

Viability of Using Enamel and Dentin from Bovine Origin as a Substitute for Human Counterparts in an Intraoral Erosion Model

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This study ascertained whether under dental erosion models that closely mimics the real-life situation enamel and root dentin from bovine origin would be reliable substitutes for human counterparts. Through a 2x2 crossover design, in a first trial, 14 volunteers wore a palatal device containing slabs of bovine and human enamel. Half of the participants ingested (4x daily, for 10 days) orange juice first, crossing over to mineral water, while the remainder received the reverse sequence. In a second trial, volunteers wore devices with slabs of bovine and human root dentin. Except for the duration of each intraoral phase, which lasted 2 rather 10 days, the experiment with root dentin run exactly as for enamel. Dental substrates were analyzed for surface microhardness. Two-way ANOVAs ($\alpha=0.05$) indicated no difference between the microhardness values recorded for human and bovine enamel ($p=0.1350$), but bovine root dentin had lower microhardness compared to its human counterpart ($p=0.0432$). While bovine enamel can reliably substitute its human counterpart in *in situ* dental erosion models, bovine root dentin does not seem to be a viable alternative to the corresponding human tissue.

Key Words: dental erosion, bovine teeth, human teeth, microhardness, intraoral.

INTRODUCTION

Owing to the increasing prevalence of dental erosion, a pathological chronic loss of dental hard tissues due to chemical influence of extrinsic and intrinsic acids without the involvement of microorganisms, a substantial effort has been devoted to modeling this lesion, both under laboratory and *in situ* conditions. However, some experimental parameters of dental erosion studies are still controversial or yet to be elucidated, including the choice of the type of hard tissue substrate. This issue has arisen mainly due to ethical constraints in using human teeth, despite the assumption that they are merited to be the most appropriate source of hard tissue substrate from the perspective of clinical relevance (1).

Although there are uncertainties in estimating whether and, if so, to what extent bovine teeth reflect the human counterparts in erosion models, the formers have gained increasingly widespread use. The rationale

for using bovine enamel is manifold: i) it is readily available (2); ii) has a more uniform composition (2); iii) its crystallite orientation matches that of human enamel (3); iv) its weight percentage calcium content is equivalent to that of human enamel and shows a similar, gradual decrease from the surface to the dentine-enamel junction (4); v) its matrix proteins are composed of amino acids that resemble that of human enamel (5). However, bovine and human enamel differ in some aspects: i) the latter has a keyhole arrangement of the prisms, while inter-row sheets or lamellar sheets tend to occur in the unguates (6); ii) crystallites of bovine enamel are 1.7 times thicker (7); iii) bovine teeth have a wider interprismatic region (8).

Despite the fact that bovine enamel has been considered as a promising substitute for human enamel (1), findings in the erosion literature have been equivocal. While in an *in vitro* study (9) surface ultrastructure of bovine and human enamel following erosive episodes

was indistinguishable, in another investigation eroded lesions have been shown to progress twice as fast in bovine than in human enamel (10). As a result, enamel originated from cattle has been suggested to wear more than human enamel under laboratory conditions (11). In addition, in a combined *in-* and *ex-situ* erosion model, although microhardness changes observed for bovine and human enamel had been of significantly different magnitudes, these substrates were considered alike (12).

Regarding root dentin, it has been suggested that by having a significantly higher tubule density, the bovine substrate seems to be a less suitable substitute for human root dentin (13). In fact, bovine root dentin has been shown to have lower microhardness than the human counterpart (14). However, even so, after a de-mineralization intraoral caries model those substrates did not differ from each other in terms of mineral loss and lesion depth (14). In the only paper comparing the erosive/abrasive wear of dentin from human and bovine origin, the latter were found to be an acceptable alternative (15). Although as an *in vitro* investigation it represents an important first step in gathering an insight into the viability of using bovine dentin, it is still unexplored if this result holds under conditions that approaches the clinical reality.

In view of the still open question on the suitability of using bovine dental substrates in erosion studies, this investigation was devised to ascertain, in a systematic fashion, through a completely *in situ* erosion model, whether the microhardness of enamel and root dentin from bovine teeth would be similar to that of human teeth.

