ORIGINAL ARTICLE

Influence of de-remineralization process on chemical, microstructural, and mechanical properties of human and bovine dentin

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Abstract

Objectives This study compared the chemical composition, microstructural, and mechanical properties of human and bovine dentin subjected to a demineralization/remineralization process.

Materials and methods Human and bovine incisors were sectioned to obtain 120 coronal dentin beams ($6 \times 1 \times 1$ mm^3) that were randomly allocated into 4 subgroups ($n = 15$) according to the time of treatment (sound, pH-cycling for 3, 7, and 14 days). Three-point bending mechanical test, attenuated total reflectance–Fourier transform infrared (ATR-FTIR), thermogravimetric (TG), and X-ray diffraction (XRD) techniques were employed to characterize the dentin samples.

Results Regarding chemical composition at the molecular level, bovine sound dentin showed significantly lower values in organic and inorganic content (collagen cross-linking, CO_3/amide I, and CO_3/PO_4; $p = 0.002$, $p = 0.026$, and $p = 0.002$, respectively) compared to humans. Employing XRD analyses, a higher mineral crystallinity in human dentin than in bovines at 7 and 14 days ($p = 0.003$ and $p = 0.009$, respectively) was observed. At the end of the pH-cycling, CI (ATR-FTIR) and CO_3/PO_4 ratios (ATR-FTIR) increased, while CO_3/amide I (ATR-FTIR), PO_4/amide I (ATR-FTIR), and %mineral (TG) ratios decreased. The extension by compression values increased over exposure time with significant differences between dentin types ($p < 0.001$, in all cases), reaching higher values in bovine dentin. However, flexural strength (MPa) did not show differences between groups. We also observed the correlation between compositional variables (i.e., PO_4/amide I, CI, and %mineral) and the extension by compression.

Conclusions Human and bovine dentin are different in terms of microstructure, chemical composition, mechanical strength, and in their response to the demineralization/remineralization process by pH-cycling.

Clinical relevance These dissimilarities may constitute a potential limitation when replacing human teeth with bovines in in vitro studies.

Keywords Human dentin · Bovine dentin · Demineralization · Remineralization · Chemical composition · Biomechanics

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Introduction

The human tooth is the preferred substrate for in vitro studies in dental research [1, 2]. However, due to the advance of minimally invasive approaches in conservative dentistry, it is becoming increasingly difficult to obtain a sufficient number of sound human teeth. Besides the difficulty in obtaining large quantities of human dental samples, there are ethical issues that prevent their use for experimental purposes [1, 3–5]. Therefore, alternative substrates have been proposed and used in dental research.

The most commonly non-human substrate used as a substitute for humans is the bovine tooth, and their employment

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has considerably increased in recent years [1]. The main reasons that motivate its utilization are the ease of obtaining a large number of samples of adequate quality and the possibility of standardizing age, diet, and other environmental conditions, which are impossible to control in human teeth [1, 2]. Despite these advantages, the chemical composition, structural, and morphological characteristics of bovine teeth are not fully comparable to human teeth [3, 5–8].

The evidence concerning the use of bovine teeth in the adhesion test is weak since several studies show different conclusions [1–3]. The results of one of those studies showed considerably lower bond strength compared to the human teeth, particularly in deep dentin [3]. This difference in mechanical behavior is probably due to structural properties related to the density and diameter of the dentinal tubules at different depths between bovine and human dentin [6, 7]. Moreover, the radiodensity of bovine coronal dentin is significantly lower compared to human coronal dentin [5, 8], most likely due to the difference in the mineral composition of both substrates. Therefore, we should consider all these features between both types of tissues to interpret the results in the experiments for dental research correctly.

From a clinical point of view, the artificial induction of carious lesions in human dentin is a key research procedure in the development of preventive and therapeutic strategies. Although different methodologies have been developed to simulate carious lesions, in vitro protocols have important limitations, since they cannot completely simulate the complex intraoral conditions involved in the natural process, such as bacterial biofilms, saliva, and similar collagen degradation [9–11]. However, the pH-cycling model can mimic the dynamics of mineral loss and gain associated with the natural process presenting similar hardness values to naturally caries-affected dentin layer, unlike the microbiological method which presents lower hardness values than those of natural lesions [9, 11]. Nevertheless, so far, no report has studied the behavior of bovine coronal dentin using the pH-cycling method and its possible differences compared to human dentin. Therefore, it is necessary to investigate their chemical and structural properties as well as their mechanical response to extrapolate the results to the human teeth and to determine their suitability as an alternative.

