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ORIGINAL ARTICLE

Characterization of the bone matrix and its contribution to tooth loss in human cadaveric mandibles

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Abstract

Objective. It is uncertain as to what extent the major bone matrix constituents, mineral and collagen, show inter-individual variation and dependence on age and sex in jawbones. The purpose of this study was to clarify this uncertainty using cadaveric mandibles and investigate the association of bone matrix with the number of existing teeth. ***Materials and methods.*** Cortical bone samples (1 × 1 cm) collected from the mental of 48 cadaveric mandibles (27 men and 21 women; age range = 56–93 years and 63–103 years, respectively) were used to quantify three bone matrix indices: mineral content, collagen content and extent of lysine hydroxylation of collagen. Associations with age and comparisons by sex were evaluated based on bone matrix indices and the numbers of existing teeth. The numbers of existing teeth were compared between the groups showing low and high bone matrix index values. ***Results.*** A great amount of inter-individual variation was seen in all bone matrix indices. No bone matrix indices were associated with age, while the number of existing teeth was negatively associated with age. The bone matrix indices and number of existing teeth did not differ by sex. The number of existing teeth was nearly twice as high in the group showing high collagen content as in the low collagen group; however, an analysis of covariance showed a significant inter-group difference not from bone matrix indices, but rather from age. Interestingly, in comparison to femoral collagen, mandibular collagen showed lower lysine hydroxylation, which can represent an aspect of bone quality. ***Conclusions.*** Mandibular bone matrix shows great inter-individual variation and is independent of age and sex, but did not show as strong a relationship with tooth loss as age. Even so, mandibular collagen may represent a unique characteristic of bone matrix and deserves to be further investigated.

Key Words: *age, collagen, lysine hydroxylation, mineral, sex*

Introduction

Bone fracture is a target area in orthopedics where extensive studies have been performed. It has been comprehensively explained that bone fracture is not only caused by decreased bone quantity, but also by impaired bone quality. In the material phase, bone quality is determined by the major bone constituents, minerals and collagen. Minerals provide bone stiffness and collagen allows for flexibility and tensile strength. More importantly, in the biological phase, minerals and fibrillar collagen are closely associated with each other, but the latter controls the former. Collagen forms fibrils and functions as a 3-dimensional template organizing the minerals' deposition and growth [1]. The organization of minerals and collagen is regulated by the nature and extent of the post-translational modifications of collagen, many of which are unique to collagen [2]. One such important modification is the hydroxylation of specific lysine (Lys) residues, since it determines the pattern of covalent intermolecular cross-linking and serves as a site for glycosylation [3,4]. Those modifications, in turn, can affect collagen maturation [4–6], fibrillogenesis and mineralization [3,4]. The organization of collagen and minerals controlled by such modifications yield the individual properties and strength of bone [7,8]. In fact, the bones of senile

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osteoporotic SAMP6 mice have shown strength reductions, concomitantly decreased collagen amounts and over-hydroxylation of collagen molecule Lys residues [9,10]. The over-hydroxylation affects the lateral packing of collagen molecules [11], thereby repressing collagen fibrillogenesis [10,12,13] and mineralization [13]. The elevated Lys hydroxylation (LH) levels are also observed in human bones with osteoporosis [14,15] and osteogenesis imperfecta [16]. Thus, a quantitative analysis of minerals and collagen could represent a partial measure of bone quality.

