

Animal and human dentin microstructure and elemental composition

Research Article

Nina Mlakar^{*1}, Zlatko Pavlica¹, Milan Petelin², Janez Štrancar³,
Petra Zrimšek⁴, Alenka Pavlič⁵

1 Clinic for Small Animal Medicine and Surgery, Veterinary Faculty, University of Ljubljana, 1000 Ljubljana, Slovenia

2 Department of Oral Medicine and Periodontology, Faculty of Medicine, University of Ljubljana, 1000 Ljubljana, Slovenia

3 Laboratory of Biophysics, EPR Centre, Solid State Physics Department, Jožef Stefan Institute, 1000 Ljubljana, Slovenia

4 Clinic for Reproduction and Horses, Veterinary Faculty, University of Ljubljana, 1000 Ljubljana, Slovenia

5 Department of Paediatric and Preventive Dentistry, Faculty of Medicine, University of Ljubljana, 1000 Ljubljana, Slovenia

Received 10 June 2013; Accepted 7 December 2013

Abstract: Animal teeth are a common model in studies on dentin adhesive materials. The aim of this study was to compare microstructural parameters (density and diameter of dentinal tubules (DT), peritubular dentin (PTD) thickness, PTD and intertubular dentin (ITD) surface area) and chemical characteristics of canine, porcine, equine, and human root dentin. The middle layers of dentin were harvested just below a cemento-enamel junction from incisors and investigated by means of scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDXS). SEM evaluation of the specimens revealed, that porcine dentin shared most similarities with human dentin. When comparing the density of DTs, canine dentin was also found to be similar to human dentin. Elemental composition of the root dentin did not differ significantly in porcine, equine and human dentin, but in canine dentin higher magnesium value in PTD compared to ITD was found. It is known that microstructural and chemical characteristics affect the strength of the adhesive bonds created among restorative materials and dentin. According to the results of this study, porcine dentin seems to be the most appropriate model to study dental materials to be used in human restorative dentistry.

Keywords: *Dentin • Energy dispersive X-ray spectroscopy • Intertubular dentin • Peritubular dentin • Scanning electron microscopy*

© Versita Sp. z o.o

1. Introduction

Dentin is an anisotropic composite material [1] which comprises the bulk of the tooth and gives it its shape. It is a non-vascular mineralized connective tissue, less mineralized comparing to overlying enamel, composed of 70% inorganic hydroxyapatite (HA) crystals, 20%

organic collagen fibers with small amounts of other proteins and lipids, and 10% water by weight [2].

Dentin is a vital tissue containing multiple closely packed dentinal tubules (DT) throughout the bulk of dentin. Dentinal tubules extend radially from the pulp to the dentino-enamel (DEJ) or dentino-cemental junction (DCJ) [2]. In the tooth crown the path of DT resembles an S curve, while in the root DT course is straighter [2,3]. In

* E-mail: nina_mlakar@siol.net

human tooth DT diameters are approximately 0.9 μm at the DEJ and 2.5 μm at the surface facing a dental pulp where DT are also packed closer together [4]. Similarly, diameters of DT decrease towards the external layers of dentin in some animals (in canine teeth from 2.1 μm to 0.9 μm [5,6] and in equine teeth from 2.5 μm to 1.0 μm) [3], while in bovine teeth DT diameters are narrower at the dentin surface facing a pulp comparing to those at the periphery [7,8] (with main DT diameters measuring 1.8 μm at a pulp side and 2.3 μm at DEJ) [7].

Dentinal tubule, which contains odontoblast's process and cytoplasmic fluid [9,10], is surrounded by hypermineralized collar of peritubular dentin (PTD) throughout dentin, except near the pulp [3,4]. In human, PTD mineral content by volume is approximately 90% [3], but if PTD contains any collagen remains controversial [11–18]. The bulk of the dentin comprises intertubular dentin (ITD), whose organic content is approximately three times higher than in PTD [14]. In ITD, collagen assembles a solid framework with fibrils arranged into incremental planes, oriented preferentially in layers perpendicular to DT and parallel to the long axis of the root [9,19–22]. The major role of interwoven collagen fibrils is to control deposition and orientation of HA crystals [20]. Data published on basic dentin microstructure characteristics and elemental composition of equine and human incisors, are summarized in Table 1.

In human, PTD is roughly 40% better mineralized than the rest of the dentin [2], while in equine a mineral content of PTD does not exceed that of ITD by more than 9% [12]. To our knowledge, data on PTD or ITD mineral contents, microstructure characteristics and elemental composition in porcine and canine incisor dentin lack.

Mineral composition of HA crystals, the major components of dentin, is mostly represented by calcium

(Ca) and phosphorus (P) [23,24], with the Ca/P ratio closely correlating to the microhardness value [25,26]. Data published on Ca and P proportions in incisors of different species are presented in Table 1. In addition to Ca and P, other elements are present in dentin, but in much smaller amounts and some of them as trace elements only [27]. Comparison of concentration of twelve elements (Ca, P, sodium (Na), magnesium (Mg), chlorine (Cl), potassium (K), manganese (Mn), aluminium (Al), zinc (Zn), copper (Cu), iron (Fe), and fluorine (F)) measured in human dentin and enamel of deciduous and permanent teeth revealed lower elements concentrations in dentin than in enamel. Mg however, was higher in the dentin compared to the enamel, and in the deciduous dentin compared to the dentin of permanent teeth [27].

