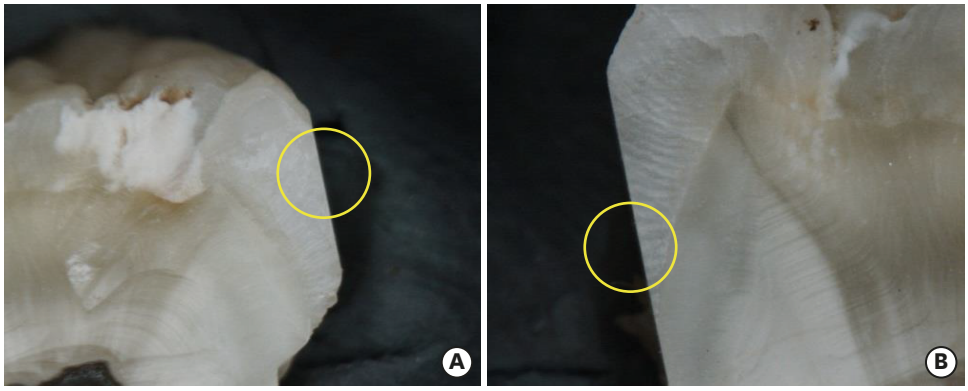

Fig. 2. Prepared enamel samples—coronal part of the tooth was sectioned in bucco-lingual direction in order to assess the enamel prism orientation. The enamel in examined area was flattened and polished to obtain a smooth surface perpendicular to a long axis of enamel prisms. The examined area of the enamel in the mid-height of the crown is marked in the circle (A). The examined area of the enamel in the cervical part is marked in the circle (B).

gold in JEE-4X apparatus (Jeol, Japan). The SEM analysis was performed using a Hitachi-3000N (Hitachi High-Tech, Japan) scanning electron microscope. In each species group, microphotographs were taken at magnifications of ×2,000 and ×5,000.

Quantitative analysis of enamel prisms

The "Met-llo" 1.1 computer program (Lodz University of Technology) was used to determine the length and width of enamel prisms.

Statistical analysis

The statistical analysis of empirical data was performed using the 2-way analysis of variance (ANOVA) along with post-hoc multiple comparison tests using SPSS 22.0 statistical software (IBM Corp., USA). Estimation of normality of distribution was skipped due to balancing of the experimental system (i.e., the same sample size in each cell). In the case of 2 factors, e.g., species of mammals and tooth area, the interactions between them were assessed. The level of significance at $\alpha = 0.05$ was considered statistically significant for all statistical procedures.

RESULTS

The results obtained in the present study are shown in **Figs. 3**-**6** and in **Tables 2** and **3**. As it was stated above in the MATERIALS AND METHODS section, 15-sec etching of porcine enamel was not efficient, since no regular etching pattern was visible after this time, regardless of the examined area or magnification (**Fig. 3**). However, 30-sec etching with orthophosphoric acid exposed the prismatic structure in these samples (**Fig. 4**).

The comparison of SEM microphotographs of effectively etched enamel samples of three studied species in both examined areas at ×2,000 magnification enabled the visual evaluation of the morphological structure, shape, orientation of enamel prisms and the type of etching pattern (**Fig. 4**).

In **Fig. 4**, the enamel samples of all species showed the same regular organization of prisms and the same etching pattern—the Silverstone's type 2 (prism sheaths were dissolved and prism cores were prominent).

