Comparison of Knoop and Vickers Surface Microhardness and Transverse Microradiography for the Study of Early Caries Lesion Formation in Human and Bovine Enamel

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Short title: Hardness vs TMR–Human vs Bovine Lesions

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Abstract

Objective:

The aims of the present laboratory study were twofold: a) to investigate the suitability of Knoop and Vickers surface microhardness (SMH) in comparison to transverse microradiography (TMR) to investigate early enamel caries lesion formation; b) to compare the kinetics of caries lesion initiation and progression between human and bovine enamel.

Design:

Specimens (90×bovine and 90×human enamel) were divided into six groups (demineralization times of 8/16/24/32/40/48h) of 15 per enamel type and demineralized using a partially saturated lactic acid solution. SMH was measured before and after demineralization and changes in indentation length (ΔIL) calculated. Lesions were characterized using TMR. Data were analyzed (two-way ANOVA) and Pearson correlation coefficients calculated.

Results:

ΔIL increased with increasing demineralization times but plateaued after 40h, whereas lesion depth (L) and integrated mineral loss (ΔZ) increased almost linearly throughout. No differences between Knoop and Vickers SMH in their ability to measure enamel demineralization were observed as both correlated strongly. Overall, ΔIL correlated strongly with ΔZ and L but only moderately with the degree of surface zone mineralization, whereas ΔZ and L correlated strongly. Bovine demineralized faster than human enamel (all techniques).

Conclusions:

Lesions in bovine formed faster than in human enamel, although the resulting lesions were almost indistinguishable in their mineral distribution characteristics. Early caries lesion

demineralization can be sufficiently studied by SMH, but its limitations on the assessment of the mineral status of more demineralized lesions must be considered. Ideally, complementary techniques to assess changes in both physical and chemical lesion characteristics would be employed.

Keywords: Demineralization; Enamel; Human Enamel; Bovine Enamel; TMR; Surface Microhardness; Knoop; Vickers

1. Introduction

Dental researchers have utilized a considerable number of analytical techniques to quantify changes in the mineral content of enamel during caries lesion formation. The most often employed techniques are transverse microradiography (TMR),[1] Knoop[2] and Vickers[3] microhardness initially used perpendicular (K-SMH, V-SMH) or later also in parallel (CSMH)[4] to the hard tissue surface, polarized light microscopy (PLM),[5] confocal laser scanning microscopy (CLSM),[6] and, more recently, quantitative light-induced fluorescence (QLF).[7] All techniques have their disadvantages, such as being e.g. destructive in nature, insensitive to small changes in mineral content, subjective in their use and interpretation of the data, indirect measures of mineral content, and/or expensive to acquire and maintain. Researchers (almost) unanimously agree that TMR is the 'gold standard' due to its ability to quantify subsurface mineral distributions and changes thereof.[8] But its inherently destructive nature, challenging specimen preparation and requirement for an X-ray source (among other reasons) mean that TMR is not necessarily the first choice and that surrogate techniques, such as SMH, are utilized. K- and V-SMH are both four-sided pyramidal diamonds and represent the most common surface hardness techniques used in dental research. The Vickers indenter is square-shaped (both diagonals are used in the calculation of hardness values), whereas Knoop is elongated with an approximate ratio between long and short diagonals of 7:1 (only the long diagonal is used for hardness value calculations). Knoop and Vickers differ considerably in their indentation depths (all are approx. values) – it is 1/30 of its length of the long diagonal for Knoop, but 1/7 of the diagonal length for Vickers. Early studies were able to demonstrate that changes in K-SMH correlate with enamel calcium loss[4] and that strong linear correlations exist between surface

Knoop indentation lengths (IL(Knoop)) and lesion depth (L) – at least for shallow lesions.[9-10] Data from more recent studies (employing different baseline lesions among them) highlighted very strong linear correlations between IL(Knoop) and integrated mineral loss (ΔZ) in situ[11] and in vitro,[12] although other studies did not.[13-14] The reasons for this discrepancy could be manifold, such as the mineral content and density of the surface zone, extensive subsurface demineralization, differences in mineral distribution, and presence of laminations etc. – none of which have been satisfactorily investigated to date. Likewise, comparative studies utilizing K-SMH, V-SMH and TMR could not be retrieved.

Bovine enamel is often employed in dental research when sourcing of human teeth is challenging – either due to local regulatory reasons or due to the limited availability of unaffected teeth. Bovine has many advantages over human enamel (e.g. larger surface area, more uniform enamel thickness), although it is not necessarily a like-for-like replacement as shown in many studies.[15-1617] Their varied dissolution characteristics may be explained by subtle differences in their microstructure and chemical composition which have been studied to some extent with these findings being summarized very recently.[18]

As there is considerable scope for further research in these areas, the aims of the present in vitro study were two-fold: a) to investigate the suitability of K- and V-SMH in comparison to TMR to investigate early enamel caries lesion formation; b) to compare the kinetics of caries lesion initiation and progression between human and bovine enamel. SMH techniques and TMR were chosen as they are complementary analytical techniques and provide information about changes in structure as well as mineral content of the forming lesions. Likewise, early caries lesion formation in bovine and human enamel substrates was investigated due to the lack of reliable data comparing both tissues. In the present authors' opinion it is important to utilize different

methods (if available) to analyze caries lesions to obtain a better understanding of changes in structure as well as mineral content.