Human teeth would generally be the substrates of choice in studies focused on dentin adhesive materials, but their use is being restricted due to ethical limitations. In studies focusing adhesion to enamel and dentine bovine incisors are commonly employed as a substitute to human teeth [28,29], since they are larger and are easily obtained. Nevertheless, the use of bovine dentin in such studies is being questioned due to different microstructural characteristics [7,8] which significantly affect the strength of the adhesive bonds created among restorative materials and dentin [30]. The aim of the study was, first of all to determine variations in microstructure of porcine, canine, equine and human dentin as analysed by scanning electron microscope (SEM). Second, the study was designed to also quantify dentin microstructure variations across the species studied. And thirdly, to estimate whether there are any differences in basic elemental composition between PTD and ITD of the species studied.

Table 1. Data on dentin microstructure, and Ca and P characteristics of human and different animal species.

Species	Reference	No. of investigated teeth	Age of subject (yrs)	Type of permanent tooth	No. of dentinal tubules (mm^{-2})	DT diameter (μm)	Ca (wt%)	P (wt%)
Equine	[3]	15	5–20	incisor	10 000–25 000	1.8–2.3	-	-
	[10]	46, 4	1.5–18	cheek teeth (premolar, molar), incisor	-	2.9 $\pm$ 0.1	-	-
	[42]	15	-	upper central incisor	-	-	29.654 ^a	14.922 ^a
Human	[45]	10	45–51	central and lateral incisor, canine	23 931 $\pm$ 1438.1	-	-	-
	[46]	60	-	anterior teeth	-	-	25.20 $\pm$ 0.83 ^b	10.39 $\pm$ 0.30 ^b

^a Energy dispersive X-ray spectroscopy

^b Inductively coupled plasma atomic emission spectrometry

2. Materials and methods

2.1. Dentin samples

Dentin samples were obtained from permanent maxillary incisor teeth of dogs, pigs, horses and humans. Only teeth free of carious or any other hard tissue disease were included in the study. Canine teeth were obtained immediately after the dogs were euthanized due to fatal illness unrelated to oral/dental health. Porcine and equine teeth were extracted immediately after slaughter under veterinary supervision in an abattoir. Human caries-free incisor teeth were obtained from patients with progressive form of periodontal disease extracted after unsuccessful treatment outcome. Data on individual teeth, belonging to an individual animal or human, are summarized in Table 2.

From each tooth a sample from primary dentin (3 mm x 4 mm x 1 mm) was cut within 24 hours after extraction, using a diamond bur (No. 848, Dendia, Feldkirch, Austria) with rotation of 300 000 rpm on a water-cooled dental handpiece (Super-Torque, 630 B, Kavo, Germany). A sample was obtained from the labial side of the cervical part of a tooth root, just below a cemento-enamel junction (CEJ) in the middle of the bulk of dentin, as is shown schematically in Figure 1. As equine teeth are hypsodont they do not have a cervical area. A sample from equine primary dentin was therefore obtained from the labial side of tooth, just below the attached gingiva. On each sample, the surface facing the DEJ was marked in order to make dentin sample orientation afterwards possible.

2.2. Dentin microstructure and elemental analyses

Samples were embedded in epoxy resin (Epofix, Struers, Copenhagen, Denmark) and left to polymerize overnight. The exposed cross-sections were polished and then placed into ultrasonic bath (Bandelin Sonorex GT 120 Transistor, Bandelin Electronics, Germany) for 5 minutes to remove the smear layer [31], dehydrated



Figure 1. A rectangle on a photograph indicates the area where the dentinal sample was obtained from. The sample was obtained from the middle of the bulk of the labial side of dentin, in dimensions of 4x3x1 mm. The thicker line of the rectangle indicates the investigated dentin sample's surface.

with 70% ethanol, and stored in a desiccator for 24 hours. Afterwards, the samples were sputter-coated in a vacuum with a thin carbon layer (Vacuum Evaporator, Type JEE-SS, JEOL, Tokyo, Japan). In all samples a surface examined was the one originally facing the pulp. Dentin microstructure was observed by scanning electron microscopy (SEM) (JEOL JSM-5610, JEOL, Tokyo, Japan), under 10 kV, with the backscattered electron (SEM-BSE) mode and magnifications from 1000x to 14 000x.

Elemental analyses were carried out with energy dispersive X-ray spectroscopy (EDXS) (EDXS detector Gresham Scientific Instruments Ltd, Buckinghamshire, UK) at working distance of 10 mm and an acceleration voltage of 10 kV. On each dentin sample elemental analyses were performed at the site of interest in PTD and in ITD at magnification of 7000x, except in canine samples, where analyses were performed at magnification of 14 000x. Semiquantitative elemental analyses consisted of estimation of Ca, P, Na and Mg, and from those data the Ca/P ratios were calculated.