https://doi.org/10.4142/jvs.2020.21.e83

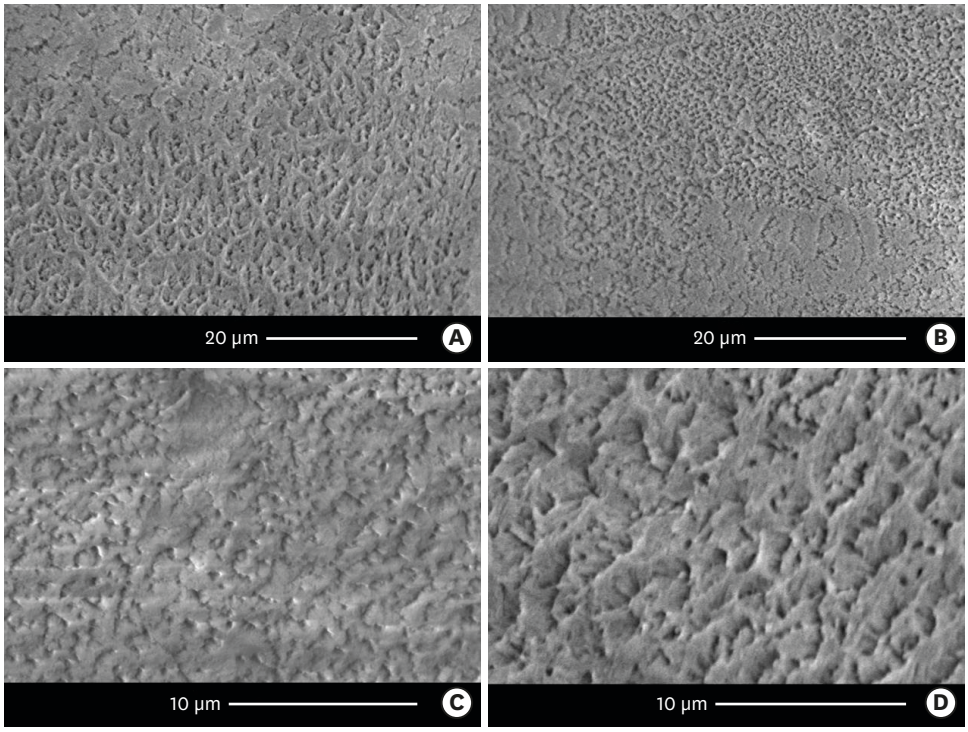

Fig. 3. Scanning electron micrographs of porcine enamel in both examined areas showing not efficient etching for 15 sec. No evident, regular etching pattern is visible with dissolved prism cores or prism sheaths. (A) Areas intact by etchant are noticeable in each micrograph. Enamel in mid-height of the crown area (magnification ×2,000, scale bar = 20 μm). (B) Enamel in the cervical area (×2,000, scale bar = 20 μm). (C) Enamel in the mid-height of the crown area (×5,000, scale bar = 10 μm). (D) Enamel in the cervical area (×5,000, scale bar = 10 μm).

Nevertheless, enamel prisms revealed differences in shape: they resembled arcades in the human teeth, were characterized by the elongated form in bovine teeth, and had an elliptical shape in porcine teeth. Human and bovine enamel prisms appeared to be more porous than the porcine ones which looked smoother (**Fig. 4**). The interrod regions of distinctive thickness were pronounced in the porcine enamel. It was particularly well visible in **Fig. 5**, where one of the interprismatic regions was marked with a circle.

Table 2. Results of comparing the mean lengths of the enamel prisms (μm) by mammal species and tooth areas

Statistical parameters	Mammal species studied			*p* value when including terms for main effects
	Human teeth (μm)	Bovine teeth (μm)	Porcine teeth (μm)	
Mid-height of the crown[a]				<0.05
Mean	**4.732**	**6.787**	**5.721**	
Median	4.696	7.094	5.332	
Minimum	3.4	4.3	4.2	
Maximum	5.8	8.8	9.2	
SE	0.216	0.213	0.205	
Cervical area[a]				<0.05
Mean	**5.929**	**8.105**	**4.597**	
Median	5.960	7.970	4.596	
Minimum	3.4	6.5	3.4	
Maximum	7.7	11.6	5.9	
SE	0.240	0.205	0.205	
p value when including terms for main effects	<0.05	<0.05	<0.05	*p* value when including terms for interactions <0.05

Mean values are bold-faced.
[a]The probability concerns the comparisons of means in rows; each mean value differs from the others.