As jaw bones rarely exhibit fractures, dentistry is more concerned with age-dependent bone resorption and tooth loss resulting from resorption progressing in an extreme manner. Researchers have extensively focused on radiographic measurements of bone quantity, but whether bone quantity is associated with bone resorption and tooth loss remains inconclusive [17]. In addition to bone quantity, the status of the extracellular bone matrix might be a factor in the association; however, an analysis of the bone matrix of human jaw bones has not been investigated previously. At present, an extensive clinical analysis of bone matrix is not practical. One reason is the uncertainty of whether the mineral and collagen contents and extent of LH of collagen depend on aging and sex. The other reason is the requirement of surgical bone sampling. In terms of ethical issues and saving time and cost, a preliminary pre-clinical investigation is preferred. Cadaveric study can resolve these difficulties, because mineral and collagen are greatly stable and durable compared with other non-collagenous proteins in bone. Minerals cannot be eroded without living osteoclasts and osteocytes. Collagen possesses a triple-helical molecular structure and forms rigid fibrils through covalent intermolecular cross-links; moreover, in bone, the fibrils are tightly packed in the minerals, exhibiting stability and resistance against collagenases. Indeed, the cross-links of collagen in ligaments and cartilage can be preserved in formalin for 6 months [18] and, even without formalin fixation, are persistent and detectable in the bones of humans who lived 3500 years ago [19]. On the basis of these findings, an analysis of minerals and collagen can be performed using human cadaveric jaw bones fixed in formalin, which provides a suitable model for a preliminary pre-clinical investigation.

As the bone matrix in the jaw has not been thoroughly analyzed, it is important to determine the inter-individual variation of the matrix and its dependence on age and sex. The aim of the present study was to first evaluate the variations and dependence of bone matrix expressed by mineral and collagen contents and the extent of collagen LH using human cadaveric mandibles. The cadavers had varying numbers of remaining teeth, which were easy to quantify. Although tooth loss is caused by many reasons including caries; periodontitis; tooth fracture; requirements for orthodontic and/or comprehensive dental treatment; iatrogenesis; life-style choices, such as smoking and taste in food; and socioeconomic reasons; it could be partly explained as a terminal phenomenon caused by excessive bone resorption induced by individual poorness of bone quality. The second purpose was to preliminarily test the association of the number of remaining teeth with age, sex and bone matrix indices for future reference in clinical investigations.

Materials and methods

Bone sample preparation

A total of 48 human cadaveric mandibles obtained from 27 men (mean age = 76.7 years; range = 56–93 years) and 21 women (mean age = 83.9 years; range = 63–103 years) were used in this study (Table I). The cadavers were donated to the Department of Morphological Biology of Fukuoka Dental College for educational and academic purposes and gave written consent in advance. The cadavers had been preserved in 15% phosphate-buffered formalin at a pH of 7.4 (Wako Pure Chemical Ind., Osaka, Japan) for 2 years, at which point they were used for educational purposes by students and immediately after which they were used for this study. All cadavers were verified as having various causes of death, with the exception of bone diseases (Table I). All procedures were in accordance with the principles of the Declaration of Helsinki of 1964 (revised in 2004). Frontal cortical bones (1 × 1 cm in area) were collected from the mental region of the mandibles (Figure 1). A cortical bone was also collected from the femoral neck of 45 cadavers only for comparisons of amino acid composition with the mandibular bone.

Table I. Characteristics of the cadavers.

	All	Men	Women	*p*
Number of subjects	48	27	21	
Age (years), mean (SD)	79.9 (9.5)	76.7 (9.0)	83.9 (8.7)	0.008*
Cause of death (*n*)				
Respiratory	19	11	8	
Cardiovascular	7	3	4	
Cerebrovascular	5	2	3	
Malignant neoplasm	5	4	1	
Renal	4	2	2	
Hepatic	2	0	2	
Others	6	5	1	

*Derived from Student's *t*-test (men vs women).

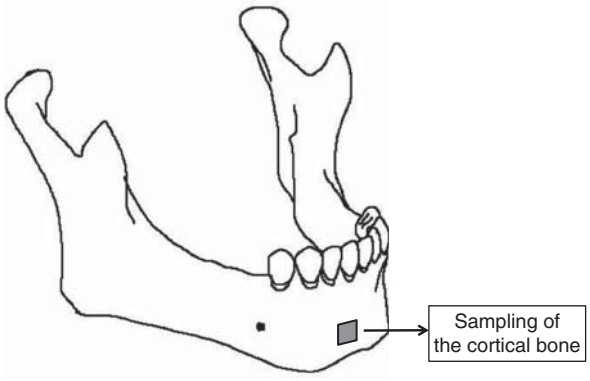

Figure 1. An illustration of cortical bone sampling site. Frontal cortical bones were collected from the mental region indicated by a dark grey area (1 × 1 cm). The collection site was far from the roots of the teeth to prevent contamination from the root and periodontal tissues. The collected bones were completely cleaned of soft and trabecular bone tissues.