2. Materials and methods

2.1. Specimen preparation

Enamel specimens were obtained from human permanent (predominantly molars and premolars, only buccal and/or lingual surfaces were used) and bovine incisor teeth (only buccal surfaces were used). Human teeth were extracted mainly for orthodontic reasons and were obtained from dental offices located in the State of Indiana, USA (water fluoridation at 1 ppm F). Bovine teeth were obtained from Tri State Beef Co. (Ohio, USA), from cattle with an average age of three years (range: 18 months to five years) and which stem from several states in the USA (personal communication with Tri State Beef Co.). Both human and bovine teeth are received at the present, first author's laboratories approximately monthly; however, determinations of origin, exact age and other characteristics of the donor (human or animal) are impossible due to the large number of teeth received.

Tooth crowns were cut into 4 × 4 mm specimens using a Buehler Isomet low-speed saw with one specimen prepared per tooth. The teeth were stored in deionized water containing thymol during the sample preparation process. Specimens were ground and polished to create flat, planar parallel dentin and enamel surfaces using a Struers Rotopol 31/Rotoforce 4 polishing unit (Struers Inc., Cleveland, Pa., USA). The dentin side of the specimens was ground flat to a uniform thickness with 500-grit silicon carbide grinding paper. The enamel side of the specimen

was serially ground using 1,200, 2,400 and 4,000 grit paper. The specimens were then polished using a 1 μm diamond polishing suspension on a polishing cloth until the enamel surface had a minimum of a 2 × 4 mm highly polished facet across the specimen. Resulting specimens had a thickness range of 1.7 – 2.2 mm. This polishing procedure ensured the removal of surface enamel (amount depending on the natural curvature of the enamel surface) which may contain relatively high concentrations of artificially introduced trace elements (e.g. F, Sr) that would otherwise compromise the comparison between tissues. The specimens were assessed under a Nikon SMZ 1500 stereomicroscope at 20 × magnification for cracks, hypomineralized (white spots) areas or other flaws in the enamel surface that would exclude them from use in the study. An experimental window, measuring approximately 1.7 × 4 mm, was created on the specimens using acid-resistant, colored nail varnish (Sally Hansen Advanced Hard As Nails Nail Polish, USA), leaving sound enamel areas on either side. Prepared specimens were stored at 100% relative humidity at 4 °C until use. All specimens were prepared by the same, well-trained technicians using standard operating procedures.

2.2. Sound enamel surface microhardness

Specimens were mounted individually on 1-inch acrylic blocks using sticky wax. A total of eight sound enamel baseline indentations (2100 HT; Wilson Instruments, Norwood, Mass., USA) were placed in the center of each specimen: four using a Knoop diamond indenter using a 50 g load (approx. 100 μm apart from each other), and four using a Vickers diamond indenter using a 200 g load (approx. 150 μm apart from each other and in close proximity (approx. 200 μm) to the Knoop indentations), each with a dwelling time of 11 s. The respective indentation loads and number of indentations per specimen were chosen based on standard operating procedures and

reflect those typically employed in the main author's laboratories.[12,19] Knoop and Vickers indentation lengths (IL_{base}(Knoop) – length of the long diagonal, IL_{base}(Vickers) – mean of both diagonals) were recorded. Human and bovine enamel specimens were each allocated to six groups (i.e. one per demineralization time) with n = 15 each based on IL_{base}(Knoop) so there was no significant difference in mean IL_{base}(Knoop) between groups within enamel types. Sample size was determined based on a preliminary study (data not shown).

2.3. Artificial caries lesion creation

In vitro incipient caries lesions were formed in the specimens by immersion into a solution of 0.05 mol/l lactic acid and 0.2% (w/v) Carbopol C907 which was 50% saturated with respect to hydroxyapatite and adjusted to pH 5.0 using KOH (modified after White[20]). The following demineralization times were chosen: 8; 16; 24; 32; 40; 48 h. These time points were chosen to understand the different phases of lesion formation from surface softening to subsurface demineralization with surface layer formation. Specimens were demineralized using 40 ml of said solution per specimen at 37°C separated by enamel type and then by demineralization time. Specimens were rinsed with deionized water after demineralization and stored at 100% relative humidity at 4 °C until further use.