https://doi.org/10.4142/jvs.2020.21.e83

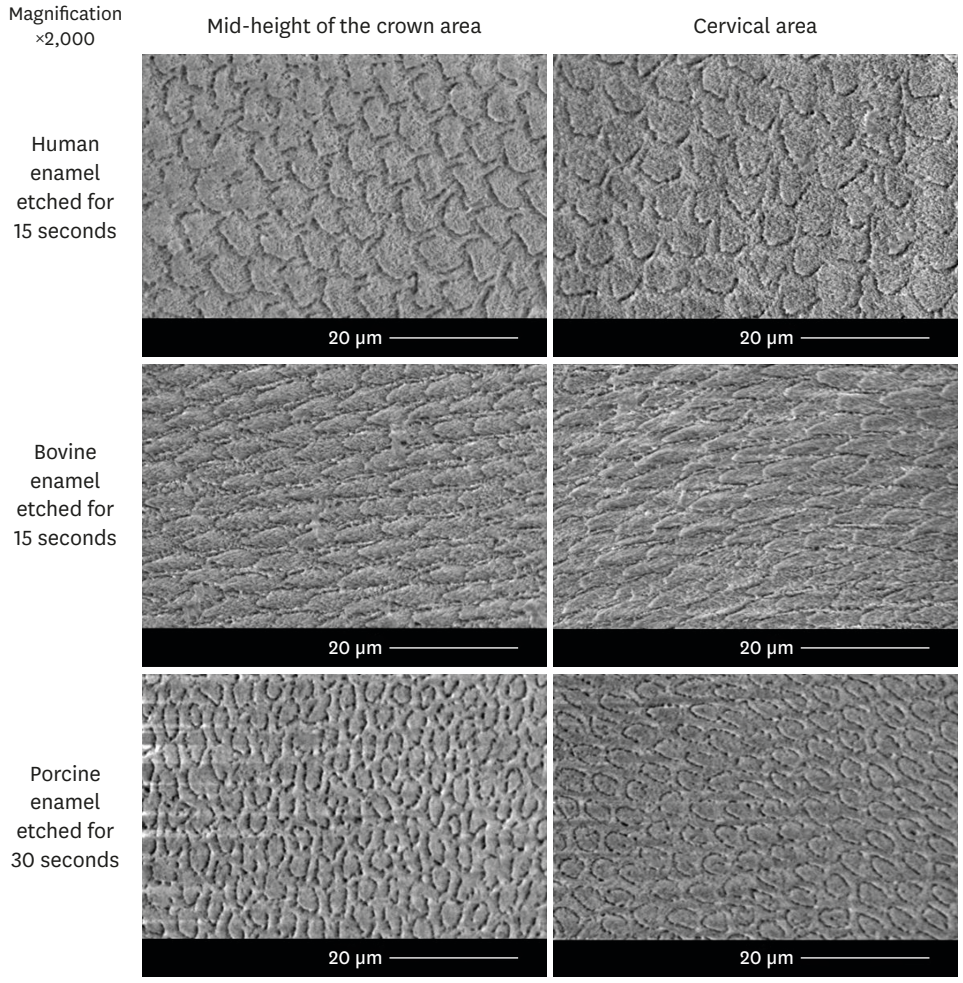

Fig. 4. The comparison of scanning electron micrographs of human, bovine and porcine effectively etched enamel in the mid-height of the crown area and cervical area. In every micrograph regular etching patterns are visible, as well as dissolved rod sheaths and prominent prism cores. The prisms differed in shapes between examined species. In both examined areas human enamel prisms resembled arcades, bovine were noticeably elongated and porcine had an elliptical shape. Human and bovine enamel prisms appeared to be more porous than the porcine ones, which looked smoother. The interrod regions of distinctive thickness were pronounced in the porcine enamel (magnification ×2,000, scale bar = 20 μm).

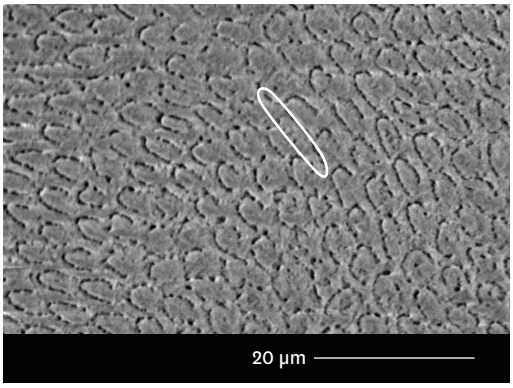

Fig. 5. Scanning electron micrograph of effectively etched porcine enamel (in mid-height of the crown area). Dissolved prism sheaths and prominent prism cores are visible. Wide rows of interprismatic enamel are marked in the circle (magnification ×2,000, scale bar = 20 μm).