2.3. Estimation on PTD and ITD proportions

An image analysis programme (ImageJ 1.45s, Image processing and analysis in Java 1.6.7_20, Bethesda,

Table 2. Data on dentin samples included in the study

Species	No. of teeth	Age of animal / human (yrs)	Type of permanent tooth
Canine	7	3–6	maxillary third incisor
Porcine	10	3–5	maxillary third incisor
Equine	10	14–18	maxillary third incisor
Human	5	45–58	maxillary central incisor

MD, USA) was used for image display selection and analysis. The density of DT per $12\,237\,\mu\text{m}^2$, depicted on image of magnification 1000x, was counted on photomicrographs. Fractions of DT partly visible on the periphery of a photomicrograph were taken as 50%. For each dentin sample 10 photomicrographs were taken and analysed, and a mean density of DT per mm^2 was calculated.

DT diameters and PTD thicknesses were measured on 10 photomicrographs of each dentin sample at magnification of 6000x. In obliquely shaped DT, the shorter diameter was measured. In equine dentin, where PTD was asymmetrically deposited, shorter and longer diameters of PTD were measured and a mean value was calculated. Surface areas of PTD and ITD for each photomicrograph, and subsequently a mean values for a specimen, were calculated.

3. Statistical analysis

Data for dentinal parameters (density of DT per mm^2 , DT diameters, PTD thicknesses, ITD, and PTD surface areas), mean amounts of Ca, P, Na, and Mg, and Ca/P ratios (calculated for PTD and ITD) were presented as mean values and standard deviation (SD). Differences between dentin parameters for different species (canine, porcine, equine, and human) were compared using One Way Analysis of Variance (ANOVA) in the case of normally distributed data and equality of the variances, and Kruskal-Wallis Analysis of Variance on Ranks in case of non-normal distribution or non-equal variances. When a significant difference was revealed, values were compared by parametric Holm-Sidak method with Bonferroni adjustment for multiple tests or non-parametric Dunn's test.

Comparisons of Ca/P ratios between PTD and ITD in each species investigated were analyzed using a paired Student's t-test in the case of normal distribution and a Mann-Whitney Rank Sum Test if data were not normally distributed. Differences with values of $P < 0.05$ were considered to be significant. SigmaStat 3.5 (SYSTAT Software Inc.) software was used for the analyses.

4. Results

4.1. Dentin microstructure analysis

Canine, porcine, equine and human dentin cross-sections revealed comparable microstructure to some extent (Figure 2). On all SEM-BSE images there was a distinct collar of brighter PTD which encircled black

DT and indicated better mineralization of the PTD compared to ITD. Additionally, on all samples ITD appeared rather granular, especially in canine dentin. The major qualitative distinction was the oval appearance of the PTD collar shape around DT exposed in all examined equine dentins (Figure 2 E-F) in contrast to the ring like structure in canine, porcine and human dentins.

Further on, statistical analysis of microstructure parameters revealed some dissimilarities between the species, with the least differences noted between human and porcine dentin (Figure 3 and Figure 4). In human dentin the largest DT diameter ($1.4 \pm 0.3\,\mu\text{m}$) was observed, which was significantly larger in comparison to

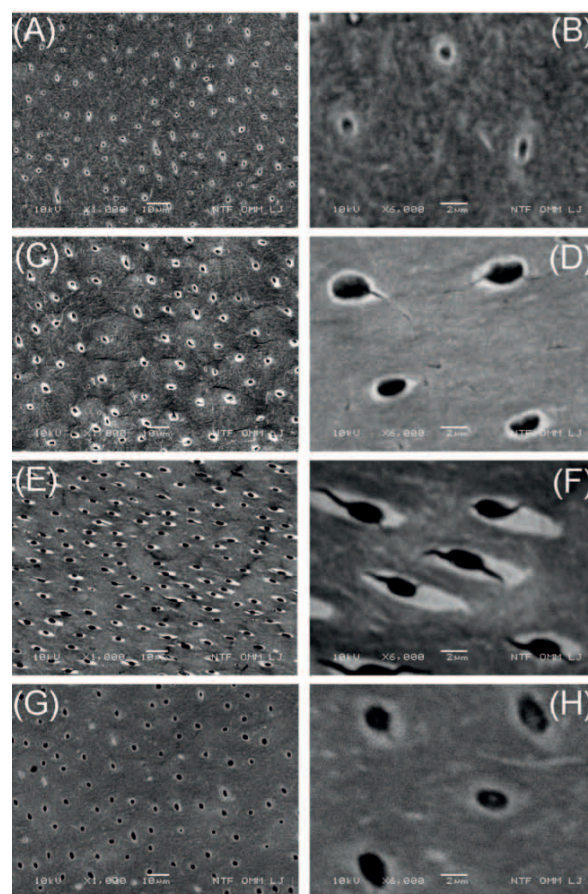


Figure 2. Dentin microstructure of cross-sections from the middle of the bulk of dentin as observed under scanning electron microscopy (SEM) in backscatter electron mode (BSE) of (A, B) canine, (C, D) porcine, (E, F) equine and (G, H) human dentin. In all species better mineralization of PTD than ITD is present. (A, B) In canine dentin DT are significantly smaller and surrounded by narrower PTD area comparing to PTD in other species. Additionally, in canine dentin density of DT per mm^2 was notably lower. (E, F) Equine dentin shows typical shape of oval PTD. On higher magnification uneven diameter of PTD is clearly seen. Density of DT per square plane and DT diameters of (C, D) porcine and (G, H) human dentin are very similar. Photomicrographs (A, C, E and G) and (B, D, F and H) are taken at magnification of 1000x and 6000x, respectively. DT, dentinal tubules; ITD, intertubular dentin; PTD, peritubular dentin.