2.4. Lesion surface microhardness

After demineralization, a second set of eight indentations were placed on each specimen, as described above and in close proximity (approx. 200 µm) to the sound enamel indentations. Indentation lengths were recorded (IL_{post}) and changes vs. IL_{base} calculated for both Knoop and Vickers indenter types as follows: $\Delta IL = IL_{post} - IL_{base}$.

2.5. Microradiography

One section per specimen, approximately 100 μm in thickness, was cut from the center of the specimen and across the lesion window and sound enamel areas after lesion creation using a Silverstone-Taylor Hard Tissue Microtome (Scientific Fabrications Laboratories, USA). Sections which were found to be thicker than 120 μm (determined using drop gauge) were hand-polished using 2,400-grit silicon carbide paper to the required thickness. The sections were mounted, with an aluminum step wedge, on high resolution glass plates Type I A, Microchrome Technology Inc., San Jose, CA) and X-rayed at 20 kV and 30 mA at a distance of 42 cm for 65 min. The film was developed in Kodak d-19 developer for 3 min, placed in a stop bath (Kodak 146-4247) for 45 s, and then fixed (Kodak 146-4106) for 3 min. All plates were then rinsed in deionized water for 15 min and air-dried. Microradiographs were examined with a Zeiss EOM microscope in conjunction with the TMR software v.3.0.0.11 (Inspektor Research Systems BV, Amsterdam, The Netherlands). A window (approx. 400 × 400 μm), representative of the entire lesion area and not containing any cracks, debris or other alterations which would introduce additional variables not inherent to the specimen but the sample preparation and analytical technique, was selected for analysis and without the intent of introducing bias. Sound enamel mineral content was assumed not only to be equivalent between tissues[21] but also 87 % v/v.[22,23] Sectioning and initial TMR analyses (plate reading) were conducted by the same technicians. Final TMR analysis (actual determination of lesion parameters) was conducted by the present, first author. The variability of the repeated TMR analysis of sections was determined in a separate study. The standard deviation of the mean for any of the reported variables was found to be lower than 4 %.

The following variables were recorded for each specimen/section: ΔZ – integrated mineral loss (product of lesion depth and the mineral loss over that depth); L – lesion depth (83 % mineral; i.e. 95 % of the mineral content of sound enamel); R – ratio of integrated mineral loss to lesion depth (ΔZ/L); SZ_{max} – maximum mineral density of the lesion surface zone.

2.6. Statistical analysis

A two-way ANOVA was used to compare the effects of enamel and demineralization time on the study variables (IL_{base}(Knoop), IL_{base}(Vickers), ΔIL(Knoop), ΔIL(Vickers), ΔZ, L, R, and SZ_{max}). The ANOVA models included terms for enamel (human, bovine), demineralization time (8; 16; 24; 32; 40; 48 h) and their interaction. A Student-Newman-Keuls test was used for all pairwise comparisons. Pearson correlation coefficients were calculated to investigate associations between all aforementioned study variables.

3. Results

Table 1 provides the data and statistical analyses for all variables and for both human and bovine enamel.

3.1. Hardness data

All groups were balanced within enamel type for IL_{base}(Knoop) and IL_{base}(Vickers). Although differences were small, sound human was found to be softer than bovine enamel when using the Knoop indenter, but opposite results were obtained using the Vickers indenter. Sound enamel

baseline indentations were still visible after all demineralization times, indicating no surface loss. ΔIL values were affected by both enamel type and demineralization time and regardless of indenter type, but their interaction was insignificant. Increasing demineralization times led to increases in ΔIL from 8 to 40 h after which they plateaued. Bovine softened significantly faster than human enamel and regardless of indenter type (overall differences between enamel types in ΔIL were 29 % for Knoop and 31 % for Vickers).

3.2. TMR data

The ΔZ data followed a similar pattern to the ΔIL data with the differences being a) values increased continuously over the 48 h demineralization period, and b) the two-way interaction between enamel type and demineralization time was significant. Bovine enamel demineralized faster than human enamel. Figure 1 shows all individual data points and corresponding linear fits. Linear regressions followed these formulas: human enamel – $y = 252 + 18.4x$; $R = 0.842$; $p<0.0001$; bovine enamel – $y = 360 + 26.4x$; $R = 0.898$; $p<0.0001$. Thus, the difference in dissolution rate between tissues was approx. 43 % (comparing slopes or overall ΔZ values). L followed a similar pattern as ΔZ and increased with increasing demineralization times with similar differences being observed between enamel types (47 % overall difference). R showed an initial increase after 16 h, but then increased only marginally which was not significant. SZ_{max} values decreased after 16 h and then remained virtually constant throughout. Human enamel exhibited small but significantly higher SZ_{max} values, whereas R values were indistinguishable. The mean mineral distribution profiles for lesions created in bovine enamel are shown in Figure 2. Virtually identical profiles were obtained for human enamel (not shown). It is evident that surface layer formation took place during demineralization and that the shorter demineralization

times resulted primarily in surface softening. Based on all individual sections with little difference between tissues observed, only approx. 30 % had an established surface zone after 8 h which increased to 63 % after 16 h, 79 % after 24 h and 100% after 32 h of demineralization. Lesion body mineralization and surface zone width were not investigated due to the mostly surface-softened nature of these lesions; however, both appeared to change little after formation of the surface layer (Figure 2).