MATERIAL AND METHODS

Experimental Design

Two independent, randomized, 2-period, crossover trials were conducted. In a 10-day crossover study, the factors examined were: 1) *dental enamel* at 2 levels (bovine and human) and 2) *consumed beverage* at 2 levels (orange juice and mineral water - control). After a 2-day lead-in period, during which the volunteers used only the toothpaste and toothbrush supplied by the researchers, the participants were randomly allocated to ingest either orange juice or mineral water during 10 working days. This was followed by a 2-day washout period. Volunteers were then crossed over to imbibe the alternate beverage for further 10 working days. Half of the participants received the sequence of orange juice first, crossing

over to mineral water, while the remainder received the reverse sequence. In second trial, the human and bovine slabs were from root dentin rather than from enamel. The experimental design was essentially the same, except that each intraoral phase lasted 2 days. The response variable was Knoop surface microhardness values (SMH).

Volunteers and Ethical Aspects

Fourteen volunteers took part in each trial. The experimental sample of the study aimed to compare human and bovine enamel comprised 11 females and 3 males (aged 20-31 years), while 7 females and 7 males (aged 21-44 years) participated in the root dentin trial. Participants were enrolled after providing written informed consent to the protocols reviewed and approved by the Ethics Committee of the Ribeirão Preto Dental School, University of São Paulo (Processes #2005.1.551.58.5 and #2006.1.762.58.7). Volunteers were eligible if they exhibited no tooth wear lesions, caries activity or periodontal disease and showed mean stimulated saliva flow rate ≥ 0.7 mL/min. Ineligible were subjects wearing fixed or removable orthodontic appliances. Women who were pregnant or breastfeeding were not included.

Preparation of Specimens

Thirty-five bovine incisors and human third molars, stored in saturated aqueous thymol solution, with no coronal cracks or enamel malformations were used in this study. Teeth were scraped of any remaining soft tissues, polished with pumice slurry, and sectioned at the cemento-enamel junction, using a low-speed water-cooled diamond saw (Isomet 1000; Buehler Ltd., Lake Bluff, IL, USA). Each tooth was cut mesiodistally and buccolingually to obtain 2 crown and 2 root slabs measuring 3 x 3 x 2 mm. Sectioned pieces were mounted on acrylic rods with sticky wax and ground on a water-cooled lapping and polishing unit (Beta Grinder-Polisher; Buehler Ltd.) with aluminum oxide abrasive papers (600- and 1200-grit) and polished with a 0.3- μ m alumina suspension. Slabs were then cleansed ultrasonically in deionized water for 10 min to remove any residues of the polishing procedure.

Specimens were sterilized with ethylene oxide at 55°C and thoroughly aerated to remove all traces of residual sterilant. A careful light microscopic examination took place on all sections to exclude any of them which did not reveal an intact surface.

To standardize the test pieces, slabs were pretested using an HMV-2 microhardness apparatus (Shimadzu Corp., Kyoto, Japan). Five Knoop microhardness indents were made in a linear fashion along the vertical center line, spaced 200 μm apart. In enamel, a 25-g indentation load was applied for 30 s, while in root dentin indents were at 10 g for 10 s. For both the enamel and root dentin trials, 56 out of the sets of 70 bovine and human sectioned pieces were selected based on the averaged SMH data.

Intraoral Procedures

In the screening visit, an experienced examiner performed oral examinations. During the same visit, an alginate impression of the maxillary and mandibular arches of each subject was taken. The impressions were then poured with die stone to fabricate the study casts and an upper acrylic removable appliance. To accommodate slabs, appliances had 2 retention slots on either side of the midline. The specimens were mounted in such a way that the sections were recessed 1.0 mm below the surface of the appliance to avoid tongue friction.

Qualifying subjects started a 2-day lead-in period for their first randomly assigned treatment leg. Each subject was instructed to use standard toothbrushes and fluoride toothpaste. At the conclusion of the lead-in period, to allow salivary pellicle formation, volunteers started wearing the appliance 1 h prior to imbibing the randomly assigned beverage (orange juice or mineral water) for 10 working days in the crossover trial with enamel or for 2 days in the study with root dentin. The subjects ingested the allocated drink as 250 mL volumes 4 times *per* day when the drink was sipped, under supervision, over a 10-min period. The drinking times were 9 a.m., 11 a.m., 1 p.m. and 3 p.m. with a window of 30 min at each time point, following the methodology described by West et al. (16). Drinks were consumed at room temperature. The commercially available, ready-to-drink orange juice (Suco Fazenda Bela Vista, Tapiratiba, SP, Brazil) had pH 3.74 and contained no added water, sugar or preservatives/additives. The mineral water (Minaura; Villas Boas Mineração Ltda, Sta Rosa de Viterbo, SP, Brazil) had pH value of 6.10.

