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Artículo Original

Analysis of the distribution of microhardness, mineral, and organic content of human, bovine, and ovine teeth

Análisis de la distribución de microdureza, contenido mineral y orgánico de dientes humanos, bovinos y ovinos

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Abstract

Objetive. Human teeth have been commonly used for in vitro and in situ studies. Currently, other animals' teeth have been purposed for dental research to overcome human teeth' problematic availability. This study aimed to investigate the enamel and dentin from human, bovine, and ovine teeth concerning the microhardness, organic, and inorganic contents via micro-Raman spectroscopy. **Methods.** Human, bovine, and ovine teeth were divided according to their type and age into seven groups: Ovine; Bovine-12 months; Bovine-24 months; Bovine-36 months; Bovine-48 months; Bovine-+60 months; Human (control). The enamel's microhardness (superficial and deep) and dentin (superficial, middle, and deep) were analyzed. The calcium/phosphate ratio and amide contents were determined by micro-Raman spectroscopy. **Results.** Overall, the microhardness of human enamel was superior to the other species. Dentin's microhardness was similar among groups. Ovine group showed lower values of calcium/phosphate ratio than human. Amide content was similar between bovine and human. The microhardness and calcium/phosphate ratio of enamel and dentin, respectively, decreased as the age of bovine teeth increased. **Conclusions.** Researchers must be aware and take into consideration the differences of ovine and bovine enamel compared to human enamel. Other alternatives that are more similar to the microhardness of human enamel should be sought. Bovine teeth of 12 and 24 months are suitable substitutes for dentin of human teeth. Researchers must also be aware of the age of the animals and specify it in the studies.

Keywords: Spectrum Analysis; Raman; Hardness; Collagen Type I; Collagen Type III; Models; Animal (MESH NLM).

Resumen

Objetivo. Los dientes humanos se han utilizado comúnmente para estudios in vitro e in situ. Actualmente, los dientes de otros animales se han destinado a la investigación dental para superar la disponibilidad problemática de los dientes humanos. Este estudio tuvo como objetivo investigar el esmalte y la dentina de los dientes humanos, bovinos y ovinos en relación con la microdureza y los contenidos orgánicos e inorgánicos a través de la espectroscopia micro-Raman. **Métodos.** Los dientes humanos, bovinos y ovinos se dividieron según su tipo y edad en siete grupos: Ovinos; Bovino-12

meses; Bovino-24 meses; Bovino-36 meses; Bovino-48 meses; Bovino-+60 meses; Humano (control). Se analizó la microdureza del esmalte (superficial y profunda) y de la dentina (superficial, media y profunda). La relación calcio/fosfato y los contenidos de amida se determinaron mediante espectroscopía micro-Raman. **Resultados.** En general, la microdureza del esmalte humano fue superior a la de otras especies. La microdureza de la dentina fue similar entre los grupos. El grupo ovino mostró valores más bajos de la relación calcio/fosfato que el humano. El contenido de amida fue similar entre bovinos y humanos. La microdureza y la relación calcio/fosfato del esmalte y la dentina, respectivamente, disminuyeron a medida que aumentaba la edad de los dientes bovinos. **Conclusiones.** El esmalte de los dientes ovinos y bovinos no es un sustituto adecuado del de los dientes humanos. Se deben buscar otras alternativas que sean similares a la microdureza del esmalte humano. Sin embargo, los dientes bovinos de 12 y 24 meses son sustitutos adecuados de la dentina de los dientes humanos. Los investigadores deben conocer la edad de los animales y especificarla en los estudios.

Palabras clave: Espectrometría Raman; Dureza; Colágeno Tipo I; Colágeno Tipo III; Modelos Animales (DeCS BIREME).

Introduction

Human teeth are commonly used for *in vitro* and *in situ* studies. Currently, the use of human teeth has been hampered due to their shortage. The progression of preventive dentistry and the development of novel dental materials have decreasing tooth extractions. Furthermore, Research Ethics Committees impose strict controls for using human source samples. Other animals' teeth have been purposed for dental research to overcome human teeth' problematic availability [1-3]. As an advantage, animal teeth are easier standardized regarding their age, degree of sclerosis, wear, caries lesion, and fluoride exposition [4-6].

Bovines' teeth are the most used due to their histomorphology similarity to humans' teeth [7]. The phosphate/carbonate ratio during the de- and remineralization processes of dentin [8], number of dentinal tubules [9], and dentin permeability [10] are similar between human and bovine teeth. Besides bovines' teeth, ovines' teeth have also been increasingly used because of their similarity with human teeth, easy handle, and wide availability [1,3,11].