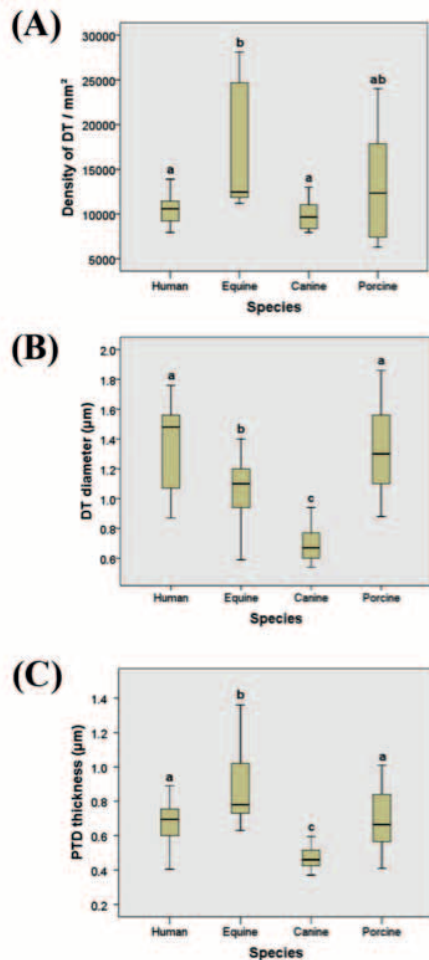


Figure 3. Box plots show median, upper and lower quartiles and the range of results of (A) density of DT per square plane, (B) DT diameter, and (C) PTD thickness of human, equine, canine and porcine dentin samples. Boxes marked with the different letters are significantly different ($P < 0.05$).

canine ($0.7 \pm 0.1 \mu\text{m}$) and equine ($1.1 \pm 0.2 \mu\text{m}$) ($P < 0.05$) but not to porcine dentin ($1.3 \pm 0.3 \mu\text{m}$) ($P > 0.05$). Between human and porcine dentin no significant differences were found in density of DT ($10\,500 \pm 1500$ and $13\,000 \pm 6000/\text{mm}^2$, respectively), PTD thickness (0.7 ± 0.1 and $0.7 \pm 0.2 \mu\text{m}$, respectively), PTD surface area (5.4 ± 1.4 and $4.7 \pm 1.0\%$, respectively) and ITD surface area (93 ± 2 and $94 \pm 1\%$, respectively) ($P > 0.05$).

In canine dentin DT diameter ($0.7 \pm 0.1 \mu\text{m}$), PTD thickness ($0.5 \pm 0.1 \mu\text{m}$) and PTD surface area ($1.9 \pm 0.5\%$) were significantly lower ($P < 0.05$), and consequently ITD surface area ($98 \pm 1\%$) was significantly larger than in other species ($P < 0.05$). The lowest mean DT density value in canine ($10\,000 \pm 1500/\text{mm}^2$) differed significantly in comparison to equine dentin only ($17\,000 \pm 6500/\text{mm}^2$) ($P < 0.05$). Equine had significantly lower ITD surface area ($86 \pm 4\%$) compared to other species ($P < 0.05$),

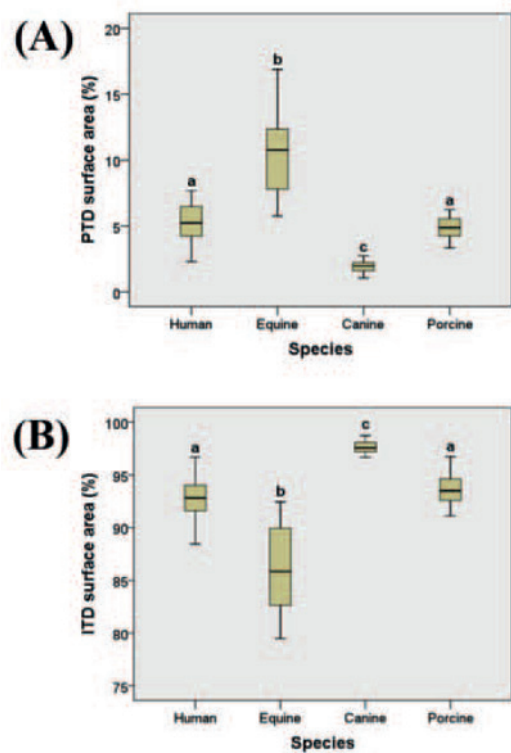


Figure 4. Box plots show median, upper and lower quartiles and the range of results of (A) PTD surface area and (B) ITD surface area of human, equine, canine and porcine dentin samples. Boxes marked with the different letters are significantly different ($P < 0.05$).

whereas PTD thickness ($0.9 \pm 0.2 \mu\text{m}$) and PTD surface area ($10.6 \pm 2.9\%$) were significantly higher in comparison to other species ($P < 0.05$). Density of equine DT ($17\,000 \pm 6500/\text{mm}^2$) was significantly higher only in comparison to canine and human dentin ($10\,000 \pm 1500$ and $10\,500 \pm 1500/\text{mm}^2$, respectively) ($P < 0.05$).