https://doi.org/10.4142/jvs.2020.21.e83

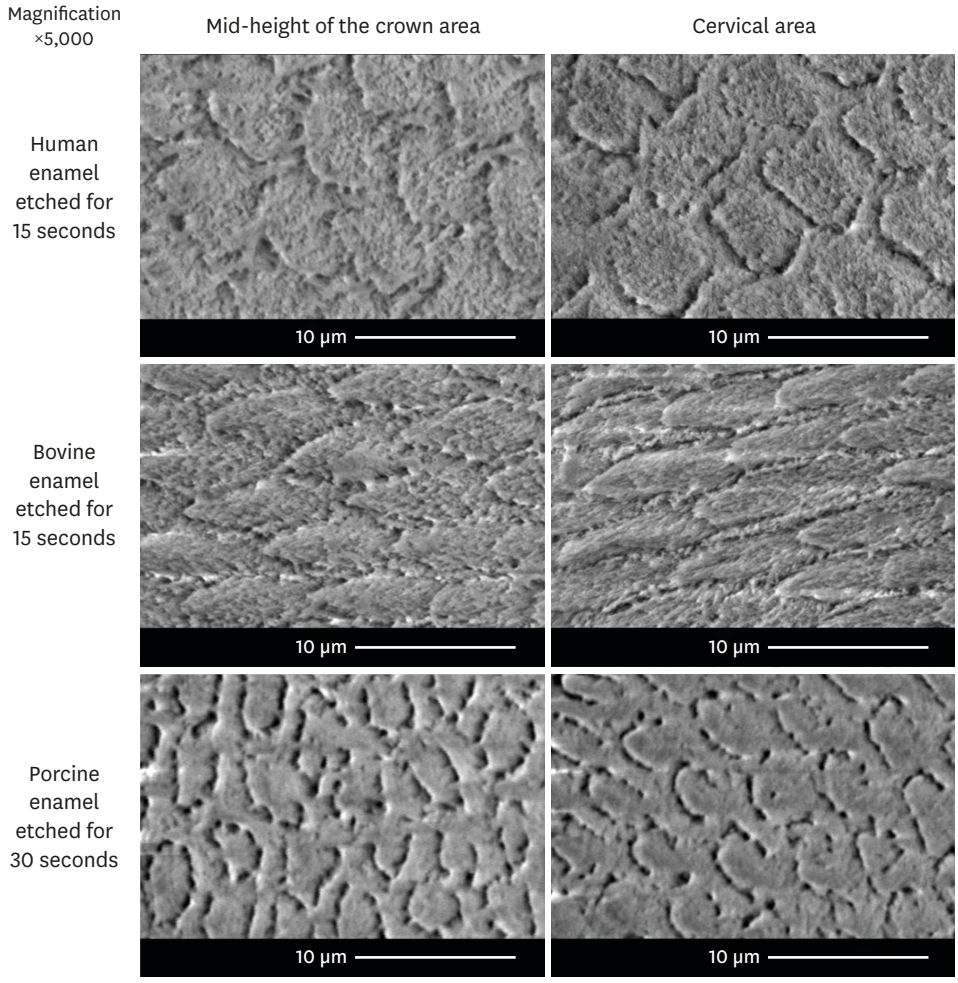

Fig. 6. The comparison of scanning electron micrographs (magnification ×5,000) of human, bovine and porcine effectively etched enamel in the mid-height of the crown area and cervical area. The prisms differed in shapes between three species. In both examined areas human enamel prisms resembled arcades, bovine were noticeably elongated and porcine had an elliptical shape. Human and bovine enamel prisms in this magnification appeared to be considerably more porous than the porcine ones, which looked smoother (scale bar = 10 μm).

Fig. 6 shows examples of SEM microphotographs of the enamel of three examined species in 2 studied areas at ×5,000 magnification. This magnification enabled us to perform precise measurements of enamel prism length and width.

The collected data was subjected to statistical analysis and presented in **Tables 2** and **3**. **Table 2** revealed statistically significant differences in the enamel prism length between all groups of samples and in both studied sites on the tooth crown ($p < 0.05$). In the mid-height of the crown, the length of human prisms appeared to be the shortest (the mean value was 4.732 μm), the porcine prisms were longer (5.721 μm) and the bovine were the longest (6.787 μm). Whereas in the cervical region, the shortest enamel prisms were found in porcine teeth (4.597 μm) followed by human prisms (5.929 μm) and the longest prisms were detected also in the bovine samples (8.105 μm).

Human and bovine prisms were longer in the cervical region than in the mid-height of the crown as compared to the porcine prisms which were longer in the mid-height of the crown.

https://doi.org/10.4142/jvs.2020.21.e83

Table 3. Results of comparing the mean widths of the enamel prisms (μm) by mammal species and tooth areas

Statistical parameters	Mammal species studied			*p* value when including terms for main effects
	Human teeth (μm)	Bovine teeth (μm)	Porcine teeth (μm)	
Mid-height of the crown[a]				<0.05
Mean	**4.942**	**3.953**	**3.128**	
Median	5.952	2.998	3.123	
Minimum	1.9	2.1	2.1	
Maximum	7.0	6.9	6.0	
SE	0.226	0.221	0.230	
Cervical area				<0.05
Mean	**5.123**[b]	**2.650**	**2.682**	
Median	5.146	2.573	2.698	
Minimum	4.1	1.7	1.9	
Maximum	5.9	3.5	3.6	
SE	0.250	0.214	0.214	
p value when including terms for main effects	0.591	<0.05	0.157	*p* value when including terms for interactions <0.05

Mean values are bold-faced.
[a]The probability concerns the comparisons of means in rows—in the mid-height of the crown area; each mean value differs from the others; [b]The probability concerns the comparisons of means in rows—in the cervical area; the mean value for human prisms is significantly higher than for bovine and porcine, which do not differ from each other.

The analysis of data from **Table 3** showed that the highest mean value of the enamel prism width at the mid-height of the crown was observed in human teeth (4.942 μm), lower width values were found in bovine (3.953 μm) and the lowest in porcine enamel prisms (3.128 μm). All these values differed statistically significantly ($p < 0.05$). The mean width of human prisms in the cervical area (5.123 μm) was significantly greater ($p < 0.05$) compared to that of bovine and porcine enamel (2.650 μm and 2.682 μm, respectively), in which no statistically significant differences were found.

The comparison between the two assessed localizations on the tooth surface revealed statistically significant differences ($p < 0.05$) only in bovine enamel—wider prisms were observed in the mid-height of the crown (3.953 μm) than in the cervical area (2.650 μm). The mean width of human and porcine enamel prisms did not differ significantly.

DISCUSSION

In experimental dental studies, human third molars extracted due to impeded eruption and less frequently premolars extracted for orthodontic reasons are usually used. As a substitute of human teeth, scientists commonly use porcine molars, resembling human molars in shape and size, and bovine mandibular incisors, whose size and shape are similar to human maxillary central incisors.

These particular groups of animal teeth are chosen due to several reasons, such as practical aspects of acquisition and preparation of samples from bovine and porcine teeth. In bovine lower central incisors, there is a large amount of enamel available for examination and their flat surface facilitates and accelerates sample preparation. An additional advantage of bovine teeth is the fact that they are easily extracted, thus, obtaining bovine teeth requires less effort and time and does not demand surgical skills from the operator.

