



Review

A paradigm shift for bone quality in dentistry: A literature review



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ABSTRACT

Purpose: The aim of this study was to present the current concept of bone quality based on the proposal by the National Institutes of Health (NIH) and some of the cellular and molecular factors that affect bone quality.

Study selection: This is a literature review which focuses on collagen, biological apatite (BAP), and bone cells such as osteoblasts and osteocytes.

Results: In dentistry, the term “bone quality” has long been considered to be synonymous with bone mineral density (BMD) based on radiographic and sensible evaluations. In 2000, the NIH proposed the concept of bone quality as “the sum of all characteristics of bone that influence the bone’s resistance to fracture,” which is completely independent of BMD. The NIH defines bone quality as comprising bone architecture, bone turnover, bone mineralization, and micro-damage accumulation. Moreover, our investigations have demonstrated that BAP, collagen, and bone cells such as osteoblasts and osteocytes play essential roles in controlling the current concept of bone quality in bone around hip and dental implants.

Conclusion: The current concept of bone quality is crucial for understanding bone mechanical functions. BAP, collagen and osteocytes are the main factors affecting bone quality. Moreover, mechanical loading dynamically adapts bone quality. Understanding the current concept of bone quality is required in dentistry.

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1. Introduction

Bone tissue, which plays an essential role in skeletal homeostasis, responds to mechanical load. A well-known anatomist Georg Hermann von Meyer and a structural engineer and mathematician Karl Culmann discovered marked similarity between the trabecular structure of the proximal femur and stress trajectory patterns in 1867 [1]. Julius Wolff also found an association between trabecular morphology and stress trajectories in 1869 [2,3]. This famous theory is referred to as “Wolff’s law,” which indicates bone adaptation to mechanical load [4]. For instance, bones of the stroke forearm and hand increase bone density, diameter, and length compared with those of the contralateral arm of professional tennis players [5]. Moreover, athletes, who perform much strength training, have greater bone mineral densities (BMDs) than non-athletes [6]. Conversely,

although astronauts who had been in space 4–6 months demonstrated decreased bone mass ranging 2–9%, their BMDs recovered up to 50% within 9 months after returning to Earth due to gravity [7]. Therefore, mechanical load positively and negatively changes skeletal bone mass (bone quantity) and BMD.

Another famous theory, “mechanostat,” was proposed by Harold Frost in 1987 [8]. This theory states that bone strains induced by mechanical load determine bone reactions. Bone strains ≥ 1500 –3000 microstrain induce bone modeling to increase cortical bone mass, while strains < 100 –300 microstrain rapidly proceed basic multicellular unit (BMU)-based remodeling, which removes existing cortical and trabecular bone. BMU couples initial bone resorption to a final bone formation process. Normal lamellar bone is fractured when bone strain reaches 25000 microstrain [9]; 1000 microstrain indicates bone length change of 0.1% compared with the original length. Bone strains are converted to various mechanical stimuli such as fluid shear stress [10,11], hydrostatic pressure [12], and direct deformation of osteocytes that reside in the bone matrix [13–15], which suggest that osteocytes, but not bone itself, regulate bone homeostasis in response to mechanical load.

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Until 2000, bone strength was considered to be synonymous with BMD. However, a new clinical parameter, “bone quality,” was proposed by the National Institutes of Health (NIH) in 2000 [16]. Bone quality, which is defined as “the sum of all characteristics of bone that influence the bone’s resistance to fracture” is completely independent of BMD. Therefore, to determine bone strength, not only BMD but also bone quality must be evaluated. A lower BMD induces greater fracture risk in bone [17]. However, the relationship between increased BMD by antiresorptive therapy and reduced fracture risk is not proportional [18], indicating that increased BMD does not always lead to decreased fracture risk. The NIH defines bone quality as comprising bone architecture, bone turnover, bone mineralization, and micro-damage accumulation [19] (Fig. 1). In addition, recent investigations have proposed novel and promising bone quality parameters focusing on the bone microstructure such as osteocytes, biological apatite (BAP), and collagen fibers. Indeed, Nakano et al. proposed that BAP orientation is one of the major determinants of bone quality because BAP orientation strongly depends on the anatomical bone portion, especially in mandible, closely related to the *in vivo* stress distribution [20]. In addition, Ishimoto et al. clearly demonstrated that degree of BAP orientation is more strongly correlated with bone strength than BMD using a regenerative bone defect model in rabbit ulna [21]. Kuroshima et al. demonstrated that osteocytes, BAP, and collagen fibers could become new clinical parameters to evaluate bone quality in implant dentistry [22], suggesting that understanding bone quality is clinically relevant.