Although bovine and ovine teeth offer some advantages for dental research, there are different physical and chemical compositions compared to humans that must be considered. Knowledge about the divergences among teeth types is essential for properly interpreting data. Contradictory outcomes of caries lesion depth, microhardness, microleakage, and adhesive bond strength are reported when dentin or enamel of bovines and ovines are compared to human teeth [1,12,13].

Dental hardness and essential chemical composition, such as collagen content, are important factors to be analyzed among different types of teeth. The collagen content and the mineral amount of dental tissues may impact the outcomes of adhesives microtensile bond strength (μ-TBS), which is used to assist in predicting restorative failures *in vivo* [14]. Therefore, the evaluation of the organic matrix content, inorganic compounds, and mechanical properties of different types of teeth could assist in explaining variations from *in vitro* outcomes. This study aimed to investigate the enamel and dentin from human, bovine, and ovine teeth concerning the Knoop hardness, organic and inorganic contents.

Methods

Experimental design

In this *in vitro* study, three species were analyzed: ovine, bovine, and human. The dental tissues, enamel and dentin, were investigated regarding their microhardness and chemistry (phosphate/carbonate ratio, collagen I, and collagen III) via Raman spectroscopy. Figure 1 displays a flowchart of the study. The following groups were analyzed (n = 5):

- Ovine – 5 months;
- Bovine – 12 months;
- Bovine – 24 months;
- Bovine – 36 months;
- Bovine – 48 months;
- Bovine – +60 months;
- Human (control group) – patients with 18 to 60 years old.

Samples preparation

The human teeth were extracted due to periodontal diseases and obtained from a tooth bank (protocol nº 003-2010, School of Dentistry, Federal University of Rio Grande do Sul, Brazil). Informed consent was obtained for experimentation with human subjects, and privacy rights were respected. The bovine and ovine teeth were obtained from a slaughterhouse via donation.

External debris was removed with periodontal scalers, and the teeth were stored with distilled water at 4 °C for no more than three months. The teeth were sectioned below the cementum-enamel junction to remove the roots. A low-speed diamond disc was used to section

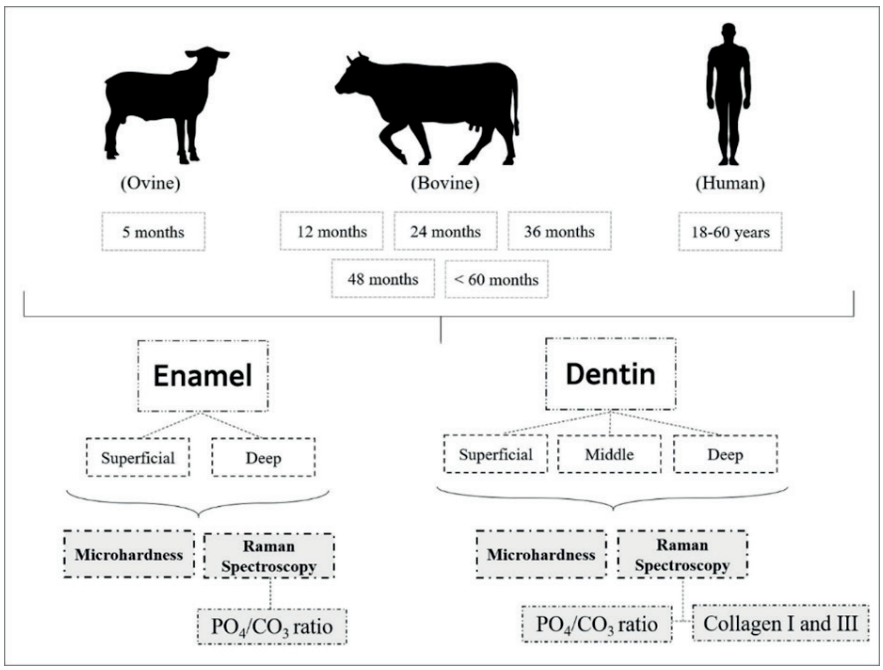

Figure 1. Flowchart of the present study. Three species were analyzed: ovine, bovine, human. The dental tissues, enamel, and dentin, were investigated via microhardness assessment and Raman spectroscopy.

each crown in two halves in the sagittal axis (mesial and distal regions) and three thirds in the axial axis (incisal, middle, and cervical regions).

The samples were embedded in self-curing acrylic resin (São Paulo, SP, Brazil) and polished (Model 3v, Arotec, Cotia, SP, Brazil) with 600, 1200, and 2000-grit silicon carbide (SiC) abrasive papers under coolant water and felt disc with alumina suspension (Alumina, 0.5 µm, Arotec, Cotia, SP, Brazil) for 2 min each step. The samples were cleaned with an ultrasonic bath for 3 min using distilled water.