Three cadavers that had received an orthopedic implant replacement were excluded from the femoral neck bone collection. The cortical bones were completely cleaned of soft and trabecular bone tissues and defatted with trichloroethylene for 6 days; the trichloroethylene was renewed every day. The specimens were dried by immersion in 100% ethanol for 3 days; the ethanol was renewed every day. The samples were pulverized under liquid N_2 using a Spex Freezer Mill (Spex, Metuchen, NJ), washed with cold distilled water and lyophilized until they formed a completely dried bone powder.

Measurements of mineral and collagen contents and the extent of LH of collagen

Approximately 10 mg of dried bone powder was ashed at 700°C for 6 h in a muffled furnace. The ashed segment was weighed and the ash weight, being the mineral content, was calculated as a percentage of the dried bone weight. To determine the collagen content and the extent of LH of collagen, amino acid analysis was performed [10]. Briefly, ~2 mg of dried bone powder was hydrolyzed with 6 N HCl and an aliquot of each hydrolysate was subjected to amino acid analysis using a high-performance liquid chromatography system (C-R7A/LC-10A; Shimadzu, Kyoto, Japan) with a strong cation exchange column (Shim-pack Amino-Li, Shimadzu, Kyoto, Japan). The amino acid composition of the bone matrix protein was expressed as residues per 1000 total amino acids. The collagen content was calculated based on the value of hydroxyproline (Hyp) and expressed as μg/mg of dried bone sample. The extent of LH of collagen was calculated as moles of hydroxylysine (Hyl) per mole of collagen (mol/mol), based on the value of 300 residues of Hyp in a collagen molecule.

Associations and comparison tests

The amino acid composition of the bone matrix protein was compared between the mandibles and femurs of the 45 cadavers described above. The existing teeth were counted, all of which possessed an intact or restored crown, without severe caries or mobility. To clarify whether there was age dependence, associations with age were evaluated based on mineral content, collagen content, extent of LH of collagen and the number of existing teeth. To identify whether there was a difference by sex, the values of the above indices were compared between men and women. To test the relationship between bone matrix indices and the number of existing teeth, the two groups were first categorized on the basis of the mean of each index: low mineral content (57.11–62.77%, n = 24) and high mineral content (62.98–73.28%, n = 24); low collagen content (100.0–158.9 μg/mg, n = 29) and high collagen content (162.9–283.8 μg/mg, n = 19); and low levels of LH (8.30–10.46 mol/mol, n = 22) and high levels of LH (10.54–12.84 mol/mol, n = 26). Then, the numbers of existing teeth were compared between the two groups.

Statistical analysis

All statistical analyses except analysis of covariance (ANCOVA) were performed using GraphPad Prism 5.02 software (GraphPad Software Inc., La Jolla, CA). Associations were tested by determining Pearson correlation values. Inter-group comparisons were performed using Student's t-test, Welch's t-test or a Mann-Whitney U-test. Data are expressed as the mean (SD). In addition, comparisons of the existing teeth between the low and high bone matrix index groups were performed using ANCOVA while controlling for age, sex and the other bone matrix indices as covariates. The data were analyzed using SPSS version 20.0 (IBM Corp., Armonk, NY). Associations and comparisons were considered to be significant at $p < 0.05$.

Results

Amino acid composition of the bone matrix of the mandibles and femurs

Table II shows the amino acid compositions of the mandibles and femurs expressed as residues per 1000 total amino acids. The profiles of the mandible and femur samples showed a similar pattern, except for histidine, Hyl and Lys, indicative of a collagen-rich matrix in the two bones, as a high number of Hyp residues specific for collagen was observed. The similarity of the number of Hyp and proline (Pro) residues between the mandibles and femurs shows that the two bones have a similar proportion of

Table II. Amino acid composition of the bone matrix of the mandibles and femurs.