Thus, the objectives of the current research were to induce a demineralization-remineralization (de-re) process into human and bovine dentin by the pH-cycling method with different exposure times and to compare their overall properties at different physicochemical scales. For this purpose, the dentin samples were characterized by chemical (ATR-FTIR and thermogravimetric), microstructural (XRD), and mechanical (3-point bending test) analysis. The proposed null hypothesis was that there are no differences (i.e., mechanical, microstructural, and/or chemical properties) between human and bovine dentin and that the pH-cycling time does not influence these differences.

Materials and methods

Sample preparation

The current research was approved by the Ethics Committee of the University of Granada, Spain (#1006-2019). Ten sound human upper incisors (from 50- to 70-year-old adult patients), extracted for periodontal reasons, and eight sound bovine lower incisors, extracted immediately after slaughtering (at 1–2 years old) the animals in a local abattoir, were cleaned and stored in 0.1% thymol solution until sample preparation.

The incisors were fixed with natural wax in acrylic resin molds to be sectioned perpendicularly to the long axis at 2 mm below the cementoenamel junction, cutting the root using an IsoMet 11/1180 low-speed precision microtome (Buehler, Coventry, UK) and a diamond disk XL 12205 (Benetec Limited, London, UK). The enamel and superficial dentin were removed by a cylindrical diamond bur (Komet #6881-314-016, Komet-Brasseler, Lemgo, Germany) in a high-speed handpiece (Kavo Supertorque Lux 660B. Kaltenbach & Voigt GmbH, Biberach, Germany) under constant water irrigation. The diamond burs were replaced after every five uses. The complete removal of enamel and the presence of dentin on the entire surface were checked with an SZ-TP stereomicroscope (Olympus, Tokyo, Japan). Subsequently, the dentin specimens were sectioned from the mid-coronal dentin with a diamond disk on an Accutom 50 cutting machine (Struers A/S, Ballerup, Germany), underwater cooling, to obtain $6 \times 1 \times 1\ mm^3$ dentin beams. Sixty beams for both human and bovine dentin were obtained. Samples were randomly allocated to two experimental groups according to the type of substrate, human or bovine dentin, and four subgroups ($n = 15$) based on the time of pH-cycling treatment: #1 = sound (control); #2 = pH-cycling for 3 days; #3 = 7 days; and #4 = 14 days. The control group was stored in distilled water until their evaluation. The rest of the specimens were subjected to a de-re process.

pH-cycling

The de-re process was created by a pH-cycling method modified by Marquezan et al. [9]. The specimens were immersed in 1 ml of a demineralizing solution containing 2.2 mM $CaCl_2$, 2 mM NaH_2PO_4, and 50 mM acetic acid adjusted to a pH of 4.8 for 8 h. Subsequently, samples were immersed in a 1 ml remineralizing solution formulated based on 1.5 mM $CaCl_2$, 0.9 mM NaH_2PO_4, and 0.15 M KCl and adjusted to a pH of 7.0 for 16 h. This procedure was carried out for 3, 7,

and 14 days, depending on the group, renewing the solutions daily, without agitation and at room temperature.

Sample analysis

Three-point bending test

The samples were inspected under a microscope to confirm the absence of microcracks or fractures, and their dimensions were measured (i.e., length, width, and thickness). Subsequently, the flexural strength was determined by a mechanical tester, Instron 3345 (Instron Co., Canton, Massachusetts, USA) using a 500 N load cell on a 2-point support, with a length of 4.0 mm between points. The bending tests were carried out at a loading speed of 1 mm/min until the samples fractured. To characterize the mechanical properties of the dentin samples, the flexural strength (MPa) and the extension by compression (mm) were determined.

Attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR) analyses

Samples were analyzed using an FTIR JASCO 6200 spectrometer equipped with a diamond-tipped ATR accessory (ATR Pro ONE, JASCO Inc., Maryland, USA). The spectra were recorded at a resolution of 2 cm^{-1} with 32 accumulations using a spectral range of $400–4000 \text{ cm}^{-1}$ in absorption mode. The relative amounts of protein (mainly corresponding to collagen), phosphate, and carbonate mineral molecular groups in the dentin samples were determined from the peak area of the absorption bands associated with each chemical component [12–14]. The overlapping of peaks was resolved, and their integrated areas were measured using curve fitting software (Peakfit v4.12, Systat Software, San Jose, CA, USA). The second derivative method was used to resolve the peak calculations within the spectrum region. A variation of the maximum amplitude and position 5% and $\pm 2 \text{ cm}^{-1}$, respectively, was allowed. The degree of smoothing was adjusted to 10% (Savitzky-Golay algorithm), and a mixed Gaussian-Lorentzian function was used to adapt the contours of the bands within the region. The curve fitting was accepted when r^2 achieved values higher than 0.995.

From the peak area measurements, the following compositional parameters were determined to define dentin chemical properties: the relative amount of mineral to the organic matrix (PO_4/amide I) determined as the ratio of the main phosphate (v_1, v_3 PO_4; $900–1200 \text{ cm}^{-1}$) to the amide I band area ratio ($1590–1710 \text{ cm}^{-1}$). The carbonate substituted to the mineral content (CO_3/PO_4) determined as the ratio between the carbonate substituted (v_3 CO_3; $1390–1440 \text{ cm}^{-1}$, type B substitution) to the main phosphate band area ($900–1200 \text{ cm}^{-1}$). The amount of carbonate mineral (v_3 CO_3) to the organic matrix (CO_3/amide I). The mineral crystallinity index (CI)

determined as the ratio between phosphate sub-band areas at 1030 cm^{-1} (high crystalline apatite phosphates) to 1020 cm^{-1} (poorly crystalline apatite phosphates) [13, 15]. The amount of collagen crosslink was determined as the ratio between the amide I sub-bands areas at 1660 to 1690 cm^{-1} [16].

Thermogravimetric (TG) analyses

The water, organic matter, and mineral content of the dentin were determined by heating dentin samples at different temperatures using a Carbolite furnace (CWF 1100). The weight losses were measured at different temperature ranges: 25–200 °C for water content, 200–600 °C for organic matter content (OM), and up to 600 °C for the mineral content (%mineral) [17–19].

X-ray diffraction (XRD) analyses

The X-ray diffraction pattern of the powdered samples was obtained with an X'Pert Pro diffractometer (PANalytical, Almelo, Netherlands) using CuKα radiation produced at 40 mA and 45 kV. The scans were acquired between 20 and 75° (2θ values) with a step of 0.0042° and a time/step of counting of 5.08 s. The average crystallite size (D) of the apatite crystals was calculated using the XPowderX software (Martín-Ramos, 2004) with Scherrer equation (Schreiner and Jenkins, 1983): $D (002) = K\lambda/(B \cos \theta)$, where K is a constant that varies with the crystalline habit and is chosen as 0.9 for elongated crystals of apatite (Klug and Alexander, 1959), λ is the wavelength CuKα radiation ($\lambda = 1.5406$ Å), B is the full width at half maximum (FWHM) of the 002 apatite diffraction peak, and θ is the corresponding diffraction angle for this reflection. The width of an XRD line represents a measure of the average coherent crystal size domains along a specific crystal direction (i.e., apatite c-axis) and can also be used as an index of crystallinity of materials [13, 15, 20, 21].

Statistical analysis

Means and standard deviations were calculated for each of the parameters analyzed. The normal distribution of the data for all the variables was verified by the Shapiro-Wilk test. For the tests in which the data did not adjust to a normal distribution, pairwise comparisons between sound specimens, pH-cycling for 3, 7, and 14 days were performed using the Mann-Whitney U test. Likewise, the comparisons between the different types of dentin (human and bovine) were made with the Mann-Whitney U test. When the data followed a normal distribution, comparison between groups within a tooth type was performed using one-way ANOVA test and the comparison between the two types of teeth the Student T test. Spearman's correlation analysis was used to study the relationships between the different chemical properties of dentin and dentin

mechanical properties. All statistical analyses were performed using the software SPSS 24.0 (SPSS Inc., Chicago, USA) and OriginPro (OriginLab Corporation, Massachusetts, USA). A level of significance of $p < 0.05$ was established.

Results

Three-point bending test

The flexural strength (MPa) showed no significant differences between the treatment groups (sound and pH-cycling) and types of dentin (Fig. 1A). The extension by compression (mm) increased with the time of exposure to pH-cycling in both substrates (Fig. 1B), showing statistically significant differences between human and bovine dentin in all treatments ($p < 0.001$ in all cases), with bovine dentin obtaining the higher values.