Microhardness evaluation

The mesial-half of the specimens were subjected to a microhardness test in two sub-regions of enamel (superficial and deep) and three sub-regions of dentin (superficial, middle, and deep) of each dental third (incisal, middle, and cervical). The mean value of Knoop hardness (KHN) was obtained after three indentations (50 g/10 s) on each sub-region of enamel and nine indentations (25 g/10 s) on each sub-region of dentin, 100 µm apart from each other. The indentations were assessed using a digital microhardness tester (HMV 2, Shimadzu, Tokyo, Japan). The calculation of the hardness value was carried out by equation 1:

Equation 1:

$$\text{Knoop hardness} = \frac{14228.\,c}{d^2}$$

**14,228 is a constant, c is the load in grams, and d is the largest diagonal length in micrometers.*

The results were processed to obtain digitalized images (Figure 2 – Figure 3) of the microhardness values using Sigma Plot version 12.0 for Windows (Systat Software Inc, San Jose, CA, USA).

Evaluation of phosphate/carbonate ratio and amide content

The distal-half of the specimens were subjected to vibrational analysis by micro-Raman spectroscopy using Senterra equipment (Bruker Optics GmbH, Ettlingen, Baden-Württemberg, Germany) with three coadditions for 5 s of irradiation using a 100 mW diode laser with 785-nm wavelength and 50 x 1000 µm aperture size. The spectra were obtained with a resolution of ~3.5 cm^{-1} from 440 cm^{-1} to 1800 cm^{-1}. The analysis was performed using the same indentation mapping of microhardness evaluation. The carbonate (CO_3^{2-} v_1), phosphate (PO_4^{3-} v_1), and pyrrolidine rings of proline and hydroxyproline (type I and III collagen) were attributed to the peaks 1071, 960, and 1440-1474 cm^{-1} [15]. The phosphate/carbonate ratio and amide averages were processed to obtain digitalized images using Sigma Plot (Systat Software Inc, San Jose, CA, USA).

Statistical Analysis

The statistical analysis was performed in Sigma Plot Software (Systat Software Inc, San Jose, CA, USA). The normality of data was tested using the Kolmogorov-Smirnov test ($p > 0.05$). The statistical analyses were performed using One-way ANOVA and Tukey's post hoc at the 0.05 level of significance.

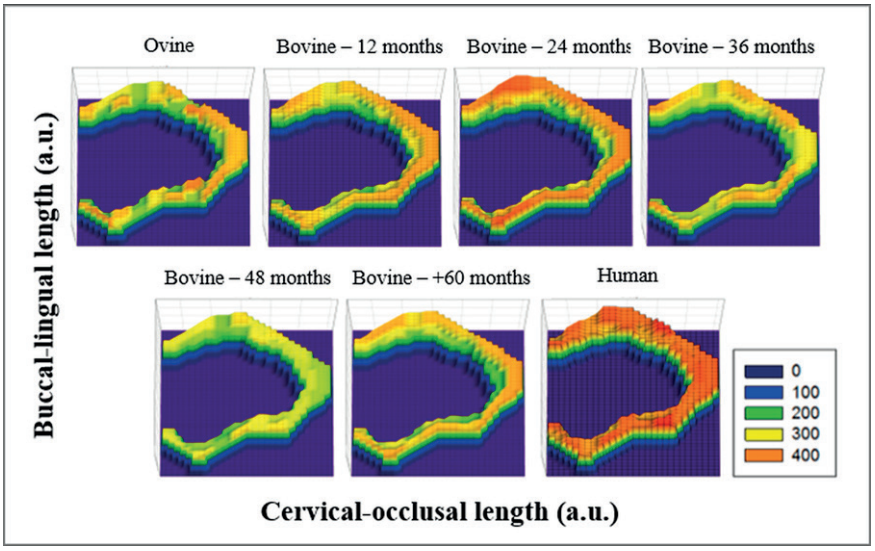

Figure 2. Representation of the Knoop hardness values of the analyzed enamel (superficial and deep) from buccal to lingual region and from cervical to occlusal region, considering the halve of teeth. From purple (0) to orange (400), the Knoop hardness values increase.