Amino acid	Mandible	Femur	p*
Aspartic acid	47.2 (2.5)	47.5 (5.6)	NS
Hydroxyproline (Hyp)	80.8 (8.4)	82.2 (9.6)	NS
Threonine	14.3 (1.3)	14.7 (2.2)	NS
Serine	32.3 (2.0)	32.6 (3.6)	NS
Glutamic acid	75.8 (3.8)	76.8 (9.0)	NS
Proline (Pro)	126.0 (2.9)	123.7 (13.8)	NS
Glycine (Gly)	329.2 (23.3)	327.2 (73.1)	NS
Alanine	149.6 (10.3)	148.0 (15.4)	NS
Valine	21.8 (5.3)	23.1 (5.9)	NS
Methionine	ND	ND	
Isoleucine	6.6 (1.9)	6.9 (2.2)	NS
Leucine	25.6 (1.6)	27.0 (4.7)	NS
Tyrosine	ND	ND	
Phenylalanine	14.2 (0.9)	14.0 (2.4)	NS
Histidine	4.8 (0.8)	5.3 (1.1)	0.040
Hydroxylysine (Hyl)	2.8 (0.2)	4.1 (4.1)	< 0.0001
Lysine (Lys)	20.9 (1.5)	19.7 (4.3)	0.049
Arginine	48.2 (3.1)	47.2 (9.2)	NS

The mean values (SD) (n = 45) are shown as relative amounts in 1000 total residues.
*Derived from a Mann-Whitney U-test.
ND, no detection; NS, no significant difference.

collagen content among bone matrix proteins and comparable amounts of Pro hydroxylation, one of the important post-translational modifications of collagen. Interestingly, the mandibles exhibited a significantly lower number of Hyl residues, but a higher number of Lys residues than the femurs, implying that Lys hydroxylation, another important post-translational modification of collagen, occurs less in the mandible than in the femur.

Inter-individual variation in the bone matrix indices, the number of existing teeth, and their associations with age

There was great variation among individuals for all indices, including mineral content (57.11–73.28%, Figure 2A), collagen content (100.0–283.8 μg/mg, Figure 2B), extent of LH (8.30–12.84 mol/mol, Figure 2C) and number of existing teeth (0–14, Figure 2D). No significant association with age was seen for mineral content (r = 0.24, p = 0.11), collagen content (r = −0.03, p = 0.84) or extent of LH (r = 0.01, p = 0.93), implying the independence of all bone matrix indices from aging. In contrast, the number of existing teeth exhibited a significant negative association with age (r = −0.45, p = 0.001, Figure 2D), indicating that the number of teeth tends to decrease with increasing age. The tendencies of association with age did not differ by sex. The mineral and collagen contents were not significantly correlated (r = 0.16, p = 0.29).