ATR-FTIR analyses

Figure 2 shows the ATR-FTIR spectra comparison between human and bovine dentin of the control group (sound sample) and at 14 days of pH-cycling.

In the results obtained with the peak area calculations, for both types of dentin, the relative phosphate mineral content associated to the organic matrix (PO_4/amide I) (Fig. 3A) and the substituted carbonate (type B) content associated to the organic matrix (CO_3/amide I) (Fig. 3B) decreased significantly from the control to the pH-cycling 14-day group ($p < 0.001$ for both cases). Human dentin showed a higher content of PO_4/amide I at 7 days ($p = 0.007$) as well as CO_3/amide I at sound ($p = 0.026$) and 7 days ($p = 0.001$) than bovine. Figure 3C shows that the substituted carbonate (type B substitution) in the mineral (CO_3/PO_4) increased at the end of pH-

cycling, and bovine dentin obtained statistically significant lower values than human in the control group ($p = 0.002$). The crystallinity index (CI) (Fig. 3D) increased from control to the 14-day group for both human and bovine dentin ($p = 0.001$ human, $p < 0.001$ bovine). A higher CI was observed in human than bovine dentin at 7 days of pH-cycling with significant differences ($p = 0.024$). The collagen cross-linking (Fig. 3E) decreased from control to the 14-day group without significant differences. A higher degree of collagen cross-linking in humans than bovine dentin in the control group ($p = 0.002$) was observed.

Thermogravimetric analyses

The thermogravimetric measurements (Table 1) show that there were no differences between the groups nor between the types of dentin concerning the water content. In both types of dentin, the percentage of organic matter (OM) was increasing between the control and the 14 days of cycling (human $p = 0.001$, bovine $p < 0.001$), and the human dentin had higher values than bovine at 3 days ($p = 0.010$). Moreover, human dentin did not show differences between the groups with respect to mineral content, while bovine dentin had a lower percentage ($p < 0.001$) at 14 days of pH-cycling than the control group. In addition, there were differences in the mineral content between human and bovine dentin at 14 days ($p = 0.039$).

Correlations between chemical and mechanical variables

Table 2 shows the relationships between some chemical variables and the mechanical properties of human and bovine dentin.
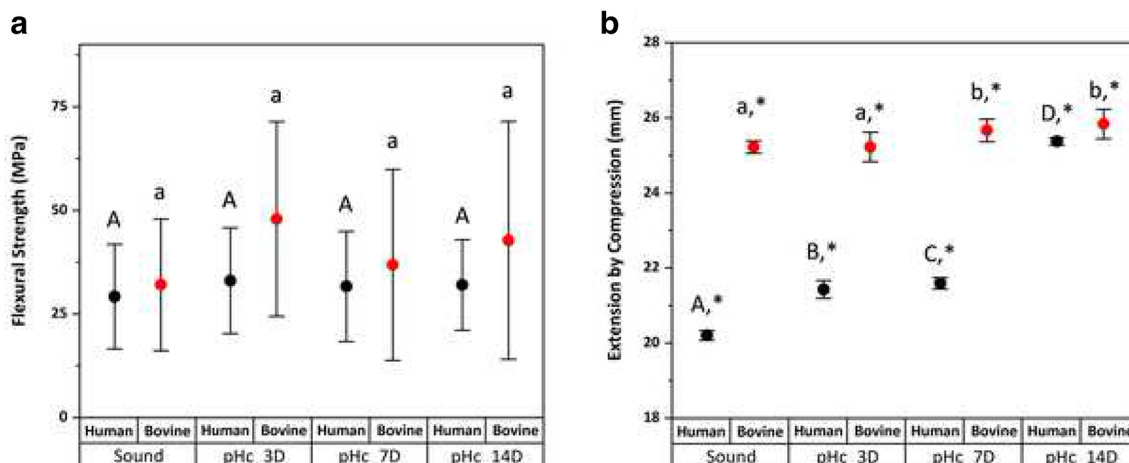

Fig. 1 Three-point bending mechanical tests. Different uppercase letters indicate significant differences between the human dentin groups. Different lowercase letters indicate significant differences between the bovine dentin groups. Asterisks indicate significant differences between the types of dentin (human and bovine) in each pH-cycling time (significance $p < 0.001$)