On the contrary, these animals' teeth from other functional groups are not suitable for investigations. The anatomy of bovine molars hinders the microscopic observations as

there is very little amount of enamel on the occlusal surface, which is available for the investigations. On this surface of bovine molars there are infundibulas, which develop from the enamel organ. They are crescentic and partially filled with cementum and blackened feed residue. The vestibular surface of bovine molars is irregular as well, there is no appropriate amount of flat area of enamel which is necessary to conduct SEM studies. Other authors also disqualified bovine molars for *in vitro* research due to their morphology, and they conducted studies on lower incisors. Bovine upper incisors are also not available for the dental experiments because they occur in a form of a compact connective tissue bulge covered with highly cornificated stratified pavement epithelium, whereas porcine incisors are not appropriate due to their small size [27].

The purpose of this study was to examine and compare those permanent animal teeth, which are easily accessible, and gaining them does not involve any special breeding and sacrifice of animals. Thus, the age of bovine and porcine teeth was about 36 months, and it was dictated by the rules of food industry. Moreover, other scientists, who carried out their investigations on these species also used teeth of animals of similar age [11,13,16].

Human, bovine and porcine enamel is a highly mineralized tissue and consists predominantly of calcium phosphate salts in the form of hydroxyapatites. The chemical composition of the enamel of these species is very similar. Enamel hydroxyapatites may be incorporated with various anions and cations such as sodium, potassium, magnesium, fluoride, chloride and many other trace elements [28-30]. Hydroxyapatite crystals are bundled to form prisms [1].

A study by Silverstone et al. [31] has shown that the exposure of human dental enamel to acid solutions *in vitro* reveals its prismatic structure and produces three basic etching patterns. In type 1 etching pattern, the prism core was preferentially removed leaving the prism peripheries relatively intact. In type 2 etching pattern, the reverse pattern was observed. The peripheral regions of prisms (sheaths) were removed preferentially, leaving prism cores relatively unaffected. In type 3 etching pattern, there was a combination of previous patterns together with regions in which the pattern of etching could not be related to prism morphology.

In the present study, the etching pattern obtained for human, bovine, and porcine enamel was similar. All SEM microphotographs showed the dissolution of the prism sheaths, giving a “cobblestone” appearance—the Silverstone's type 2 etching pattern [31]. These observations are consistent with the reports of other authors regarding human and bovine teeth [3,4,32]. As far as porcine teeth are concerned, Lopes et al. [33] obtained the Silverstone's type 1 pattern. However, this is the only study in which porcine enamel was analyzed and the authors applied a different methodology compared to our study.

Various factors may affect the enamel etching i.e. chemical composition (especially fluoride content), the type and concentration of an etchant and duration of etching, as well as the prismatic or aprismatic structure [4,13]. Literature provides even information that the etching pattern depends on the functional group of the tooth being etched [34].

In our study, the duration of enamel etching seems to be a particularly relevant factor that distinguishes porcine teeth from those of the other examined species. Pre-evaluation of SEM microphotographs showed that the etching of porcine enamel for 15 sec with 35%

orthophosphoric acid was not sufficient to reveal any type of etching pattern. Therefore, the etching time for porcine enamel was prolonged to 30 sec. The same etching time (30 sec) was used by Lopes et al. [33] for human and porcine teeth in order to achieve a satisfactory image of the surface. Based on these findings, we suggest that porcine enamel should be etched for minimum 30 sec in materials or dental studies which require demineralization. On the other hand, when dissolution time and tissue loss are investigated and compared with human teeth, porcine teeth do not seem to be a good substitute for human teeth, which is in agreement with Field et al. [35] studies.

A visual comparison of the etched enamel at the mid-height of the crown and in the cervical area in human, bovine, and porcine teeth did not reveal any evident differences in the prism structure. In both areas irrespective of the species, a regular etching pattern was found. This observation does not correspond with other authors' outcomes, in which etching pattern was irregular in the cervical area (aprismatic enamel) of human enamel [34]. This difference may arise from the necessity of flattening and polishing the sample surface before etching in our study. Such a procedure might have resulted in the loss of the enamel layer above ca. 20–80 μm, which corresponds to the thickness of aprismatic enamel in this area [35]. It is difficult to compare the results obtained in this study with others as we could not find any research analyzing bovine or porcine enamel in different anatomical areas and it seems that there are no reports confirming the presence of aprismatic enamel in these animals.

An important factor which affects etching is a direction of cutting the sample before etching. Namely, crystals tend to dissolve easier when an etchant is applied along the long axis of the crystal, contrary to etching performed perpendicularly [1,22]. This can be easily observed in the interprismatic region, where hydroxyapatite crystals are arranged at a different angle compared to the central parts of prism heads, and thus they are more difficult to be etched [30,36]. In SEM microphotographs of porcine enamel, wide interprismatic areas were observed and rows of prisms were clearly separated (**Fig. 5**). Our data compares favorably with that of Reis et al. [4] and Popowics et al. [37], who stated that pig's interrod enamel crystallites were organized into sheets or partitions between rows of enamel prisms. Moreover, interrod crystallites differed strongly in orientation from rod crystallites. A great amount of interprismatic enamel may explain a longer time required for etching porcine teeth in our investigation and supports the thesis that this enamel should be used with caution in *in vitro* studies.