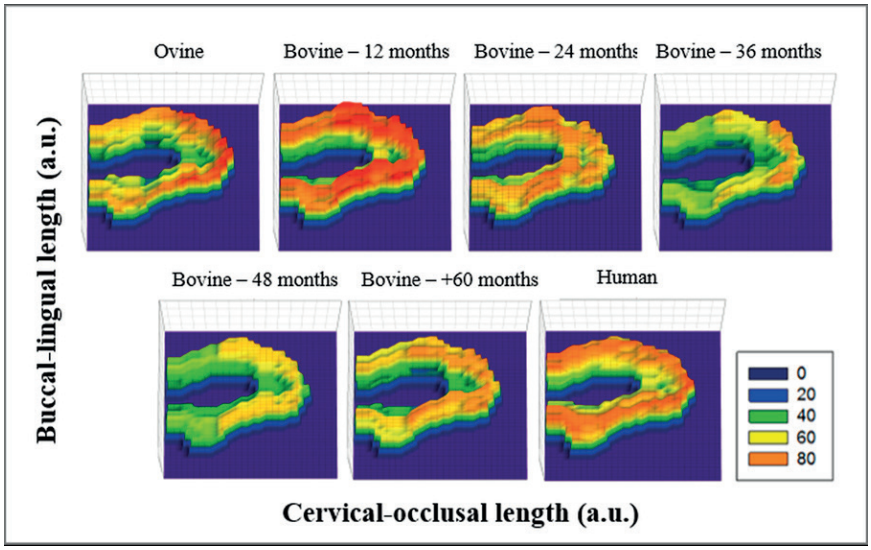

Figure 3. Representation of the Knoop hardness values of the analyzed dentin (superficial, middle, and deep) from buccal to lingual region and from cervical to occlusal region, considering the halve of teeth. From purple (0) to orange (80), the Knoop hardness values increase.

Results

The results for microhardness of each third of enamel are presented in Table 1 and Figure 2. Higher microhardness was observed for humans' enamel. There were statistically significant differences for human enamel (control group) compared to all other groups ($p < 0.05$), except for Bovine, 24 h - months, in incisal and middle thirds at superficial and deep sub-regions, respectively ($p > 0.05$). In the deep sub-region of the cervical and incisal thirds and the superficial sub-region of the middle third, all animal groups were different compared to the control group ($p < 0.05$). A significant difference was found at the superficial sub-region of the cervical third between Bovine – 36 months (267.93 ± 4.02) and the control group (320.00 ± 24.06) ($p < 0.05$).

Table 2 and Figure 3 show the results of microhardness for the different thirds sub-regions of dentin. There were statistically significant differences in the incisal third within the superficial sub-region among human dentin (control group), Bovine – 36 months, and Bovine – 48 months ($p < 0.05$), and in the middle sub-region between human dentin (58.59 ± 5.25) and Bovine – 12 months (71.83 ± 2.06). In the middle third, significant difference was shown within the superficial sub-region between human dentin (63.84 ± 6.19) and Bovine – 36 months (54.65 ± 1.78) groups. Yet in the the middle third, significant difference was observed within middle sub-region between human dentin and all animal groups except Bovine – 24 months and Bovine – +60 months groups, and within deep sub-region between

Table 1. Knoop hardness values of the different thirds of enamel among groups.

Group	INCISAL		MEDIUM		CERVICAL	
	Superficial	Deep	Superficial	Deep	Superficial	Deep
Ovine	282.91±30.00^{CD}	291.66±40.45^{B}	272.13±24.40^{B}	253.26±23.85^{B}	297.41±30.18^{ABC}	272.91±29.76^{B}
Bovine – 12 months	308.06±3.75^{BC}	269.53±4.93^{BC}	283.53±14.25^{B}	249.13±12.98^{B}	292.33±12.06^{BC}	272.26±22.40^{B}
Bovine – 24 months	320.00±8.95^{AB}	256.44±18.22^{BC}	329.58±18.63^{AB}	258.41±23.92^{A}	329.58±14.63^{A}	258.41±23.92^{B}
Bovine – 36 months	287.33±13.52^{BCD}	256.83±9.95^{BC}	281.16±25.26^{B}	265.83±5.49^{B}	294.73±16.59^{ABC}	250.66±1.33^{B}
Bovine – 48 months	263.08±12.25^{CD}	259.33±2.75^{BC}	263.50±16.79^{B}	250.50±19.21^{B}	267.93±4.02^{C}	252.53±7.33^{B}
Bovine – +60 months	296.73±12.46^{BCD}	232.93±4.97^{C}	288.20±9.75^{B}	250.26±11.98^{B}	298.53±7.98^{ABC}	263.13±7.14^{B}
Human	350.83±22.48^{A}	341.00±19.27^{A}	342.66±8.85^{A}	310.20±29.33^{A}	320.00±24.06^{AB}	332.08±19.69^{A}

Different letters mean statistical differences for the same column.

Table 2. Knoop hardness values of the different thirds of dentin among groups.