Fig. 2 ATR-FTIR spectra comparison between human and bovine dentin of control group (sound) and at 14 days of pH-cycling. Redline = human dentin (H), black line = bovine dentin (B)
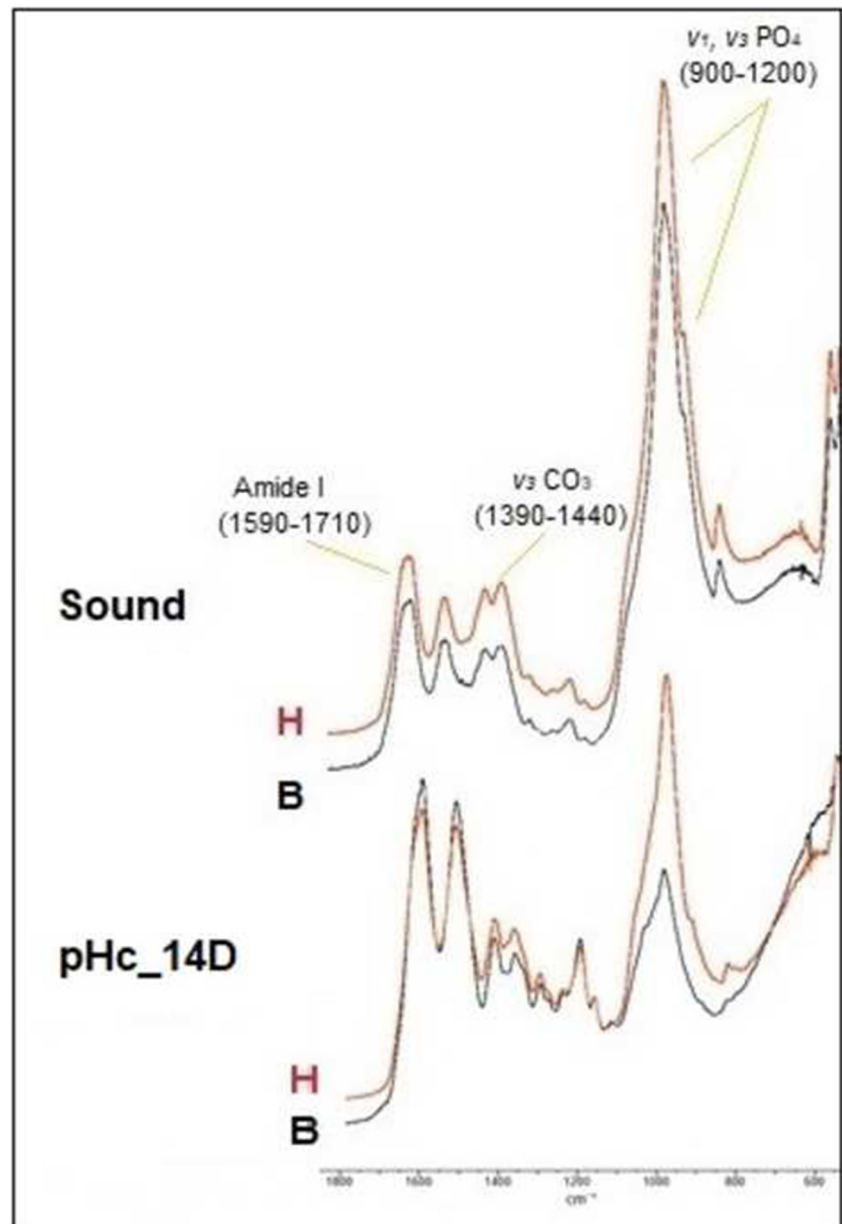
XRD analyses

Table 3 summarizes the crystallite size measurements (obtained from (002) diffraction peak) for apatite crystals. Although it was observed a tendency for the mineral crystallinity to increase throughout the pH-cycling treatment, there were no statistically significant differences between the groups for each type of dentin. Human dentin showed a larger apatite crystallite size than bovine at 7 ($p = 0.003$) and 14 days ($p = 0.009$) of pH-cycling. No correlations between crystallite size and chemical composition or mechanical variables were observed.