Our analysis of SEM microphotographs confirmed that prisms differ in shape between species. Bovine prisms were distinctly elongated, contrary to human and porcine prisms which had a more regular form—in human teeth they resembled arcades and in porcine teeth were elliptical. This observation was confirmed by other reports in the literature as Nakamichi et al. [3] also defined bovine enamel prisms as oval and narrow. Phylogenetic analyses have shown that the type of diet affects the development of enamel prism orientation. This may explain some differences found in the bovine enamel morphology compared to that of humans and porcine, as cattle are typical herbivores [38]. Similar findings are described by Wang et al. [30] who reported differences in the arrangement of prisms, decussations and interprismatic region between human and bovine teeth.

A comparison of enamel prism dimensions obtained in our study with data from the available literature seems rather difficult because most of the authors have measured only the diameter of prisms. As far as human teeth are concerned, such an approach seems reasonable, whereas

in case of other species, especially cattle, it seems necessary to consider two dimensions, length and width, as bovine prisms can be three times longer than wider. According to the literature, the diameter of human enamel prisms ranges between 3 to 7 μm [22,36,39,40]. The reported values are similar to those obtained in our research (4.732–5.929 μm), which confirms the reliability of our study.

Only few authors [3, 22] measured bovine prisms. Sanches et al. [22] proved that bovine prism diameter ranged between 3.7 and 8.8 μm. Our results of mean length and width values of bovine prisms were within this wide range of measurements (except for the width in the cervical area), but they differed significantly in comparison to human teeth. This is contrary to Sanches et al. [22] reports, where values of bovine prism diameter did not differ significantly from human teeth.

The results of the present study indicated also significant differences ($p < 0.05$) between dimensions of human and porcine enamel prisms. Human prisms in the cervical area were longer (**Table 2**) and wider than porcine ones (**Table 3**), whereas human prisms in the mid-height of the crown were shorter (**Table 2**) and wider than porcine (**Table 3**). The only publication where the size of porcine teeth was mentioned gives just the information that the diameter of "swine prisms seems to be half of human prism diameter" [33], which corresponds to the results of our study (2.682 μm vs. 5.123 μm).

Analyzing the results of our research in terms of use of breeding animals' enamel in experimental dental research, it should be emphasized that the same organization of prisms and the same etching pattern obtained in our investigation could suggest the similarity of these tissues allowing human teeth to be substituted by bovine and porcine ones. This thesis is in agreement with the report of Nakamichi et al. [3], who compared the suitability of human and bovine teeth in the studies on adhesion of restorative materials to tooth tissues. The authors stated that the different shape of prisms of those mammals did not significantly affect the strength of the composite-enamel interface. Other authors' findings [5] reveal however that the values of adhesive strength between bovine enamel and composite or glass-ionomer materials were considerably lower (about 40–44%) than those occurring between human enamel and the same materials. Authors explained this fact by the presence of larger crystal grains and a greater damage to the crystal lattice caused by faster maturation of bovine teeth [5]. These reports together with our data suggest that the results obtained in the study on enamel of different animal species may not be comparable with the results obtained on human teeth.

Additional doubts in this matter are also raised by the individual feature of porcine enamel, namely the presence of almost smooth prism cores. This is contrary to human and bovine teeth, where some porosities created by hydroxyapatite crystals could be visualized at a greater magnification. Our finding is not in agreement with Reis et al. [4], who investigated the adhesion of composite material to human, bovine, and porcine enamel and performed SEM analysis of those tissues. They reported the highest bond strength values for porcine enamel and described it as the most porous. According to those authors, the created irregularities contributed to better micromechanical retention and provided the best adhesion between enamel and composite. Our results also vary from those given by Field et al. [35], who studied the influence of erosive factors on human, bovine, and ovine enamel. Authors observed the smoothest surface of bovine enamel after acidic demineralization and reported higher resistance to surface changes. In conclusion, animal teeth were

disqualified in terms of substitution of human teeth in erosion studies. These variations and discrepancies indicate that the porcine and bovine enamel microanatomy needs to be further investigated to obtain unambiguous observations.

In conclusion, our research provides the comparison of microanatomy and dimensions of the prisms of human, bovine, and porcine enamel. Similarities were found in the arrangement of enamel prisms and the same etching pattern for all three species. However, the differences in the shape and dimensions of the prisms were also reported, especially in bovine enamel. In porcine teeth, significant differences in microanatomy and etching time were observed, therefore, a longer exposure of this tissue to etching agents is required in *in vitro* tests.

The reported findings indicate that the results of experimental studies carried out on bovine and porcine enamel should not be compared with the results obtained on human enamel.