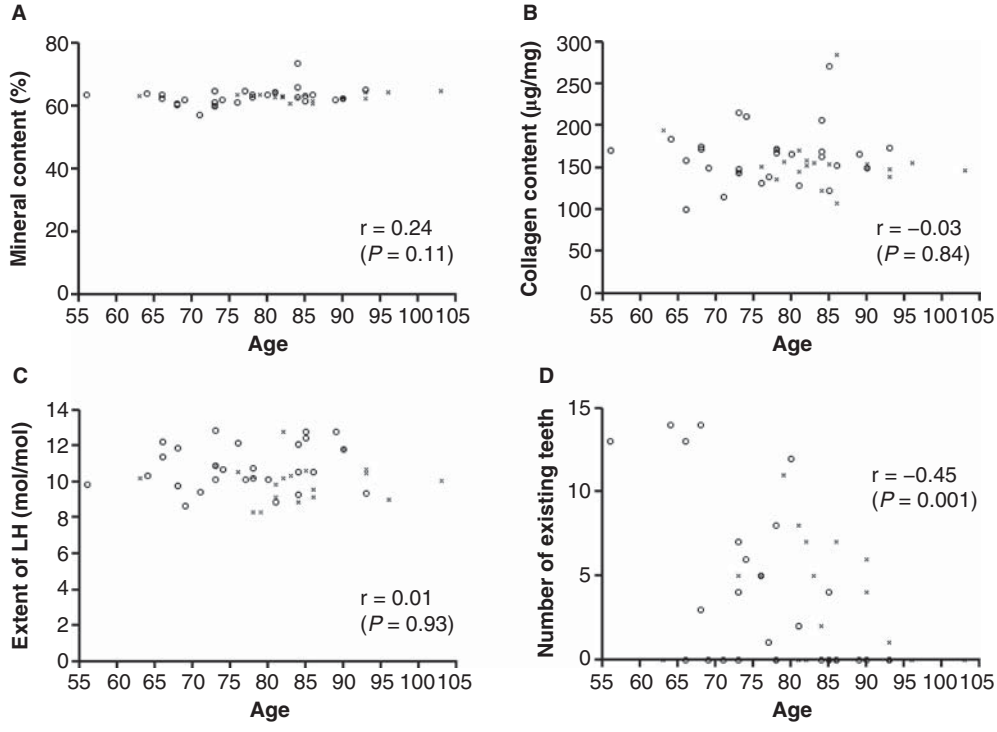

Figure 2. Associations between age and mineral content (A), collagen content (B), extent of LH (C) and the number of existing teeth (D). The open circles and crosses represent men and women, respectively.

Table III. A comparison of bone matrix indices and the number of existing teeth by sex.

	All	Men	Women	p*
Mineral content (%)	62.85 (2.22)	62.92 (2.76)	62.75 (1.27)	0.77
Collagen content (μg/mg)	160.7 (34.2)	163.7 (34.7)	156.8 (34.0)	0.49
Extent of LH (mol/mol)	10.51 (1.25)	10.81 (1.27)	10.14 (1.15)	0.07
Number of existing teeth	3.48 (4.44)	3.93 (5.11)	2.91 (3.45)	0.44

Values are expressed as the mean (SD).
*Derived from Student's or Welch's *t*-test (men vs women).

Comparisons of the bone matrix indices and the number of existing teeth by sex

There was no significant difference in mineral content ($p = 0.77$), collagen content ($p = 0.49$), extent of LH ($p = 0.07$) or the number of existing teeth ($p = 0.44$) between men and women (Table III).

The relationship between bone matrix indices and the number of existing teeth

The high mineral content and extent of LH groups showed a greater number of existing teeth than the low mineral content and extent of LH groups, but no significant difference between the two groups ($p = 0.35$ for mineral content, $p = 0.73$ for extent of LH) using Student's *t*-test (Table IV). In inter-group comparisons of collagen content, nearly twice the mean number of existing teeth were found in the high group, 4.90 (5.29), than in the low group, 2.55 (3.60); however, they were not significantly different by Student's *t*-test ($p = 0.07$). ANCOVA with age, sex and other bone matrix indices as covariates showed no significant difference in the number of existing teeth between the two groups by mineral content ($p = 0.18$), collagen content ($p = 0.30$) or extent of LH ($p = 0.61$, Table IV). Among the covariates, only age exhibited statistical significance in inter-group comparisons of mineral content ($p = 0.001$), collagen content ($p = 0.005$) and extent of LH ($p = 0.002$).

Discussion

This is the first study to quantitatively analyze the major matrix constituents of cortical bone in human mandibles and revealed that the mineral content, collagen content and extent of LH of collagen showed great variation among individuals and was independent of age and sex. The age-independence of the collagen indices shown in the present study is consistent with previous findings from animal studies in which the collagen content in rat mandibular bones remained constant from 1 month to 2 years of age [20]; LH levels of collagen remained constant during the same age range in chick cranial and mandibular bones [21]. In adult humans, these collagen indices likely remain constant throughout life, unless the individuals experience extremely adverse dietary conditions, such as vitamin D and calcium deficiencies [22,23]. The age-independence of mineral content in mandibular cortical bone was also consistent with previous findings in human skeletal bones in which ash weight per dried bone weight was constant from adulthood through later life [24,25]. If these age-independences are true, these bone matrix index values can express an inherent and intrinsic characteristic of the individual from adulthood through later

Table IV. A comparison of the number of existing teeth between the groups showing low and high bone matrix values.