Group	INCISAL			MEDIUM			CERVICAL		
	Superficial	Middle	Deep	Superficial	Middle	Deep	Superficial	Middle	Deep
Ovine	71.31±4.53^{A}	57.51±3.89^{B}	42.19±5.51^{A}	65.31±4.58^{AB}	54.62±4.43^{C}	41.73±7.85^{BC}	58.91±4.03^{AB}	55.95±2.39^{B}	45.42±8.38^{BC}
Bovine – 12 months	72.25±3.11^{A}	71.83±2.06^{A}	55.61±6.33^{A}	70.97±3.78^{A}	74.57±2.20^{A}	49.71±5.31^{AB}	3.38±3.89^{A}	67.64±2.42^{A}	43.23±3.55^{BCD}
Bovine – 24 months	57.91±3.50^{BC}	61.68±3.17^{B}	52.37±5.65^{A}	61.23±6.48^{BCD}	65.4±2.81^{B}	55.01±4.72^{AB}	61.23±6.48^{AB}	5.4±2.81^{A}	55.00±4.72^{A}
Bovine – 36 months	58.82±6.03^{C}	57.84±4.99^{B}	43.86±4.41^{A}	54.66±1.78^{D}	51.17±1.46^{C}	35.14±2.57^{C}	48.96±1.53^{C}	48.46±1.36^{C}	35.47±1.78^{D}
Bovine – 48 months	53.27±4.76^{C}	54.97±2.69^{B}	42.36±4.17^{A}	56.67±1.13^{CD}	54.96±1.78^{C}	42.77±3.00^{BC}	7.16±0.74^{C}	48.78±1.09^{C}	40.48±2.75^{CD}
Bovine – +60 months	61.27±3.16^{BC}	62.19±2.57^{B}	43.46±5.56^{A}	57.09±1.25^{BCD}	63.16±1.97^{B}	35.64±4.92^{C}	52.63±1.02^{BC}	57.24±1.33^{B}	39.49±2.46^{CD}
Human	66.06±5.35^{AB}	58.59±5.25^{B}	51.06±4.26^{A}	63.84±6.19^{ABC}	61.57±4.23^{B}	52.59±2.52^{AB}	63.63±5.78^{A}	65.47±2.60^{A}	49.78±2.90^{AB}

Different letters mean statistical differences for the same column.

human dentin (52.59 ± 2.52) and Bovine – 36 months (35.14 ± 2.57) and Bovine – +60 months (35.64 ± 4.92). In the cervical third, higher values were found for human compared to Bovine – 36 months, Bovine – 48 months, and Bovine – +60 months at the superficial and deep sub-regions, and to Ovine, Bovine – 36 months, Bovine – 48 months, and Bovine – +60 months at the middle sub-region ($p < 0.05$).

Table 3 and Figure 4 present the results of PO_4/CO_3 ratio within different thirds of enamel. There was a statistically significant difference in the superficial sub-region of incisal, middle, and cervical thirds and deep sub-region of incisal and cervical thirds between the human enaml and Bovine – 12 months ($p < 0.05$). Table 4 and Figure 5 present the results of PO_4/CO_3 ratio within different thirds of dentin. All bovine groups demonstrated a similar ratio of PO_4/CO_3 compared to human dentin, without a statistical significant difference ($p > 0.05$). G1 showed lower PO_4/CO_3 ratio values than Human in superficial and deep sub-regions of the incisal and middle third and in the deep sub-region of the cervical third ($p < 0.05$).

Table 5 shows the quantity of collagen represented by the amide bond within the different thirds of dentin. A significant increase ($p < 0.05$) of collagen was found comparing human (74.25 ± 23.47) to ovine (22.63 ± 20.9), G2 (26.35 ± 18.58), and Bovine – +60 months (19.88 ± 18.42) groups in the superficial sub-region of the incisal third. All groups of animals had lower values ($p < 0.05$) of collagen compared to Human. In the middle sub-region of the middle third, a significant increase of collagen was found in the G7 (52.81 ± 13.18) compared to Bovine – 24 months (18.30 ± 16.14) and Bovine – 36 months (20.78 ± 15.17). In the superficial sub-region of the cervical third, a higher value of collagen was found in the Human group (95.14 ± 75.10) compared to all other groups ($p < 0.05$), except for Bovine – +60 months (37.85 ± 14.21) ($p > 0.05$).

Discussion

Despite the similar elemental composition of hydroxyapatite and collagen of animals and human dental tissues, the structure and proportion of components, prisms, crystals, and tubules are different. These variations may alter the outcome interpretation of dental research. This study verified different mineral and organic concentrations and microhardness among ovine, bovine, and human teeth. Up to 24 months of age, bovine dentin showed similar microhardness, PO_4/CO_3, and collagen values compared to human dentin. Moreover, microhardness and PO_4/CO_3 ratio from enamel and dentin of bovine teeth altered significantly with increasing ages, especially over 24 months.

Bovine enamel is widely used in dental research, mainly in orthodontics and restorative dentistry, and resin composites [16].The highly compacted prisms of human

Table 3. PO_4/CO_3 ratio of the different thirds of enamel among groups.