REFERENCES

1. Chapter 12. Enamel. In: Fehrenbach MJ, Popowics T, editors. *Illustrated Dental Embryology, Histology and Anatomy*. 4th ed. Missouri: Elsevier Saunders; 2016, 147-157.
2. Lopes MB, Consani S, Gonini-Júnior A, Moura SK, McCabe JF. Comparison of microleakage in human and bovine substrates using confocal microscopy. Bull Tokyo Dent Coll. 2009;50(3):111-116.
PUBMED | CROSSREF
3. Nakamichi I, Iwaku M, Fusayama T. Bovine teeth as possible substitutes in the adhesion test. J Dent Res. 1983;62(10):1076-1081.
PUBMED | CROSSREF
4. Reis AF, Giannini M, Kavaguchi A, Soares CJ, Line SR. Comparison of microtensile bond strength to enamel and dentin of human, bovine, and porcine teeth. J Adhes Dent. 2004;6(2):117-121.
PUBMED
5. Saleh F, Taymour N. Validity of using bovine teeth as a substitute for human counterparts in adhesive tests. East Mediterr Health J. 2003;9(1-2):201-207.
PUBMED
6. Dutra-Correa M, Anauate-Netto C, Arana-Chavez VE. Density and diameter of dentinal tubules in etched and non-etched bovine dentine examined by scanning electron microscopy. Arch Oral Biol. 2007;52(9):850-855.
PUBMED | CROSSREF
7. Forssell-Ahlberg K, Brännström M, Edwall L. The diameter and number of dentinal tubules in rat, cat, dog and monkey. A comparative scanning electron microscopic study. Acta Odontol Scand. 1975;33(5):243-250.
PUBMED | CROSSREF
8. Gray SE, Burgess JO. An *in vivo* and *in vitro* comparison of two dentin bonding agents. Dent Mater. 1991;7(3):161-165.
PUBMED | CROSSREF
9. Pashley EL, Tao L, Mackert JR, Pashley DH. Comparison of *in vivo* vs. *in vitro* bonding of composite resin to the dentin of canine teeth. J Dent Res. 1988;67(2):467-470.
PUBMED | CROSSREF
10. Tanaka JL, Medici Filho E, Salgado JA, Salgado MA, Moraes LC, Moraes ME, et al. Comparative analysis of human and bovine teeth: radiographic density. Braz Oral Res. 2008;22(4):346-351.
PUBMED | CROSSREF
11. Camargo CH, Siviero M, Camargo SE, de Oliveira SH, Carvalho CA, Valera MC. Topographical, diametral, and quantitative analysis of dentin tubules in the root canals of human and bovine teeth. J Endod. 2007;33(4):422-426.
PUBMED | CROSSREF
12. Arcari GM, Baratieri LN, Maia HP, De Freitas SF. Influence of the duration of treatment using a 10% carbamide peroxide bleaching gel on dentin surface microhardness: an *in situ* study. Quintessence Int. 2005;36(1):15-24.
PUBMED

13. Attin T, Wegehaupt F, Gries D, Wiegand A. The potential of deciduous and permanent bovine enamel as substitute for deciduous and permanent human enamel: Erosion-abrasion experiments. J Dent. 2007;35(10):773-777.
PUBMED | CROSSREF

14. Balvedi RP, Versiani MA, Manna FF, Biffi JC. A comparison of two techniques for the removal of calcium hydroxide from root canals. Int Endod J. 2010;43(9):763-768.
PUBMED | CROSSREF

15. Bouillaguet S, Gysi P, Wataha JC, Ciucchi B, Cattani M, Godin C, et al. Bond strength of composite to dentin using conventional, one-step, and self-etching adhesive systems. J Dent. 2001;29(1):55-61.
PUBMED | CROSSREF

16. Camargo MA, Marques MM, de Cara AA. Morphological analysis of human and bovine dentine by scanning electron microscope investigation. Arch Oral Biol. 2008;53(2):105-108.
PUBMED | CROSSREF

17. Delbem AC, Sassaki KT, Vieira AE, Rodrigues E, Bergamaschi M, Stock SR, et al. Comparison of methods for evaluating mineral loss: hardness versus synchrotron microcomputed tomography. Caries Res. 2009;43(5):359-365.
PUBMED | CROSSREF

18. Faraoni-Romano JJ, Da Silveira AG, Turssi CP, Serra MC. Bleaching agents with varying concentrations of carbamide and/or hydrogen peroxides: effect on dental microhardness and roughness. J Esthet Restor Dent. 2008;20(6):395-402.
PUBMED | CROSSREF

19. Erickson RL, De Gee AJ, Feilzer AJ. Fatigue testing of enamel bonds with self-etch and total-etch adhesive systems. Dent Mater. 2006;22(11):981-987.
PUBMED | CROSSREF

20. Freire LG, Carvalho CN, Ferrari PH, Siqueira EL, Gavini G. Influence of dentin on pH of 2% chlorhexidine gel and calcium hydroxide alone or in combination. Dent Traumatol. 2010;26(3):276-280.
PUBMED | CROSSREF