		Number of existing teeth				Covariate				
		Low group	High group			Age	Sex	Mineral content	Collagen content	Extent of LH
Index	n Low/high group	Mean (SD)	Mean (SD)	p*	p**	p***	p***	p***	p***	p***
Mineral content	24/24	2.88 (3.54)	4.08 (5.20)	0.35	0.18	0.001	0.58		0.37	0.22
Collagen content	29/19	2.55 (3.60)	4.90 (5.29)	0.07	0.30	0.005	0.51	0.90		0.38
Extent of LH	22/26	3.27 (4.72)	3.73 (4.19)	0.73	0.61	0.002	0.77	0.85	0.31	

Low and high groups were categorized on the basis of the mean of the bone matrix index.
n, number of subjects in the low/high group.
*Derived from Student's *t*-test.
**Derived from ANCOVA with age, sex and the other bone matrix indices as covariates.
***Derived from ANCOVA for each covariate.

life. The value obtained in young adult or in middle-aged individuals can be translated in older aged individuals. It should be noted that the collagen and mineral contents express bone quality only partly or, rather, superficially. The content of collagen appears to be associated with, but not necessarily responsible for, bone strength [26]. Inter-molecular cross-links of collagen are more responsible for bone strength, the amount of which changes with age [8,26,27]. According to aging, declining bone turnover leads to a higher ratio of mature/immature enzymatic cross-links and a greater amount of non-enzymatic cross-links composed of advanced glycation end-products (AGEs), both of which could induce the mechanical deterioration of bone. Bone strength is associated with mineral content to some extent; however, the more critical factors in bone mineral composition are the substitution of carbonate and acid phosphate for hydroxyapatite, hydroxyl content, calcium/phosphate molar ratio and crystal size/perfection, all of which change with age [27]. Thus, the material phase related to bone quality includes many factors and is difficult to express completely. The bone matrix analysis performed in this study is easy to perform in a clinical setting by only using ash weight and amino acid analyses. Although it only expresses a part of the bone quality or bone matrix status, this analysis could be a powerful tool to express the age-independent and intrinsic nature of individual bones and the data can be translated to later life.

The bone matrix data in this study has several limitations in interpretation. Bone mineralized tissue remodels actively and shows heterogeneity. Briefly, it is histologically composed of cortical and trabecular bone and microscopically composed of highly and poorly mineralized (old and young osteons) and non-mineralized (osteoid) areas. The heterogeneity results in variations of mineral and collagen status in each area [7,8]. We assessed cortical bone and excluded trabecular bone, as it is difficult to completely remove formalin-fixed soft tissues from trabecular bone. The data from the sample (1×1 cm in area) used in the present study is considered to be average of the heterogeneity. The data cannot be considered to be representative of the whole mandible, but, in fact, there is no suitable site in the mandible to express it as a whole. The mental region is a preferred site for dentists to collect bone grafts, because it contains a great amount of cortical and trabecular bone and is also the area demonstrating greatest tooth survival, both in general and as seen in this study. The data from the mental region is not the representative of the whole mandible, but could be representative from a clinical view.

The pathological conditions of the cadavers also result in some limitations interpreting the data. We were informed only of the diseases causing the death of the cadavers, but not of other systemic diseases. If some had diabetes, bone strength may have deteriorated through an increase of non-enzymatic collagen cross-link AGEs under a high sugar condition [8], although it is uncertain whether the mineral and collagen contents were influenced. If the three cadavers that received an orthopedic implant replacement of the femoral neck had suffered from osteoarthritis, the Lys residues could be over-hydroxylated [28]. However, the extent of LH of the mandibles in the three cadavers (8.83, 10.14 and 10.56 mol/mol) was comparable to that of the others (8.30–12.84 mol/mol), although samples from the femoral neck could not be assessed. In normal bones, the mineral and collagen contents usually show a negative correlation [29], but they were not correlated in the present study. Occasionally, no corresponding pattern is seen from an increase of non-collagenous proteins in some situations such as ovariectomy [30] and growth hormone administration [31]. The possibility cannot be denied that the data in the present study were influenced by the pathological conditions of the individual cadavers.