4.2. Elemental analysis

The mineral concentration was analysed in PTD and ITD at the previously noted sites. Results are presented as means $\pm$ SD in Table 3. Comparing values of each element between PTD and ITD in species investigated revealed minimal statistically significant difference, apart that canine dentin showed a higher Mg concentration in PTD comparing to ITD ($P < 0.05$). However, in all species values of P were higher in PTD than in ITD. When values of a single element were compared between the species again almost no significant difference was found. In PTD Mg concentration was significantly higher in canine dentin in comparison to dentin of the other species ($P < 0.05$) and in ITD Mg concentration was significantly higher in porcine dentin than human dentin ($P < 0.05$).

Table 3. Means $\pm$ standard deviations of element content (wt%) of canine, porcine, equine, and human peritubular dentin (PTD) and intertubular dentin (ITD).

Species	PTD				ITD			
	Ca (wt%)	P (wt%)	Na (wt%)	Mg (wt%)	Ca (wt%)	P (wt%)	Na (wt%)	Mg (wt%)
Canine	61.6 $\pm$ 1.0	35.1 $\pm$ 0.5	0.4 $\pm$ 0.1	2.9 $\pm$ 0.4 ^{aA}	66.5 $\pm$ 0.4	31.8 $\pm$ 0.2	0.3 $\pm$ 0.0	1.4 $\pm$ 0.2 ^{abB}
Porcine	64.2 $\pm$ 4.1	33.7 $\pm$ 3.5	0.4 $\pm$ 0.2	1.7 $\pm$ 0.4 ^b	64.1 $\pm$ 3.6	33.3 $\pm$ 3.1	0.6 $\pm$ 0.2	2.0 $\pm$ 0.4 ^a
Equine	66.3 $\pm$ 1.0	32.1 $\pm$ 1.2	0.3 $\pm$ 0.1	1.7 $\pm$ 0.3 ^b	65.9 $\pm$ 1.4	31.6 $\pm$ 0.9	0.5 $\pm$ 0.1	1.6 $\pm$ 0.1 ^{ab}
Human	67.6 $\pm$ 1.2	31.8 $\pm$ 1.0	0.3 $\pm$ 0.1	1.3 $\pm$ 0.3 ^b	66.6 $\pm$ 0.6	30.7 $\pm$ 0.4	0.4 $\pm$ 0.1	1.2 $\pm$ 0.1 ^b

Values marked by the different lowercase letters in columns and uppercase letters in rows for each element are significantly different ($P < 0.05$).

Table 4. Means $\pm$ standard deviations of Ca/P ratios of canine, porcine, equine, and human peritubular (PTD) and intertubular dentin (ITD).

Species	Ca/P	
	PTD	ITD
Canine	1.8 $\pm$ 0.1	2.1 $\pm$ 0.0
Porcine	1.9 $\pm$ 0.3	1.9 $\pm$ 0.3
Equine	2.1 $\pm$ 0.1	2.1 $\pm$ 0.1
Human	2.1 $\pm$ 0.1	2.2 $\pm$ 0.0

Ca/P ratios are not significantly different between the species ($P > 0.05$) or between the PTD and ITD ($P > 0.05$).

Results of Ca/P ratios are presented as means $\pm$ SD in Table 4. When comparing Ca/P ratios of PTD and ITD between the investigated species, no significant differences were found ($P > 0.05$).

In canine, porcine and equine dentin the Ca/P ratios were equal in PTD and ITD and in human dentin the Ca/P ratio was lower in PTD than in ITD, nevertheless the difference was not significant ($P > 0.05$).

5. Discussion

With the aim of this study to compare microstructural parameters and chemical characteristics of canine, porcine, equine, and human root dentin, some interesting results were obtained. The most striking finding was the great microstructural and chemical resemblance of porcine and human dentin with no significant differences found in DT diameter, density of DT, PTD thickness, PTD or ITD surface areas and all investigated elements in PTD and ITD except for Mg concentration in ITD.

Results obtained on DT density in equine and in human dentin are in agreement with previously reported by Muylle and co-workers [3] and Chu and co-workers [32]. In canine dentin, however, the results of the

present study demonstrated much lower density of DT per square plane compared to previous reports [5,6]. Partially these variations could be explained by different dog breed as well as different tooth types included in the studies [6].