21. Hara AT, Queiroz CS, Paes Leme AF, Serra MC, Cury JA. Caries progression and inhibition in human and bovine root dentine *in situ*. Caries Res. 2003;37(5):339-344.
PUBMED | CROSSREF

22. Sanches RP, Otani C, Damião AJ, Miyakawa W. AFM characterization of bovine enamel and dentine after acid-etching. Micron. 2009;40(4):502-506.
PUBMED | CROSSREF

23. Sfondrini MF, Gatti S, Scribante A. Effect of blood contamination on shear bond strength of orthodontic brackets and disinclusion buttons. Br J Oral Maxillofac Surg. 2011;49(5):404-408.
PUBMED | CROSSREF

24. Souza RO, Ozcan M, Mesquita AM, De Melo RM, Galhano GA, Bottino MA, et al. Effect of different polymerization devices on the degree of conversion and the physical properties of an indirect resin composite. Acta Odontol Latinoam. 2010;23(2):129-135.
PUBMED

25. Walter R, Miguez PA, Pereira PN. Microtensile bond strength of luting materials to coronal and root dentin. J Esthet Restor Dent. 2005;17(3):165-171.
PUBMED | CROSSREF

26. Wiegand A, Vollmer D, Foitzik M, Attin R, Attin T. Efficacy of different whitening modalities on bovine enamel and dentin. Clin Oral Investig. 2005;9(2):91-97.
PUBMED | CROSSREF

27. Soana S, Bertoni G, Gnudi G, Botti P. Anatomo-radiographic study of prenatal development of bovine fetal teeth. Anat Histol Embryol. 1997;26(2):107-113.
PUBMED | CROSSREF

28. Ortiz-Ruiz AJ, Teruel-Fernández JD, Alcolea-Rubio LA, Hernández-Fernández A, Martínez-Beneyto Y, Gispert-Guirado F. Structural differences in enamel and dentin in human, bovine, porcine, and ovine teeth. Ann Anat. 2018;218:7-17.
PUBMED | CROSSREF

29. Teruel JD, Alcolea A, Hernández A, Ruiz AJ. Comparison of chemical composition of enamel and dentine in human, bovine, porcine and ovine teeth. Arch Oral Biol. 2015;60(5):768-775.
PUBMED | CROSSREF

30. Wang C, Li Y, Wang X, Zhang L, Tiantang, Fu B. The enamel microstructures of bovine mandibular incisors. Anat Rec (Hoboken). 2012;295(10):1698-1706.
PUBMED | CROSSREF

31. Silverstone LM, Saxton CA, Dogon IL, Fejerskov O. Variation in the pattern of acid etching of human dental enamel examined by scanning electron microscopy. Caries Res. 1975;9(5):373-387.
PUBMED | CROSSREF

32. Tong LS, Pang MK, Mok NY, King NM, Wei SH. The effects of etching, micro-abrasion, and bleaching on surface enamel. J Dent Res. 1993;72(1):67-71.
PUBMED | CROSSREF

33. Lopes FM, Markarian RA, Sendyk CL, Duarte CP, Arana-Chavez VE. Swine teeth as potential substitutes for *in vitro* studies in tooth adhesion: a SEM observation. Arch Oral Biol. 2006;51(7):548-551.
PUBMED | CROSSREF

34. Hobson RS, Rugg-Gunn AJ, Booth TA. Acid-etch patterns on the buccal surface of human permanent teeth. Arch Oral Biol. 2002;47(5):407-412.
PUBMED | CROSSREF

35. Field JC, Waterhouse PJ, German MJ. The early erosive and abrasive challenge: a profilometric, electron microscopic and microhardness study using human, bovine and ovine enamel. Eur J Prosthodont Restor Dent. 2017;25(2):93-100.
PUBMED

36. Braly A, Darnell LA, Mann AB, Teaford MF, Weihs TP. The effect of prism orientation on the indentation testing of human molar enamel. Arch Oral Biol. 2007;52(9):856-860.
PUBMED | CROSSREF

37. Popowics TE, Rensberger JM, Herring SW. Enamel microstructure and microstrain in the fracture of human and pig molar cusps. Arch Oral Biol. 2004;49(8):595-605.
PUBMED | CROSSREF

38. Koenigswald WV, Clemens WA. Levels of complexity in the microstructure of mammalian enamel and their application in studies of systematics. Scanning Microsc. 1992;6(1):195-217.
PUBMED

39. Cuy JL, Mann AB, Livi KJ, Teaford MF, Weihs TP. Nanoindentation mapping of the mechanical properties of human molar tooth enamel. Arch Oral Biol. 2002;47(4):281-291.
PUBMED | CROSSREF

40. De Menezes Oliveira MA, Torres CP, Gomes-Silva JM, Chinelatti MA, De Menezes FC, Palma-Dibb RG, et al. Microstructure and mineral composition of dental enamel of permanent and deciduous teeth. Microsc Res Tech. 2010;73(5):572-577.
PUBMED | CROSSREF