Group	INCISAL		MEDIUM		CERVICAL	
	Superficial	Deep	Superficial	Deep	Superficial	Deep
Ovine	0.037±0.001^{B}	0.045±0.001^{B}	0.045±0.01^{AB}	0.048±0.001^{A}	0.046±0.012^{AB}	0.051±0.012^{AB}
Bovine – 12 months	0.067±0.008^{A}	0.066±0.005^{A}	0.059±0.01^{A}	0.061±0.004^{A}	0.058±0.009^{A}	0.064±0.007^{A}
Bovine – 24 months	0.045±0.01^{B}	0.057±0.009^{AB}	0.040±0.004^{B}	0.057±0.007^{A}	0.044±0.003^{AB}	0.048±0.021^{AB}
Bovine – 36 months	0.032±0.017^{B}	0.040±0.02^{B}	0.048±0.019^{AB}	0.065±0.027^{A}	0.042±0.004^{AB}	0.050±0.005^{AB}
Bovine – 48 months	0.039±0.001^{B}	0.056±0.004^{AB}	0.038±0.003^{B}	0.058±0.005^{A}	0.049±0.014^{AB}	0.052±0.008^{AB}
Bovine – +60 months	0.048±0.007^{B}	0.049±0.008^{AB}	0.039±0.004^{B}	0.050±0.003^{A}	0.041±0.001^{AB}	0.055±0.005^{AB}
Human	0.032±0.004^{B}	0.045±0.002^{B}	0.037±0.003^{B}	0.048±0.005^{A}	0.030±0.018^{B}	0.035±0.018^{B}

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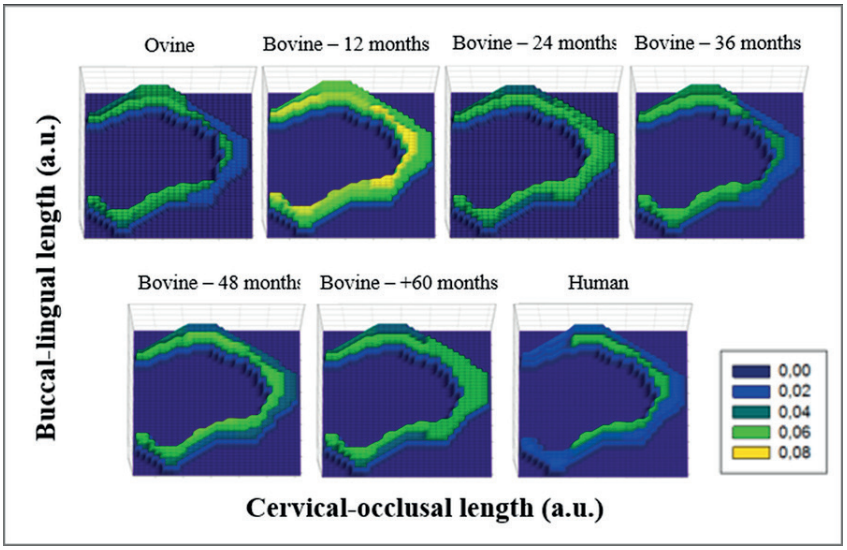

Figure 4. Images of Raman spectroscopy reflecting the analyzed areas of enamel from buccal to lingual region and from cervical to occlusal region, considering the halve of teeth. The colors are related to the intensity (quantity) of PO_4/CO_3 ratio for the different areas (superficial and deep) of enamel among groups. From purple (0,00) to yellow (0,08) the PO4/CO3 ratio increases.

Table 4. PO_4/CO_3 ratio of the different thirds of dentin among group.

Group	INCISAL			MEDIUM			CERVICAL		
	Superficial	Middle	Deep	Superficial	Middle	Deep	Superficial	Middle	Deep
Ovine	0.041±0.012^{C}	0.030±0.025^{B}	0.025±0.021^{B}	0.046±0.012^{B}	0.026±0.018^{B}	0.028±0.017^{B}	0.059±0.022^{A}	0.055±0.038^{A}	0.040±0.023^{B}
Bovine – 12 months	0.079±0.006^{A}	0.076±0.004^{A}	0.067±0.010^{A}	0.075±0.001^{A}	0.073±0.004^{A}	0.062±0.005^{A}	0.073±0.002^{A}	0.067±0.005^{A}	0.056±0.004^{AB}
Bovine – 24 months	0.067±0.007^{AB}	0.075±0.003^{A}	0.074±0.003^{A}	0.075±0.001^{A}	0.070±0.002^{A}	0.067±0.004^{A}	0.064±0.013^{A}	0.071±0.002^{A}	0.062±0.004^{A}
Bovine – 36 months	0.056±0.019^{B}	0.063±0.006^{AB}	0.057±0.015^{A}	0.070±0.002^{A}	0.063±0.003^{A}	0.063±0.005^{A}	0.070±0.002^{A}	0.072±0.008^{A}	0.062±0.006^{A}
Bovine – 48 months	0.071±0.004^{AB}	0.071±0.013^{A}	0.066±0.002^{A}	0.073±0.007^{A}	0.074±0.012^{A}	0.069±0.009^{A}	0.068±0.011^{A}	0.072±0.007^{A}	0.065±0.006^{A}
Bovine – +60 months	0.064±0.005^{AB}	0.073±0.006^{A}	0.063±0.005^{A}	0.066±0.01^{A}	0.068±0.011^{A}	0.059±0.006^{A}	0.073±0.004^{A}	0.073±0.007^{A}	0.064±0.006^{A}
Human	0.066±0.005^{AB}	0.065±0.012^{A}	0.069±0.006^{A}	0.074±0.005^{A}	0.072±0.006^{A}	0.074±0.005^{A}	0.063±0.004^{A}	0.065±0.006^{A}	0.067±0.005^{A}