Density of DT varied between species included. Similar results were obtained in other studies [5,6,33]. DT density depends upon a tooth type [4,34] and anatomical location in a tooth [30,32,34,35]. Also, the density of DT per square plane decreases from pulp towards outer tooth surface and from crown towards apical part of a tooth root [30,32]. However, by harvesting all the dentinal samples from the same area of the teeth in this study, this variable was minimized, and the results can be truly ascribed to interspecies variations.

Likewise DT diameters and the size of PTD thickness varied across different species. As also dentin sample anatomical locations in a tooth [10,32] and specimen preparation procedures [6,33,36,37] may influence results notably, all these variables were controlled as much as possible in this study to ascertain that differences/similarities were truly the result of interspecies variations. Ultrasonic bath of 0.27 M EDTA for 5 minutes was reported in dentin samples prepared by Schilke [33] and Robb [6] research groups. In order to remove smear layer effectively EDTA irrigation is recommended, but when utilized EDTA irrigation for longer than one minute it can cause excessive PTD and ITD erosion [36]. Muylle research group [3], on the other hand, etched equine incisor dentin with 20% phosphoric acid. The application of phosphoric acid on dentin surface also results in smear layer removal, but it also causes demineralization of the PTD and ITD [37]. In this study no irrigation agents or acids were used and this may be the reason for smaller DT diameters established than reported in previous studies.

Canine and human DT diameter decreases towards the tooth periphery, while in contrast, PTD thickness increase in this direction [5,32]. Similarly, PTD thickness

increases outwards in equine dentin: from 200–250 nm to 2.5 μm at the DEJ [3]. In human, PTD thickness does not increase in a linear fashion; its increasing is more pronounced from the middle of the bulk of dentin outwards [32]. Differences between mean PTD thickness in human dentin obtained in the study (0.7 μm) and the mean value of PTD thickness of 0.20 μm (0.00–0.50 μm) reported by Chu and co-workers [32] could be, at least partly, explained by different tooth types (i.e. incisor teeth versus molar teeth).

The other interesting feature found was the shape of PTD collar in equine dentin. In all species studied, except equine, almost circular PTD outline surrounded DT. Equine dentin, however, had a distinct oval shape of PTD outline, which appeared to lengthen in the coronal direction. This phenomenon has been reported before with disagreement whether PTD asymmetry increases or decreases towards the DEJ [10,38].

In all species investigated ITD represented the major proportion of dentin, yet similar ITD surface area ratios were observed between porcine and human dentin only. In human ITD proportion raises from 12% near pulp to 96% at DEJ [39]. Comparing ITD surface area proportions between the species of this study revealed the largest ITD surface area in canine and the smallest in equine dentin. This is on the contrary to the results of a previous study which demonstrated canine teeth to have smaller ITD surface area compared to human [6], and could again be explained by the different tooth types included (molar and canine teeth in previous study and incisor teeth in the present study).

Some information on mineralization degree was already obtained from the SEM-BSE mode. Differences in intensity and contrast on SEM-BSE image reflect the number of electrons emitted from the target and proportions of atoms with higher atomic number [40]. Atoms with higher atomic number appear brighter and those with lower atomic number darker. In dentin brighter PTD indicates mainly higher concentration of Ca and P ions and thus a higher degree of mineralization comparing to darker ITD [14,18,25]. As expected, EDXS measurements of PTD and ITD confirmed that the main dentin constitutive elements were Ca and P. Interestingly, in all species investigated higher amounts of P were assigned in PTD compared to ITD. Yet, except for Mg in canine dentin, statistical analysis did not confirm any significant differences between PTD and ITD in porcine, equine or human dentin for any element analysed.

Further on, except in porcine dentin, a higher Mg concentration was confirmed in PTD compared to ITD. Similar results were obtained in some other studies [15,18,24]. The overall Mg concentrations reported for canine, equine, and human dentin are 1.545% [41],

0.63% in PTD and 0.56% in ITD [25], and 0.858% [42], respectively.

Essentially, Ca/P ratio indicates crystallization of HA crystals [24]. Results of the study revealed no significant difference between the mean Ca/P ratios of PTD and ITD of all species. Moreover, the Ca/P ratios in PTD and in ITD compared between the species were statistically insignificant. Similar results were reported in human dentin by Yoshiyama and co-workers [43] and in equine dentin by Kodaka's research group [25].

Microstructure characteristics affect significantly the strength of the adhesive bonds created between restorative materials and dentin [30]. Due to lower density of DT [7], DT diameter declining in pulpal direction [7,8] and thicker PTD [7] the use of bovine dentin as a model for dental research is questioned. On the other hand, the great similarity in microstructural and chemical composition of porcine and human dentin points to suitability of porcine dentin use in human dental research. Indeed, no significant difference is found between the bond strength of human and porcine dentin to a number of resin cements [43], and a similar outcome is reported after etching of either of dentin, porcine and human [44].

In conclusion, observations of this study defined microstructural variations of canine, porcine, equine and human dentin. However, no differences were found in all microstructural parameters and majority of the elements investigated between porcine and human dentin. Porcine dentin can therefore be considered a suitable experimental substrate for dental restorative materials testing studies.