3.3. Correlation between variables

Table 2 provides all Pearson correlation coefficients and corresponding *p* values for all comparisons between variables (combined human and enamel data). The strongest correlations (i.e. $r > 0.7$) were observed for ΔZ vs. L, ΔIL(Knoop) vs. ΔIL(Vickers), ΔIL(Vickers) vs. ΔZ, ΔIL(Knoop) vs. ΔZ, and ΔIL(Vickers) vs. L. Several other interactions were found to be of significance, although their corresponding *r* values were comparatively low (≤ 0.5). For better visualization of one of the association between variables, Figure 3 presents all raw data (combined human and bovine enamel data), linear fit and prediction intervals for ΔIL(Knoop) vs. ΔZ ($r = 0.782$). Pearson correlation coefficients for the human ($r = 0.702$) and bovine ($r = 0.753$) enamel data were virtually identical (both $p < 0.001$).

4. Discussion

Hardness is commonly defined as a ‘material’s resistance to deformation’. Hardness, however, is not an intrinsic property as a material’s hardness value is a consequence of a distinct

measurement procedure. This perhaps explains the subtle differences in IL_{base} between human and bovine enamel when measured with either Knoop or Vickers indenters (Table 1), which should not be overinterpreted. Considering the 40 h demineralization time point for bovine enamel specimens for example, the mean IL after demineralization were 113.6 µm (Knoop) and 86.4 µm (Vickers) which would equate to mean indentation depths of 3.8 µm (Knoop) and 12.3 µm (Vickers) – a ratio of approx. 1:3. Bearing in mind that the hardness of a multi-layered material (such as a caries lesion) is affected by usually 7-10 times the indentation depth,[24] both indenter types (when used at present loads) should be suitable to study very shallow lesions ($L \leq 40$ µm) with Vickers being the more appropriate of the two to study slightly deeper lesions ($L \leq 100$ µm). However, no difference was observed in their ability to discriminate between demineralization times (which is also supported by their strong correlation – Table 2) – neither Knoop was more sensitive at early demineralization times, nor was Vickers for deeper, more demineralized lesions, as both data sets showed some plateauing after 40 h. This is further complicated by the fact that the plateauing was observed for human enamel at $L \sim 40$ µm, whereas it was $L \sim 55$ µm for bovine enamel – an observation that may perhaps be at least partially explained by their differences in prism arrangement[25] and also the increasing contribution of a surface zone at this stage of lesion formation. In hindsight, it would have been advantageous to include demineralization times in excess of 48 h to investigate the plateauing of IL values in more detail. However, this disconnect between ΔIL and L with increasing demineralization times was shown previously[10] and is in line with what one would expect. Perhaps the most pertinent question is – what does the SMH of a caries lesion actually describe? Based on the present data, it is quite obvious that SMH is a measure of the structural integrity of (almost) the entire lesion; i.e. it is a combined measure of surface layer presence, its degree of

mineralization and thickness, extent of subsurface (de)mineralization and mineral distribution. Any of these parameters can be the determining factor; e.g. hypomineralization or lamination could give the false impression of remineralization, or differences in mineral distribution between lesions could lead to differences in SMH when in fact ΔZ values may be very similar. Is it fair to argue then that SMH cannot be used to study demineralization or remineralization? Yes and no. Despite the plateauing which is likely due to surface layer formation rather than extensive subsurface demineralization, very strong correlations were observed between ΔIL vs. ΔZ and L (Table 2) presently. This suggests that SMH techniques have the ability to quantify changes in enamel structure during lesion formation in spite of the shallowness of their indentations. Furthermore, data from other, aforementioned studies[11-12] showed very strong correlations and under more physiologically relevant conditions. Thus, SMH can be used in caries research, provided model characteristics are understood and correlations to gold standard techniques have been previously established. To ignore or dismiss the value of SMH altogether as it does not directly measure mineral content would be foolish; SMH measures an important parameter with structural integrity that cannot necessarily be derived from TMR variables (ΔZ, L, SZ_{max}) or additional calculated variables (e.g. R = ΔZ/L). SMH should therefore be seen as a complementary measure to TMR as it provides additional information. Indeed, during studies into the de- and remineralization of the dental hard tissues, it is desirable to understand concurrent changes in both physical[26] and also chemical[27] parameters.