Different letters mean statistical difference for the same column.

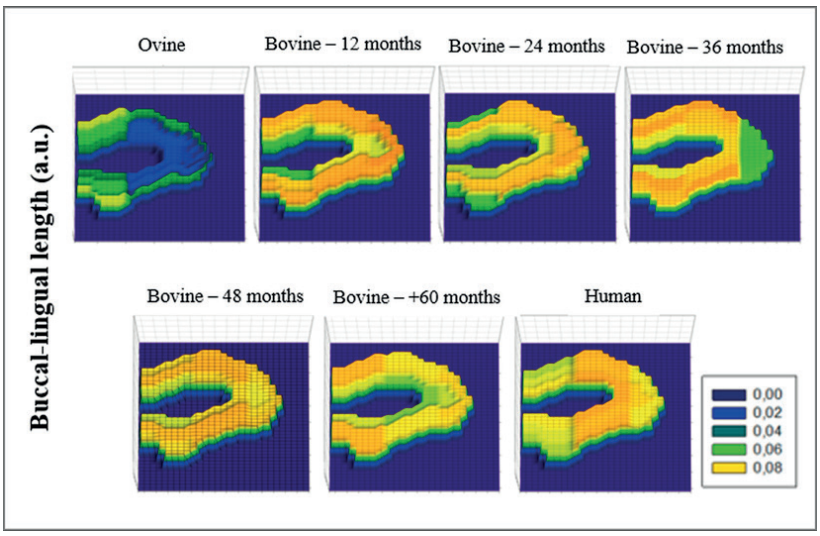

Figure 4. Images of Raman spectroscopy reflecting the analyzed areas of dentin from buccal to lingual region and from cervical to occlusal region, considering the halve of teeth. The colors are related to the intensity (quantity) of PO_4/CO_3 ratio for the different thirds (superficial, middle, and deep) of dentin among groups. From purple (0,00) to yellow (0,08) the PO_4/CO_3 ratio increases.

Table 5. Collagen content in the different thirds of dentin.

Group	INCISAL			MEDIUM			CERVICAL		
	Superficial	Middle	Deep	Superficial	Middle	Deep	Superficial	Middle	Deep
Ovine	22.63±20.90^{B}	30.23±22.92^{A}	36.91±21.38^{A}	21.40±11.51^{B}	30.28±18.61^{AB}	40.77±21.74^{A}	30.56±19.00^{B}	30.74±19.88^{A}	39.31±20.24^{A}
Bovine – 12 months	26.35±18.58^{B}	22.18±18.09^{A}	31.18±25.38^{A}	26.37±16.62^{B}	25.82±16.57^{AB}	32.40±19.58^{A}	23.71±11.81^{B}	26.65±21.52^{A}	37.90±25.04^{A}
Bovine – 24 months	29.34±14.76^{AB}	37.35±21.22^{A}	29.17±14.27^{A}	21.72±16.18^{B}	18.30±16.14^{B}	25.36±25.91^{A}	23.31±16.13^{B}	5.46±13.22^{A}	30.58±14.71^{A}
Bovine – 36 months	43.01±41.61^{AB}	32.06±35.38^{A}	32.95±22.89^{A}	27.52±17.04^{B}	20.78±15.17^{B}	35.95±23.57^{A}	28.79±16.09^{B}	24.76±17.63^{A}	33.45±22.13^{A}
Bovine – 48 months	29.37±15.20^{AB}	38.72±18.63^{A}	38.72±18.63^{A}	30.98±12.51^{B}	23.01±14.77^{AB}	42.96±19.94^{A}	21.14±18.15^{B}	17.72±18.04^{A}	24.31±21.38^{A}
Bovine – +60 months	19.88±18.42^{B}	14.22±13.95^{A}	18.06±14.24^{A}	22.16±15.69^{B}	22.65±17.38^{AB}	23.59±17.74^{A}	37.85±14.21^{AB}	7.46±12.98^{A}	27.48±15.94^{A}
Human	74.25±23.47^{A}	52.72±19.28^{A}	43.60±16.72^{A}	74.19±23.34^{A}	52.81±13.18^{A}	50.86±14.54^{A}	95.14±75.10^{A}	64.44±42.01^{A}	65.40±40.74^{A}