Formalin fixation is a well-established method for preserving bone matrix, but requires caution when interpreting the data. Boskey et al. [32] described that mineral dissolution would not be expected if the formalin is buffered at a neutral pH. Although the phosphate-buffered solution might be expected to alter the mineral composition by the adsorption of excess phosphate, the phosphate would tend to suppress mineral dissolution by shifting the equilibrium between mineral and calcium and phosphate ions to the left, leaving the composition unchanged. In fact, fixation in a formalin solution for 11 days did not alter the calcium/phosphate molar ratio or ash weight in bones compared to those frozen at -20°C for the same period [32]; and the crystallinity of the apatite was also unaltered [33]. Although the effects of longer periods such as 2 years have not been reported, the mineral content obtained in the present study is probably reliable.

Formalin cross-links proteins; it has been reported that collagen molecules became more closely packed within the fibrils without alterations in the collagen fibril distribution [34]. Boskey et al. [32] reported that formalin fixation for 3 days increased the amount of Hyp in bones compared to those frozen at -20°C for the same period and the amount was not altered by further fixation for 11 days. They speculated that the increase in the amount of Hyp likely results from the fact that formalin fixation prevents the loss of collagen biosynthesis products and degradation that occurs when processing fresh tissue for analysis. Indeed, formalin did not alter the amount of Hyp in nasal cartilage after a 9-week fixation [35]. Hyp is a specific residue of collagen. The fibrillar collagen molecule is composed of three α chains. In order for the α chains to form a triple helix, repetitive sequences of [glycine

(Gly)-X-Y]n amino acids are required; X and Y can be any amino acid, but often X is Pro and Y is Hyp. Gly, the smallest amino acid, is situated such that it can fit in the center of the triple helix in a very restricted space. Hyp is essential for the folding of procollagen α chains into triple helical molecules and to stabilize the triple helical conformation by providing hydrogen bonds and water bridges [36]. Hyp is the product of the hydroxylation of Pro catalyzed by prolyl hydroxylase. Most Hyp is 4-Hyp located at the Y position, produced by the action of prolyl 4-hydroxylase specific to the sequence X-Pro-Gly and a very small amount of Hyp is 3-Hyp at the X position, produced by the action of prolyl 3-hydroxylase specific to the sequence Pro-4-Hyp-Gly; in total, 40–45% of Pro is hydroxylated. In pure collagen in bone, Hyp amounts to ~100 residues per 1000 total amino acids [37]. In intact bone, because of the inclusion of non-collagenous proteins, it generally amounts to ~80 residues [38], comparable to that shown in the present study: 80.8 residues in the mandibles and 82.2 residues in the femurs. Although not an assessment of pure bone collagen, the calculated ratio of hydroxylation of Pro (Hyp/Hyp + Pro) was close to 40%: 39.1% in the mandibles and 39.9% in the femurs. Taken together, the assessment of Hyp and Pro in the present study does not appear to be degraded by formalin fixation.

Formalin cross-links some amino acids and turns them into derivatives. A previous amino acid analysis using bovine cartilage indicated that 10% phosphate-buffered formalin fixation for 9 weeks decreased the amount of tyrosine (from 5.1 to 0.6 residues per 1000 total amino acids), Hyl (from 17.6 to 10.4 residues) and Lys (from 5.1 to 2.5 residues), although it did not alter the amounts of other amino acids, including Hyp and Pro [35]. We did not detect tyrosine or methionine; if any were present, it would be a very small amount, 4–15 residues [37,38]. The former may be diminished because of formalin's cross-linking [35] and the latter may be oxidized from long-term preservation [39]. The most critical issue is the possibility that formalin fixation underestimates the amounts of Hyl and Lys. In fact, the total Hyl and Lys residues shown in the present study were 23.8 in the mandibles and 23.7 in the femurs, less than the 30–40 shown in previous studies from fresh bone [37,38]. Hyl and Lys amounts from formalin-fixed bones cannot be compared to those from fresh bones; however, it is thought that bones from different sites under the same fixation conditions can be compared [32]. The mandibles and femurs showed substantially similar amino acid compositions, indicative of similar bone matrix protein compositions. The two bone sites had similar amounts of total Hyl and Lys residues; additionally, the mandibles showed a smaller amount of Hyl and a greater amount of Lys. This implies that mandibles exhibit a lower hydroxylation of Lys from collagen compared to femurs. As fibrillar collagen in bone is mostly type I collagen with trace amounts of type III [14] and V collagens [40], mandibles likely have a lesser hydroxylation of Lys from mostly type I collagen and, if any, contain a smaller amount of type V collagen, which undergoes high Lys hydroxylation [40].