Discussion

The characteristics of the mineralized tissues in the tooth structures determine the response to specific treatments employed in the dental clinic. Furthermore, the chemical composition, microstructural, and mechanical properties of these tissues may vary between animal species. The comparative study of the teeth properties from different species is fundamental for in vitro dental research and its extrapolation to human teeth. Our research focuses on analyzing the differences between human and bovine dentin in a demineralization-remineralization (de-re) process by pH-cycling at various exposure times.
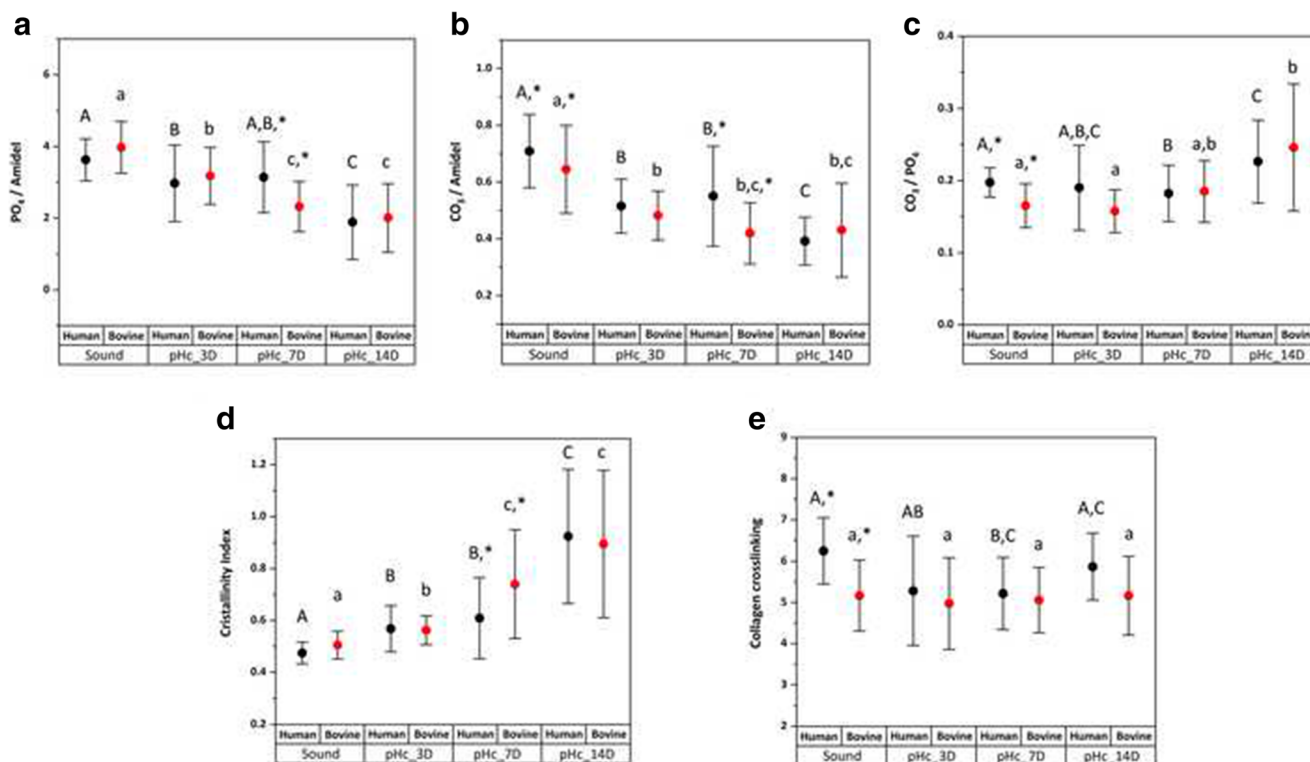
Fig. 3 Chemical composition parameters obtained by ATR-FTIR analyses. Different uppercase letters indicate significant differences between the human dentin groups. Different lowercase letters indicate significant differences between the bovine dentin groups. Asterisks indicate significant differences between the types of dentin (human and bovine) in each pH-cycling time (significance $p < 0.001$)

In the current study, the chemical composition at the molecular level of the control group (i.e., sound samples) of bovine dentin showed significantly lower values than humans in mineral components (phosphates and carbonates) and collagen crosslinking (Figs. 2 and 3). During pH-cycling experiments, dentin mineral content (measured as PO_4/amide I and CO_3/amide I ratios) decreases, whereas its mineral crystallinity (CI) increases (Fig. 3). Besides that, at the end of the pH-cycling (14 days), compared with the control group, the %mineral content (TG; Table 1; for bovine dentin) was also significantly lower. On the other hand, dentin mechanical properties, determined by 3-point bending tests, showed notable differences between human and bovine dentin in the

extension by compression values. During the pH-cycling, these bovine dentin values remain nearly constant, whereas human dentin values gradually increase (Fig. 1B). We also observed correlation relationships between the measured mechanical properties (i.e., the extension by compression) and dentin compositional parameters (PO_4/amide I, CI, and %mineral), corroborating that changes in the dentin chemical composition affect their mechanical properties (Table 2).