Different letters mean statistical difference for the same column.

enamel compared to the parallel disposition of prisms in bovine enamel [17], in addition to the high number of "crossing of enamel rods" may have contributed to the higher values (310 - 350) of enamel microhardness at deep sub-region of middle third and superficial sub-region of incisal third compared to bovines. The values observed in this study are in accordance with the literature [18], which showed 342 – 348 Knoop hardness for human enamel.

The Bovine – 24 months group demonstrated similar microhardness and PO_4/CO_3 ratio compared to humans, although the Bovine – 12 months group demonstrated higher PO_4/CO_3 ratio values and lower microhardness. Ovine and older ages over 24 months of bovine enamel presented similar inorganic compounds, despite lower microhardness values than human enamel. This outcome's rationale may be the higher microhardness with smaller sizes of enamel carbonated apatite nanocrystals [19] or higher concentrations of proteins that may decrease crystal size, inhibiting crystal growth [20]. Following a study [21], higher carbonate concentrations presented into bovine enamel substrate increase its susceptibility to acid etching. Besides, heterogeneity for bovine enamel's elemental composition was recently evidenced [22]. Therefore, except for the superficial sub-region of bovine – 24 months, our study's results indicate the precaution of substituting humans by bovine or ovine enamel due to similarity to humans.

One of the advantages of using bovine instead of human dentin is that the bovine lower incisors' size allows the preparation of control and treatment samples from the same tooth [23]. This study's results showed different microhardness and PO_4/CO_3 among dentinal thirds. Attempting to minimize sample selection bias, researchers may concentrate their samples within the same area. Although the bovine dentinal tubule lumen

displayed a larger mean diameter and a lower density than humans, dentin microhardness found in this study was similar comparing human vs. Bovine - 24 months group, and it decreased with older ages of bovine and deep regions with the proximity of pulp chamber.

Such differences comparing human, ovine, and bovine dentin above 24 months may be because the dentin in bovine incisors presents larger dentinal tubules and is more porous in the intertubular dentin than human molars, as demonstrated previously [25,26]. Hence, dentin at older ages is characterized by greater mineral deposition and collagen fibril modifications [27]. According to previous studies, the results of dentin microhardness in this study presented lower values of microhardness in the deep sub-regions, 10-15 KHN less, according to previous studies [28,29]. As previously shown, a more significant presence of dentin tubules near the pulp chamber [26] and less mineralized peritubular and intertubular dentin [30] may result in this outcome. Nonetheless, the PO_4/CO_3 ratio found within each third was similar for bovine and human dentin.

The organic compound presented in dentin substrate corresponds to the peak of amide I and III stretching modes and the bending and stretching modes of CH groups of lipids and proteins [15,31]. Furthermore, collagen's dentin organic matrix is made up of collagen that provides elasticity [32], and its fibril cross-links and proteoglycan-rich matrix contribute mechanical strength and distensibility. The collagen matrix is the critical component of acid-etched dentin, forming a hybrid layer after priming and adhesive application procedures, increasing mechanical retention to composite resin [33].

In this study, human dentin presented a similar amount of collagen compared to bovine, except in the superficial sub-region, and an increased amount compared to ovine dentin, especially within the superficial sub-region. Previous studies demonstrated a higher percentage of dentin tubules in humans compared to bovines. Although dentinal tubules' density and length have been associated with bond strength variations [25], resin restorations' long-term failure is not limited to this variable only. Degradation of denuded collagen fibrils along the bottom of hybrid layers and hydrolysis and leaching of the adhesive resin is related to the increased failure rate of resin composite restorations [34]. Thus, lower percentages of collagen do not necessarily mean the limitation of using bovine dentin in adhesive studies. This is also according to the great variability of collagen amount found within this study. Based on the collagen quantity results, we encourage further studies evolving bovine and ovine dentin to use middle and deep sub-regions to achieve a similar comparison to human dentin.

Conclusion

Superficial enamel and all sub-regions of dentin of bovine - 24 months age were similar to humans. Bovine and ovine teeth should be used carefully to replace human teeth in dental research due to the variability that different regions of these teeth may present compared to humans. Overall, the group with the highest similarity to the human was the Bovine - 24 months group.

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