SMH is often used in cross-section (CSMH) to overcome SMH's limitations regarding lesion depth and to study deeper lesions. The present study was not concerned with CSMH due to the shallowness of the employed lesions, its inability to characterize the lesion surface and as the 'gold standard' technique TMR was utilized. CSMH was studied in the past to determine lesion

mineral content, using different approaches, and, not surprisingly, different conclusions were reached.[13282930] Parallels can be drawn again as hardness, used either perpendicular or parallel, measures first and foremost the mechanical properties and structural integrity which *may* also be directionally proportional to the mineral content. It should also be mentioned that apart from the investigations by Buchalla et al.,[30] the comparison between (C)SMH and TMR assumes lesion homogeneity as these measurements occur in different areas on the same specimen, thus adding additional variability.

Bovine was found to demineralize faster than human enamel and at a rate of approx. 1.4:1, which together with their linear dissolution behavior highlights that the two tissues behave similarly but are not necessarily like-for-like replacements. The varied dissolution rates may be explained by differences in microstructure (e.g. bovine enamel is more porous[31]) and chemical composition (e.g. bovine enamel contains more carbonate[15] but less fluoride[32] than human enamel). Further differences between tissues and similarities to present findings were discussed very recently[18] and are therefore not mentioned here.

Future studies on dental hard tissue substrates should explore the correlation between different analytical techniques more comprehensively and by studying e.g. lesions with different mineral distributions, laminations, with hypomineralized surface layers in comparison to natural white spot lesions over a broader range of ΔZ and by investigating changes in lesion microstructure and chemical composition throughout the de- and remineralization process to develop a more thorough understanding of dental caries in general.

In conclusion, lesions in bovine in comparison to human enamel formed faster, although with the resulting lesions being almost indistinguishable in their mineral distribution characteristics. Early caries lesion demineralization can be sufficiently studied by SMH as these data correlated well

with TMR variables ΔZ and L. However, the limitations of SMH on the assessment of the mineral status of more demineralized lesions must be considered. Likewise, it must be borne in mind that SMH does not necessarily measure mineral content but mechanical properties and structural integrity instead. Ideally, complementary techniques to assess changes in both physical and chemical lesion characteristics would be employed.

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Conflict of interest statement

The authors declare no conflict of interest.

Ethical approval

Not required

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Table 1. Least square means, standard error of the least square means and results of the statistical analyses for all study variables

Enamel	Demineralization time [h]	IL_{base}(Knoop) [µm]	ΔIL(Knoop) [µm]	IL_{base}(Vickers) [µm]	ΔIL(Vickers) [µm]	ΔZ [vol%min×µm]	L [µm]	R [vol%min]	SZ_{max} [vol%min]
Human	8	42.5	29.6 D[1]	33.3	10.5 D	403 D	16.7 E	23.1 B	63.8 A
	16	42.5	40.7 C	33.5	21.7 C	**556** C	21.2 D	26.3 A	59.9 B
	24	42.5	46.7 B	33.3	28.8 B	**697** B	27.7 C	25.4 A	59.3 B
	32	42.6	48.1 B	33.4	31.0 B	**799** B	30.4 C	26.5 A	58.3 B
	40	42.6	55.8 A	33.5	40.9 A	**1025** A	37.4 B	27.3 A	55.6 B
	48	42.6	54.0 A	33.4	39.4 A	**1132** A	41.0 A	27.6 A	57.1 B
	overall	**42.5**	**45.8**	**33.4**	**28.7**	**769**	**29.1**	26.1	**59.0**
Bovine	8	42.0	36.2	33.5	15.4	526 f	25.7	22.3	55.0
	16	42.0	53.0	33.7	28.2	**803** e	30.6	26.4	49.6
	24	42.0	60.8	33.8	38.1	**1059** d	41.3	25.9	48.1
	32	42.0	62.9	33.6	41.1	**1189** c	46.9	25.8	53.0
	40	42.0	71.7	33.7	52.6	**1427** b	52.1	28.0	50.7
	48	42.0	69.4	33.4	50.3	**1605** a	59.5	27.3	53.2
	overall	**42.0**	**59.0**	**33.6**	**37.6**	**1102**	**42.7**	25.9	**51.6**
SEM[2]		0.3	2.0	0.1	1.4	45	2.2	0.9	1.5
Enamel[3]		***<0.001***	***<0.001***	***0.003***	***<0.001***	***<0.001***	***<0.001***	*0.847*	***<0.001***
Demineralization time		*0.999*	***<0.001***	*0.522*	***<0.001***	***<0.001***	***<0.001***	***<0.001***	***0.002***
Enamel × Demineralization time		*1.000*	*0.205*	*0.549*	*0.099*	***0.003***	*0.215*	*0.955*	*0.077*

[1]Significant differences between demineralization times within enamel types are highlighted by different letters (capital letters for human, small letters for bovine enamel), and differences between enamel types within demineralization times or overall in bold. For variables where both factors were significant but the two-way interaction was not (ΔIL(Knoop), ΔIL(Vickers), L, R, SZ_{max}), the individual means are presented for information only. Here, the results of the statistical analysis are irrespective of the enamel type and are therefore only presented once ('human enamel' rows). Likewise, as IL_{base}(Knoop) and IL_{base}(Vickers) were only affected by enamel type and R by demineralization time, these individual means are presented for information only.