Previous research has shown that human dentin is more mineralized than other animal species [22, 23]. However, other studies comparing different types of animal dentin did not reveal significant differences in their chemical composition (i.e., Ca (%) and P (%) or Ca/P ratio) [24, 25]. In the present

Table 1 Means and standard deviations for the compositional variables obtained from TG analysis of human and bovine dentin at the sound, pH-cycling for 3, 7, and 14 days

		Sound	pHc_3days	pHc_7days	pHc_14days
Water (%)	H	11.59 ± 4.07 [a]	10.15 ± 0.74 [a]	9.57 ± 1.26 [a]	10.45 ± 0.54 [a]
	B	10.92 ± 1.92 [a]	11.13 ± 1.08 [a]	11.67 ± 2.30 [a]	13.09 ± 1.47 [a]
OM (%)	H	18.94 ± 1.18 [a]	20.52 ± 0.80 [ab,*]	20.34 ± 2.38 [ab]	21.99 ± 1.34 [b]
	B	18.14 ± 1.90 [a]	18.98 ± 1.88 [a,*]	20.58 ± 1.57 [ab]	23.12 ± 3.10 [b]
Mineral (%)	H	69.47 ± 3.35 [a]	69.33 ± 1.09 [a]	70.09 ± 3.63 [a]	67.57 ± 1.49 [a,*]
	B	70.94 ± 3.55 [a]	69.89 ± 2.60 [a]	67.75 ± 3.12 [ab]	63.79 ± 3.58 [b,*]

Different letters indicate significant differences between the same dentin type groups in the row. *Significant differences between the types of dentin (H = Human and B = Bovine) of each variable in the columns (significance $p < 0.001$)

Table 2 Spearman's correlation analysis between mechanical (extension by compression) and chemicals properties for both human and bovine dentin

Extension by compression (mm)	Human dentin		Bovine dentin	
	P (Spearman)	p	P (Spearman)	p
PO_4/amide I	− 0.533	< 0.001	− 0.493	< 0.001
CI	0.694	< 0.001	0.520	< 0.001
%Mineral	− 0.448	0.005	− 0.332	0.044

study, bovine dentin showed lower mineral content associated with molecular groups (i.e., carbonates and phosphates) than human dentin (Figs. 3B, C). A previous study using FT-Raman analysis [26] also showed significant differences between human and bovine dentin in the amount of inorganic and organic components. Also, Soares et al. reported that untreated human dentin samples showed a higher content of v_4 PO_4, v_1 CO_3, amide III, CH_2, and amide I than bovine ones [26].

In our study, the pH-cycling, during the de-re process, resulted in that, at the end of the process (i.e., 14 days), the degree of mineralization measured as PO_4/amide I ratio (for ATR-FTIR) and %mineral (for TG) were significantly lower compared to sound dentin (Fig. 3A and Table 1). Furthermore, there is a progressively increased carbonate to phosphate mineral substitution (CO_3/PO_4; Fig. 3C) with pH-cycling. It is known that the solubility of phosphate groups increases in a more acidic environment [27], while carbonates (preferably in crystalline positions, type B) decrease their solubility at a lower pH [28]. Moreover, the carbonate ions are replacing (type B substitution) the phosphate ions into the crystalline structure of apatite on the demineralization process [29, 30]. Consequently, the ratio of the substituted carbonate in the mineral seems to increase as the mineral matures [31, 32]. These compositional changes could be related to the observed increased crystallinity of the dentin mineral.