Despite the small number of cadaveric specimens, age was negatively associated with the number of existing teeth, suggesting that age is a strong contributor to tooth loss. The findings in the present study suggest that bone matrix indices are not a major contributor to tooth survival, as is age, but cannot deny the possibility that they are a minor contributor, in consideration of the many causes of tooth loss, small sample size and most of the cadavers who were old and had small numbers of teeth. If younger cadavers with greater numbers of teeth had been utilized, the association might be clearer, but remains to be investigated. Despite these limitations, especially for collagen content, the high group did not show a significantly higher value ($p = 0.07$ by Student's t-test, $p = 0.30$ by ANCOVA), but did show nearly twice the number of existing teeth than the low group. Collagen content appears to be only a partial or superficial contributor to bone quality, but has been reported to be associated with bone strength [9,26]. Collagen content can reflect an intrinsic property of individuals which could influence resistance to bone damage by bacterial infection and/or occlusal load, preservation of alveolar bone level and survival of teeth. The extent of LH of collagen can represent an aspect of bone quality. The hydroxylation of Lys, specific for collagen, is catalyzed by lysyl hydroxylases. The over-hydroxylation of Lys affects the lateral packing of collagen molecules [11], thereby suppressing collagen fibrillogenesis [10,12,13] and mineralization [13]. An elevated extent of LH is particularly observed in some bone diseases such as osteoporosis [14,15], osteogenesis imperfecta [16] and osteoarthritis [28]. The extent of LH observed in the present study may show a normal range, 8.3–12.8 mol/mol of collagen, quite lower than the ~25–40 mol/mol reported in osteoporotic bones [14,15]. However, it is not the time to make a judgement. The formalin fixation did not appear to influence the amounts of Hyp and Pro as described above; as a result, the amount of collagen based on 300 residues of Hyp was similarly uninfluenced. Therefore, the underestimated amount of Hyl caused by the fixation can be directly linked to the extent of LH. If fresh bones had been used, the findings would be convincing and the index could be attractive to detect bone matrix abnormalities.

The most relevant tissue that anchors teeth is the periodontal ligament and is beyond the scope of this study; bone should be considered as secondary.

Nevertheless, a relationship between mandibular bone mass and the loss of teeth and their surrounding bone has been extensively investigated because changes in oral bone mass and structure in accordance with aging may be a partial influence [23]. The present study focused on the mandibular bone matrix, which has not been previously investigated, and we envisage a clinical investigation examining the relationship of collagen indices in the bone matrix, not only with respect to the loss of teeth and their surrounding bone, but also the loss of dental implants and their surrounding bone. As the amino acid analysis required a 2-mg bone sample, the most frequent opportunity to collect bone samples will be during implant placement. Although it is uncertain if the bone matrix indices are a minor contributor, the great inter-individual variation of each index in the mandibular bones is an attractive finding for further investigation of the possibility. A future clinical study needs to collect fresh bones and information regarding other contributors to the loss of teeth and bones and systemic diseases.

In conclusion, human cadaveric mandibular bones showed great inter-individual variation in mineral and collagen content and in the extent of LH of collagen, but were independent of age and sex. Within the limitations of sample size, there was a significant association between the number of existing teeth and age, but not with bone matrix indices. These results suggest that, unlike age, bone matrix is not a major contributing factor to tooth loss.

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