[2]Standard error of the least square mean (SEM) for each study variable. As the SEM was identical within groups for each variable (two-way ANOVA will yield a pooled SEM), this value is presented only once per column for better clarity.

[3]*p* values for each factor and interaction between factors.

Table 2. Pearson correlation coefficients for all comparisons between study variables (combined human and bovine enamel data)

	ΔIL(Knoop)	IL_{base}(Vickers)	ΔIL(Vickers)	ΔZ	L	R	SZ_{max}
IL_{base}(Knoop)	-0.093	**0.272**[1]	0.010	0.027	0.035	0.002	-0.003
	p=0.214	***p<0.001***	*p=0.893*	*p=0.728*	*p=0.652*	*p=0.982*	*p=0.964*
ΔIL(Knoop)		**0.304**	**0.905**	**0.782**	**0.650**	**0.440**	**-0.502**
		p<0.001	***p<0.001***	***p<0.001***	***p<0.001***	***p<0.001***	***p<0.001***
IL_{base}(Vickers)			**0.220**	**0.196**	**0.171**	0.112	**-0.320**
			p=0.003	***p=0.010***	***p=0.024***	*p=0.143*	***p<0.001***
ΔIL(Vickers)				**0.854**	**0.724**	**0.438**	**-0.412**
				p<0.001	***p<0.001***	***p<0.001***	***p<0.001***
ΔZ					**0.918**	**0.382**	**-0.485**
					p<0.001	***p<0.001***	***p<0.001***
L						0.023	**-0.415**
						p=0.768	***p<0.001***
R							**-0.338**
							p<0.001

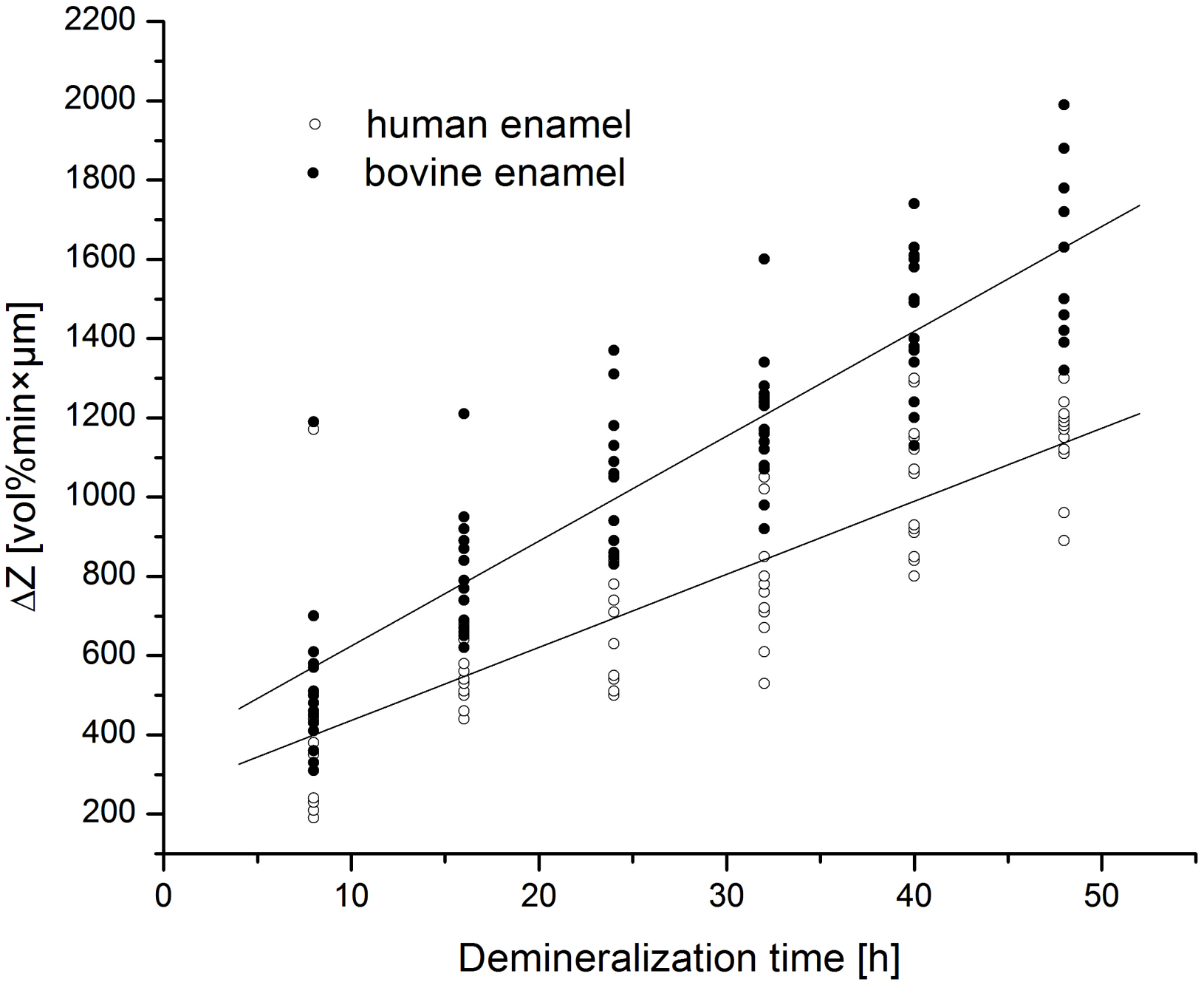
[1]Statistically significant interactions between variables are highlighted in bold.