These previous results explained how the mechanism of the de-re process mainly affected the inorganic component of dentin tissues. Regarding the organic matrix content, the relative amount of amide I (Fig. 2) and OM (Table 1) increases during the de-re process [33, 34]. On the other hand, no

difference was found between the two dentin types concerning the collagen crosslinks with the pH-cycling process (Fig. 3E). These results confirm how the process mainly affects the mineral composition of the tissue without altering the properties related to organic matter, mostly collagen, in dentin. Certainly, according to the principles of conservative dentistry, this is an important reason to keep the caries-affected dentin, in the cavity preparation, to be remineralized.

A comparison between different species (i.e., human, bovine, porcine, and ovine) concluded that human dentin is composed of apatite with larger crystallite sizes [29]. It should be noted that the XRD and FTIR techniques characterize different properties associated to mineral crystallinity, relating the arrangement of the apatite crystal structure (i.e., crystalline domains in the c-axis direction) for XRD, and phosphate molecular groups corresponding to high and poor crystalline environments for FTIR analyses [13, 20]. In our results, the mineral crystallinity measured by ATR-FTIR and XRD (i.e., CI and crystallite size, respectively) increased during the exposure time to pH-cycling (Fig. 3D and Table 3), although this trend is not statistically significant for XRD analyses. These differences can be explained by the higher solubility rates of phosphates of lower crystallinity during the de-re process [35, 36].

To date, no studies have evaluated the flexural strength of bovine coronal dentin and compare it to human coronal dentin on the de-re process. In our study, bovine and human dentin showed marked differences in their mechanical properties (i.e., extension by compression), having bovine dentin higher values than humans. This could be explained by the higher mineral content of human dentin, making the latter have lower flexibility. Also, the pH-cycling, during the de-re process, affected the evolution of these properties differently for each dentin, with more notable variations in humans (Fig. 1B). The mechanical properties of dentin are strongly influenced by its chemical and microstructural characteristics [37]. Specifically, we observe a strong correlation between the extension by compression and several compositional parameters (Table 2). As expected, as the degree of mineralization decreases (i.e., PO_4/amide I, %mineral) due to mineral loss, the dentin flexibility (measured as the extension by compression) increases. We also notice that the extension by compression and mineral crystallinity (measured as CI by ATR-FTIR) co-varied

Table 3 Means and standard deviations for crystallite size measurements by XRD analysis of human and bovine dentin at the sound, pH-cycling for 3, 7, and 14 days

	Sound	pHc_3days	pHc_7days	pHc_14days
H	14.60 ± 1.66	15.31 ± 0.44	16.73 ± 0.74*	16.93 ± 0.95*
B	12.48 ± 0.78	13.97 ± 1.02	13.04 ± 0.49*	13.64 ± 0.73*

No significant differences between the groups in the row. *Significant differences between the types of dentin (H = Human and B = Bovine) of each variable in the columns (significance $p < 0.001$). Values are expressed in nm

($p < 0.001$, for human and bovine dentin). During the pH-cycling, there is a preferential removal of less crystalline phosphate mineral so that the remaining mineral has an increased crystallinity (Fig. 3D) [36]. Besides that, there are morphological and microstructural differences between bovine and human dentin, expressed in the dissimilarity of the number and size of dentinal tubules and the inter-tubular dentin [6–8, 38, 39], that may influence the chemical behavior during de-re process and at the same time explain the observed differences in the mechanical properties of these dentitions.

Some meta-analysis, systematic, and literature reviews [1, 2, 40] conclude that the bovine dentin can be a suitable alternative for replacement human teeth in dental research. However, further investigations about the morphology, chemical composition, and structural characteristics as well as possible differences in physical properties (i.e., mechanical response), and taking into account the age of the patients and animals, should be considered for the correct interpretation of the data obtained in studies employing bovine teeth as a model for human dental materials.

Hence, with our results, the null hypothesis should be partly rejected considering that we report some differences in mechanical, microstructural, and chemical composition properties between human and bovine dentin. Furthermore, the pH-cycling process influences the chemical composition at the molecular level, its mineral crystallinity, and the mechanical properties quite differently in these two types of dentin.

Conclusions

We found some relevant differences between human and bovine dentin related to chemical composition and structural characteristics that determine their different mechanical response against the demineralization/remineralization process by pH-cycling at 3, 7, and 14 days. These disagreements may be a possible limitation when replacing human teeth for bovines in in vitro studies.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

Declarations Approval was obtained from the ethics committee of University of Granada, Spain (#1006-2019). The procedures used in this study adhere to the tenets of the Declaration of Helsinki.

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