Acknowledgments

The authors would like to sincerely thank to Prof. Ladislav Kosec and Dr. Aleš Nagode for their welcome advice and help in setting up the study, and to Mrs. Nika Breskvar for her expert technical assistance. We would also like to sincerely thank Dr. Ana Nemec for proofreading of the manuscript.

The study was supported in part by the Slovenian Research Agency grant (J3-2270-0406).

Conflict of interests

The authors declare they have no conflict of interests.

Ethical approval

Not required.

References

- [1] Rasmussen S.T., Patchin R.E., Fracture properties of human enamel and dentin in an aqueous environment, *J. Dent. Res.*, 1984, 63(12), 1362-1368
- [2] Nanci A., Ten Cate's Oral Histology: Development, Structure and Function, 6th ed., St. Louis, Mosby, 2003
- [3] Muylle S., Simoens P., Lauwers H., A study of the ultrastructure and staining characteristics of the »dental star« of equine incisors, *Equine Vet. J.*, 2002, 34(3), 230-234
- [4] Garberoglio R., Brännström M., Scanning electron microscopic investigation of human dentinal tubules, *Arch. Oral Biol.*, 1976, 21(6), 355-362
- [5] 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
- [6] Robb L., Marx J., Steenkamp G., van Heerden W.F., Pretorius E., Boy S.C., Scanning electron microscopic study of the dentinal tubules in dog canine teeth, *J. Vet. Dent.*, 2007, 24(2), 86-89
- [7] Dutra-Correa M., Anauate-Netto C., Arana-Chavez V.E., Density and diameter of dentinal tubules in etched and non-etched bovine dentin examined by scanning electron microscopy, *Arch. Oral Biol.*, 2007, 52, 850-855
- [8] Lopes M.B., Sinhoreti M.A., Gonini Júnior A., Consani S., McCabe J.F., Comparative study of tubular diameter and quantity for human and bovine dentin at different depths, *Braz. Dent. J.*, 2009, 20(4), 279-283
- [9] Kinney J.H., Pople J.A., Marshall G.W., Marshall S.J., Collagen orientation and crystallite size in human dentin: a small angle X-ray scattering study, *Calcif. Tissue Int.*, 2001, 69(1), 31-37
- [10] Kilic S., Dixon P., Kempson S., A light microscopic and ultrastructural examination of calcified dental tissues of horses: 3. Dentin, *Equine Vet. J.*, 1997, 29, 206-212
- [11] Dai X.F., Tencate A.R., Limeback H., The extent and distribution of intratubular collagen fibrils in human dentin, *Arch. Oral Biol.*, 1991, 36(10), 775-778
- [12] Magne D., Guicheux J., Weiss P., Pilet P., Daculsi G., Fourier transform infrared microspectroscopic investigation of the organic and mineral constituents of peritubular dentin: a horse study, *Calcif. Tissue Int.*, 2002, 71(2), 179-185
- [13] Qin Q.H., Swain M.V., A micro-mechanics mode of dentin mechanical properties, *Biomaterials*, 2004, 25(20), 5081-5090
- [14] Xu C., Wang Y., Chemical composition and structure of peritubular and intertubular human dentin revisited, *Arch. Oral Biol.*, 2012, 57(4), 383-391
- [15] Gotliv B.A., Robach J.S., Veis A., The composition and structure of bovine peritubular dentin: mapping by time of flight secondary ion mass spectroscopy, *J. Struct. Biol.*, 2006, 156(2), 320-333
- [16] Gotliv B.A., Veis A., Peritubular dentin, a vertebrate apatitic mineralized tissue without collagen: role of a phospholipid-proteolipid complex, *Calcif. Tissue Int.*, 2007, 81(3), 191-205
- [17] Habelitz S., Rodriguez B.J., Marshall S.J., Marshall G.W., Kalinin S.V., Gruverman A., Peritubular dentin lacks piezoelectricity, *J. Dent. Res.*, 2007, 86(9), 908-911
- [18] Gotliv B.A., Veis A., The composition of bovine peritubular dentin: matching TOF-SIMS, scanning electron microscopy and biochemical component distributions. New light on peritubular dentin function, *Cells Tissues Organs*, 2009, 189(1-4), 12-19
- [19] Sögaard-Pedersen B., Boye H., Matthiessen M.E., Scanning electron microscope observations on collagen fibers in human dentin and pulp, *Scand. J. Dent.*, 1990, 98(2), 89-95
- [20] Linde A., Goldberg M., Dentinogenesis, *Crit. Rev. Oral Biol. Med.*, 1993, 4(5), 679-728
- [21] Muylle S., Simoens P., Lauwers H., Tubular contents of equine dentin: A scanning electron microscopic study, *J. Vet. Med.*, 2000, 47, 321-330
- [22] Wiesmann H.P., Meyer U., Plate U., Höhling H.J., Aspects of collagen mineralization in hard tissue formation, *Int. Rev. Cytol.*, 2005, 242, 121-156
- [23] Hong H., Tie L., Jian T., The crystal characteristics of enamel and dentin by XRD method, *J. Wuhan. Univ. Technol. Mater. Sci. Ed.*, 2006, 21(1), 9-12
- [24] Arnold W.H., Konopka S., Gaengler P., Qualitative and quantitative assessment of intertubular dentin formation in human natural carious lesions, *Calcif. Tissue Int.*, 2001, 69, 268-273
- [25] Kodaka T., Debari K., Yamada M., Physico-chemical and morphological studies of horse dentin, *J. Electron. Microsc.*, 1991, 40(6), 385-391
- [26] Sakoolnamarka R., Burrow M.F., Swain M., Tyas M.J., Microhardness and Ca:P ratio of carious and Carisolv treated caries-affected dentin using an ultra-micro-indentation system and energy dispersive analysis of x-rays-a pilot study, *Aust. Dent. J.*, 2005, 50(4), 246-250
- [27] Lakomaa E.L., Rytömaa I., Mineral composition of enamel and dentin of primary and permanent teeth in Finland, *Scand. J. Dent. Res.*, 1977, 85(2), 89-95