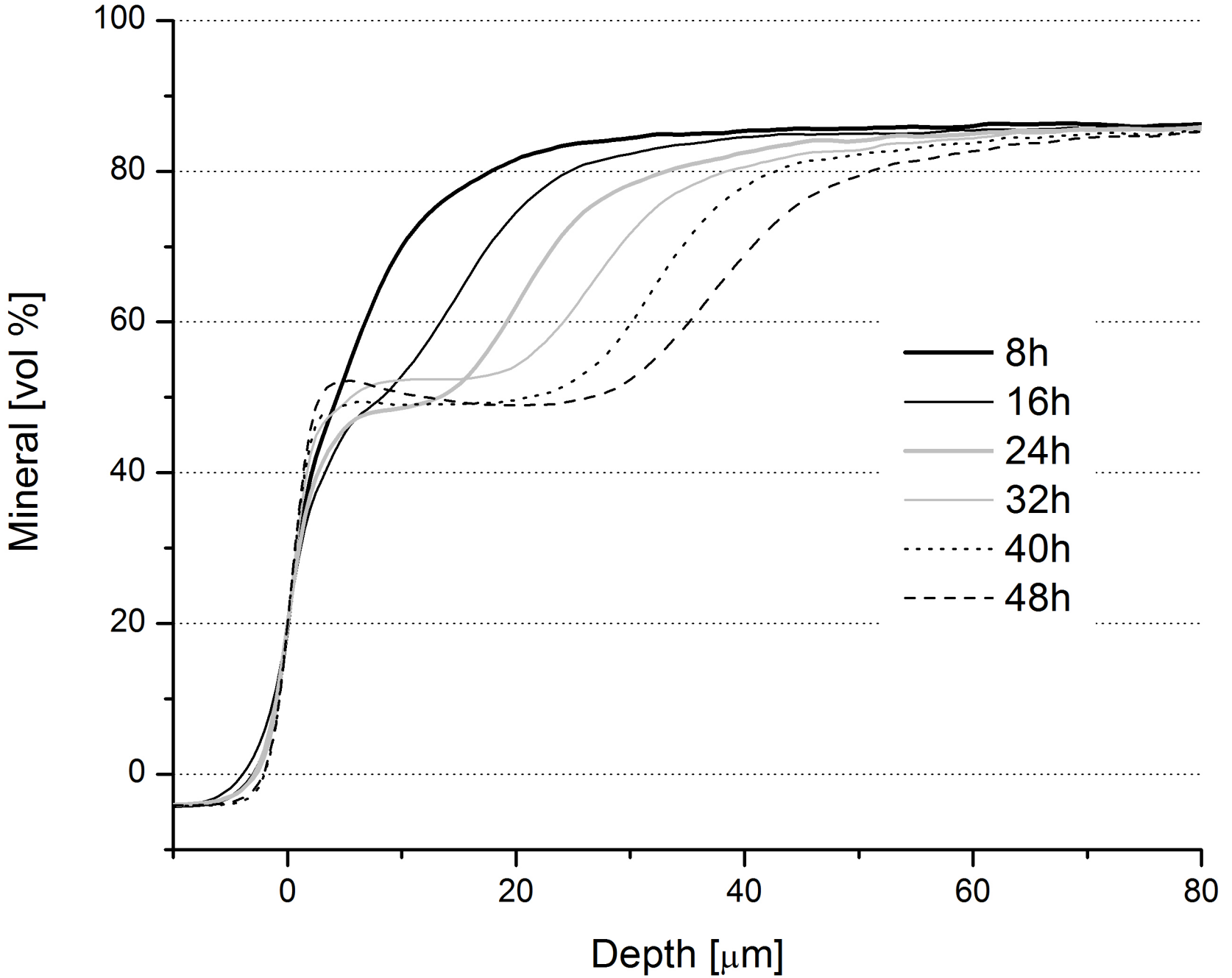
Figure legends

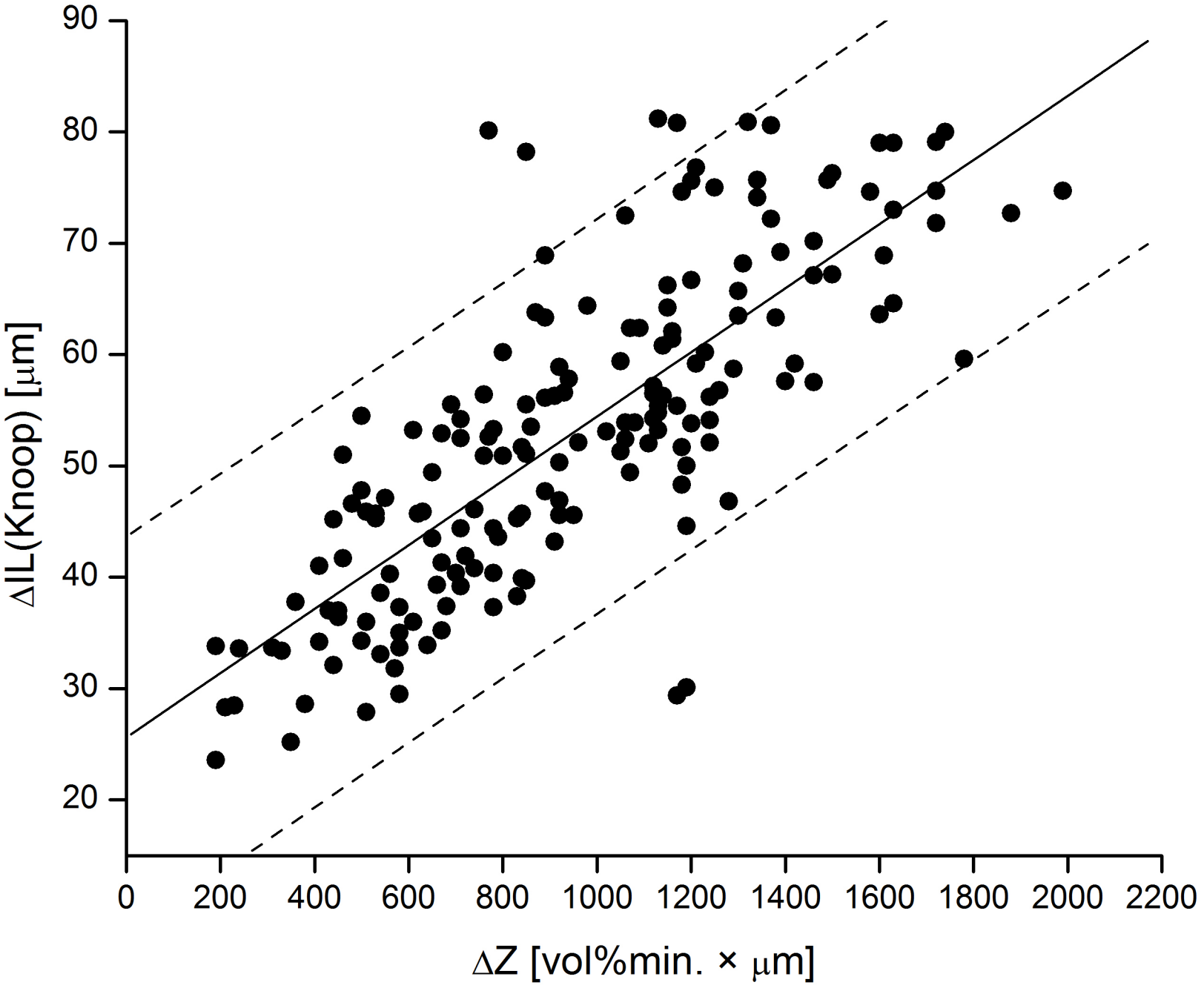
Fig. 1 – Human and bovine enamel demineralization as a function of demineralization time with corresponding linear fits.

Fig. 2 – Mean mineral distribution for bovine enamel lesions as a function of demineralization time.

Fig. 3 – Scatter plot of ΔIL(Knoop) against ΔZ. Solid line = linear regression; dashed lines = 95% prediction interval boundaries.


human enamel
bovine enamel
ΔZ [vol%min×µm]
Demineralization time [h]
2200
2000
1800
1600
1400
1200
1000
800
600
400
200
0
10
20
30
40
50


Mineral [vol %]
100
80
60
40
20
0
0
20
40
60
80
Depth [μm]
8h
16h
24h
32h
40h
48h


90
80
70
60
50
40
30
20
ΔIL(Knoop) [μm]
0
200
400
600
800
1000
1200
1400
1600
1800
2000
2200
ΔZ [vol%min. × μm]