Prevention of plaque accumulation was achieved by soaking the appliances, with contained specimens, in 0.2% chlorhexidine mouthrinse for 3 min at the beginning and end of each study day.

On completion of period one, the specimens were removed for analysis and appliances were refilled with

a new set of human and bovine enamel or root dentin specimens, depending on the trial. Volunteers were then crossed over to ingest the alternate beverage for further 10 or 2 days (for enamel and root dentin studies, respectively). A washout period of 2 days was allowed between the first and second periods.

While the appliances were in place, the subjects were only permitted to ingest the assigned beverage. Volunteers wore their appliances from 8 a.m. to 5 p.m. continuously except during mealtimes or carrying out oral hygiene procedures. Appliances and contained specimens were maintained on moist paper lining in sealed containers when removed from the mouth or overnight. Participants were instructed to refrain from using any fluoridated products or mouthrinses.

SMH Measurement

Microhardness measurements at the post-beverage intake stage were performed as outlined previously, but located 500 μm right from the midline.

Statistical Analysis

All statistical procedures were performed with Statgraphics Centurion XV at a significance level of $\alpha=0.05$. After checking if no carry-over or period effects existed, homogeneity of variance and normal distribution of errors had been confirmed for both series of experiment, split-plot ANOVA were carried out to check for the existence of significant effects of the *dental substrate, consumed beverage* and their interaction.

RESULTS

Microhardness data (mean values and standard deviations), expressed in KHN, are summarized in Table 1.

At the pre-beverage intake stage, no difference was found between human and bovine slabs for either enamel ($p=0.2346$) or root dentin ($p=0.0901$).

At the post-beverage intake, for the series of experiment with enamel, there was neither carry-over ($p=0.1697$) nor period effect ($p=0.3220$). Split-plot ANOVA ($\alpha=0.05$) indicated no interaction between the main factors (dental substrate and consumed beverage) and no difference between the microhardness values recorded for human and bovine enamel ($p=0.1350$). There was a difference between the microhardness of the enamel samples as a function of the different

drinks ($p < 0.0001$); orange juice resulted in significantly more softening than mineral water. Volunteers differed significantly from each other ($p = 0.0478$).

In the series of experiment with root dentin, no carry-over or period effect was observed ($p = 0.0980$ and $p = 0.1980$, respectively). There was no interaction between the main factors ($p = 0.0791$). Significantly lower SMH values were observed for specimens exposed to orange juice ($p > 0.0001$). Bovine root dentin exhibited lower SMH values than the human counterpart ($p = 0.0432$).

DISCUSSION

The choice of the hard tissue substrate remains one of the outstanding issues in *in situ* dental erosion models. This problem formed the basis of the present randomized crossover trials, which were designed to test the main hypothesis that human enamel and root dentin would be suitably replaceable by their bovine counterparts. To reach this goal, this investigation employed the validated *in situ* enamel erosion method designed by West et al. (16), and an adapted version of their protocol to create erosive lesions in root dentin. This methodology was preferred over any other combined in- extraoral model because it mimics more appropriately the real-life situation. Unlike under *in-ex-vivo* conditions, in which slabs have total contact with the beverages before taking the appliance back in the mouth, causing exaggerated effects, in this exclusively *in situ* study specimens were exposed to a passing acid fluid mixed with saliva.