- [28] Coradazzi J.L., Silva C.M., Pereira J.C., Francischone C.E., Shear bond strength of an adhesive system in human, bovine and swinish teeth, *Rev. Fac. Odontol. Bauru.*, 1998, 6(4), 29-33
- [29] Krifka S., Börzsönyi A., Koch A., Hiller K.A., Schmalz G., Friedl K.H., Bond strength of adhesive systems to dentin and enamel—human vs. bovine primary teeth in vitro, *Dent. Mater.*, 2008, 24(7), 888-894
- [30] Marshall G.W. Jr., Marshall S.J., Kinney J.H., Balooch M., The dentin substrate: structure and properties related to bonding, *J. Dent.*, 1997, 25(6), 441-458
- [31] Inoue T., Saito M., Yamamoto M., Debari K., Kou K., Nishimura F., et al., Comparison of nanohardness between coronal and radicular intertubular dentin, *Dent. Mater. J.*, 2009, 28(3), 295-300
- [32] Chu C.Y., Kuo T.C., Chang S.F., Shyu Y.C., Lin C.P., Comparison of the microstructure of crown and root dentin by a scanning electron microscopic study, *J. Dent. Sci.*, 2010, 5(1), 14-20
- [33] Schilke R., Lisson J.A., Bauss O., Geurtsen W., Comparison of the number and diameter of dentinal tubules in human and bovine dentin by scanning electron microscopic investigation, *Arch. Oral Biol.*, 2000, 45(5), 355-361
- [34] Schellenberg U., Krey G., Bosshardt D., Nair P.N., Numerical density of dentinal tubules at the pulpal wall of human permanent premolars and third molars, *J. Endod.*, 1992, 18(3), 104-109
- [35] Ferrari M., Mannocci F., Vichi A., Cagidiaco M.C., Mjör I.A., Bonding to root canal: structural characteristics of the substrate, *Am. J. Dent.*, 2000, 13(5), 255-260
- [36] Calt S., Serper A., Time-dependent effects of EDTA on dentin structures, *J. Endod.*, 2002, 28(1), 17-19
- [37] Van Meerbeeck B., Inokoshi S., Braem M., Lambrechts P., Vanherle G., Morphological aspects of the resin-dentin interdiffusion zone with different dentin adhesive systems, *J. Dent. Res.*, 1992, 71, 1530-1540
- [38] Muylle S., Simoens P., Lauwers H., The distribution of intratubular dentin in equine incisors: a scanning electron microscopic study, *Equine Vet. J.*, 2001, 33(1), 65-69
- [39] Pashley D.H., Dynamics of the pulpo-dentin complex, *Crit. Rev. Oral Biol. Med.*, 1996, 7(2), 104-133
- [40] Lloyd G.E., Atomic number and crystallographic contrast images with the SEM: a review of back-scattered electron techniques, *Mineral. Mag.*, 1987, 51, 3-19
- [41] Murray M.M., The chemical composition of teeth: The calcium, magnesium and phosphorus contents of the teeth of different animals. A brief consideration of the mechanism of calcification, *Biochem. J.*, 1936, 30(9), 1567-1571
- [42] Ballal N.V., Mala K., Bhat K.S., Evaluation of decalcifying effect of maleic acid and EDTA on root canal dentin using energy dispersive spectrometer, *Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod.*, 2011, 112(2), 78-84
- [43] Yoshiyama M., Noiri Y., Ozaki K., Uchida A., Ishikawa Y., Ishida H., Transmission electron microscopic characterization of hypersensitive human radicular dentin, *J. Dent. Res.*, 1990, 69, 1293-1297
- [44] Lopes F.M., Markarian R.A., Sendyk C.L., Duarte C.P., Arana-Chavez V.E., Swine teeth as potential substitutes for in vitro studies in tooth adhesion: a SEM observation, *Arch. Oral Biol.*, 2006, 51(7), 548-551
- [45] Mannocci F., Pilecki P., Bertelli E., Watson T.F., Density of dentinal tubules affects the tensile strength of root dentin, *Dent. Mater.*, 2004, 20(3), 293-296
- [46] Ari H., Erdemir A., Effects of endodontic irrigation solutions on mineral content of root canal dentin using ICP-AES technique, *J. Endod.*, 2005, 31(3), 187-189