At the pre-beverage intake stage, both in human

and bovine enamel, the Knoop hardness amounted to some 420 KHN, values slightly higher than those previously reported (17,18), which may be attributed to the fact that the enamel outer surface was only minimally removed during specimen preparation. In effect, it has already been demonstrated that microhardness of enamel varies with depth (18). At the post-beverage intake stage, despite the higher porosity of bovine enamel, this substrate showed microhardness values indistinguishable from that of the human counterpart. This result corroborates previous observations by Meurman and Frank (9), who did not find any noticeable morphological difference between eroded enamel specimens originated from cattle or human beings. On the other hand, the present finding is inconsistent with the results of an *in vitro* study in which progression of erosive lesions in bovine enamel was observed to occur twice faster (10). This may be ascribed to the fact that although bovine enamel may have been more susceptible to demineralization due to its wider interprismatic region, intraorally this structural difference may not have played a role. This is because differently from the *in vitro* condition, dilution, clearance, neutralization and buffering by saliva took place shortly after the beverage was in contact with dental tissues (19). In addition, other protective mechanisms, such as the one provided by the acquired salivary pellicle and by the calcium, phosphate and fluoride ions in saliva (19), may also have limited acidic diffusion through bovine enamel, despite its higher porosity.

Regarding root dentin, in the pre-beverage intake phase microhardness values (51 KHN) of human origin was in close agreement with a previous work, but those observed for bovine specimens were somewhat higher (14). At the post-beverage intake stage, root dentin of bovine origin differed from that of human provenience, signaling that the higher tubule density of bovine root dentin may be decisive for the progression of erosion. However, one should bear in mind that such result may not hold for other methods used to measure erosion. In fact, in terms of wear, evidence exists that bovine dentin can be considered an acceptable substitute for human counterparts (15), at least from an *in vitro* study which though may not reflect the clinical reality (20).

Although the present investigation was run in a highly controlled fashion, especially through the monitoring of beverage intake by volunteers, erosion lesion formation was subjected to interindividual variations. This may have been due to the extent to

Table 1. Microhardness data (mean values and standard deviations) at the pre- and post-beverage intake stage for each experimental group, expressed in KHN.

Substrate	Stage		
	Pre-beverage intake	Post-water intake	Post-orange juice intake
Human enamel	425 (23)	418 (28)	265 (104)
Bovine enamel	413 (47)	373 (45)	253 (108)
Human root dentin	51 (8)	51 (8)	20 (8)
Bovine root dentin	48 (6)	43 (8)	19 (6)

Vertical lines connect means that do not differ significantly.

which salivary protective factors come into play during an erosive challenge in the different volunteers. Another worth mentioning aspect is that even testing the groups in duplicate to lower the variability, the coefficient of variation of eroded substrates was of the order of 39%, as indicated by the standard deviations. This is unlikely to have occurred due to biological variation of the specimens, as variability among them was minimized by the selection of pretested slabs, whose deviations did not exceed 16% of the mean values. An explanation for the dispersion of the data may then be found in the fact that the drinking patterns and tongue positioning during drinking may vary among subjects.

In conclusion, this study has demonstrated the suitability of using bovine enamel as a substitute for its human counterpart in intraoral erosion models. Conversely, bovine root dentin did not seem to be a viable alternative to the corresponding human tissue.

RESUMO

Este estudo visou avaliar, sob um modelo de erosão dental que se aproxima da realidade clínica, se o esmalte e a dentina radicular bovinos seriam substitutos viáveis aos correspondentes substratos de origem humana. De acordo com um delineamento crossover 2x2, 14 voluntários utilizaram dispositivos palatinos contendo fragmentos de esmalte humano e bovino. Metade dos participantes ingeriu suco de laranja (4x/dia, por 10 dias) e, a seguir, alternou para a ingestão de água mineral, enquanto os demais voluntários receberam a seqüência reversa. Em um segundo experimento, os sujeitos da pesquisa fizeram uso do dispositivo palatino contendo fragmentos de dentina radicular bovina e humana. Exceto pela duração de cada uma das duas fases experimentais (2 ao invés de 10 dias), utilizou-se o mesmo protocolo empregado no estudo em que se comparou o esmalte. Os substratos dentais foram avaliados quanto a sua microdureza superficial. ANOVAs a dois critérios ($\alpha=0,05$) não indicaram diferença entre os valores de microdureza observados para o esmalte humano e bovino ($p=0,1350$), porém a dentina radicular apresentou microdureza inferior à humana ($p=0,0432$). Enquanto o esmalte bovino é um substituto fidedigno do substrato humano em modelos *in situ* de erosão dental, a dentina radicular bovina não parece ser uma alternativa viável ao tecido humano correspondente.

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