In dentistry, the effect of mechanical load on jaw bone has been well documented. Jaw bones constitutively receive functional loads such as mastication and swallowing and parafunctional loads such as grinding, clenching and tapping. Mandibular and palatal tori, which are bone outgrowths, are associated with mechanical stresses such as functional and parafunctional loads [23,24]. Orthodontic force via natural teeth dynamically proceeds bone modeling and remodeling around the teeth [25]. Occlusal force often acts as traumatic occlusion in patients with periodontitis, resulting in the destruction of periodontal tissue and alveolar bone [26]. Moreover, it is believed that occlusal overload may lead to bone loss around stable dental implants [27]. Some of the confusion surrounding the term “bone quality” is that this term has already been used in dentistry. In contrast to the current concept of “bone quality,” which is independent of BMD, “bone quality” in dentistry has largely been synonymous with BMD based on radiographic and sensible evaluations [28–30]. Although, the paradigm of bone quality has already shifted from BMD-based assessments to microstructural evaluations of bone, BMD-based diagnosis remains the gold standard in dentistry. Hence, accepting and understanding the current concept of bone quality is actually required in dentistry. However, further research based on this concept of bone quality is necessary prior to its clinical application in prosthodontic dentistry.

The aim of this literature review is to present the current concept of bone quality according to collagen, BAP orientation, and bone cells, such as osteoblasts, and osteocytes, to consider bone strength in dentistry and discuss innovative dental research based on the current concept of bone quality.

2. Bone quality based on collagen

2.1. Collagen in bone

Collagen is the main component of bone organic constituents. The composition and structure of collagen components have long

been recognized as important contributors to bone quality [31–33]. The importance of collagen components in bone mechanical properties has been demonstrated through irreversible collagen manipulations, such as formalin fixation [34], heat denaturation [35,36], and X-ray irradiation [37,38]. Studies demonstrate that collagen manipulations affect bone mechanical properties; collagen contributes predominantly to bone toughness, whereas mineral contents contribute to stiffness and strength [39–41].

Osteogenesis imperfecta (OI) is a genetic bone disorder characterized by fragile bones. Approximately 90% of OI cases are caused by dominant mutations in the genes encoding type I collagen (i.e., *COL1A1* and *COL1A2*), which affects the amount and structure of bone collagen. It has been reported that polymorphisms in type I collagen genes increase the risk of fractures, independent of changes in BMD [42]. For many years, no causative genes were identified for the remaining 10% of OI cases; however, other mutations causing recessive forms of OI have been recently discovered. Part of them are genes related to post-translational modifications of type I collagen [43].

2.2. Post-translational modifications of type I collagen

Type I collagen comprises approximately 90% of the organic component of bone. It is also the major protein in the skin, tendons, ligaments, cornea, and blood vessels. Although type I collagen is the predominant organic component in these tissues, the characteristics of these tissues, including mechanical properties, vary. Such differences occur, at least partially, because of differences in the post-translational modifications of type I collagen, which determine variances in covalent cross-linking patterns [44].

Biosynthesis of type I collagen is a long and complex process that includes a series of post-translational modifications [45]. Intra- and extra-cellular post-translational modifications are crucial to the formation of covalent cross-links and the function of collagen fibrils. A number of unique collagen-modifying enzymes and molecular chaperones are involved in these processes [46,47]. Various factors affect post-translational modifications of type I collagen, including aging [48], systemic diseases (e.g., osteoporosis, osteopetrosis, OI, and diabetes) [32,49], and mechanical stress [50–54].

Lysine hydroxylation of specific lysine residues in type I collagen are regulated by a family of enzymes called lysyl hydroxylases (LHs), which have three isoforms (LH1–3). Substrate specificities of LHs are still not clearly established; however, accumulating evidence indicates that LH1 is triple-helix specific, LH2 is telopeptide specific, and LH3 is a multi-functional enzyme with LH, hydroxylysyl galactosyltransferase, and galactosylhydroxylysyl glucosyltransferase activities. Collagen cross-linking forms among the specific lysine or hydroxylysine residue either at the triple-helical or telopeptide region; therefore, lysine hydroxylation is crucial for the determination of the cross-link types. The extent of lysine hydroxylation in type I collagen varies from tissue to tissue and pathophysiological conditions [45,55,56].

PLOD2, which encodes a telopeptide-specific LH2, is a causative gene for Bruck syndrome, which is characterized by osteoporosis, joint contracture at birth, fragile bones, and short stature due to under hydroxylation of lysine residues in telopeptides of type I collagen in bone. However, cartilage and ligament collagen show normal hydroxylation of telopeptide lysine and normal cross-linking patterns [57,58]. In line with this observation, it has recently been reported that the expression of LH2 increases in response to mechanical loading on the bone-associated, but not the cementum-associated, side of the periodontal ligament [53]. *FKBP65*, a peptidyl-prolyl *cis-trans* isomerase, is essential for the dimerization and enzymatic activity of LH2. In addition, mutations

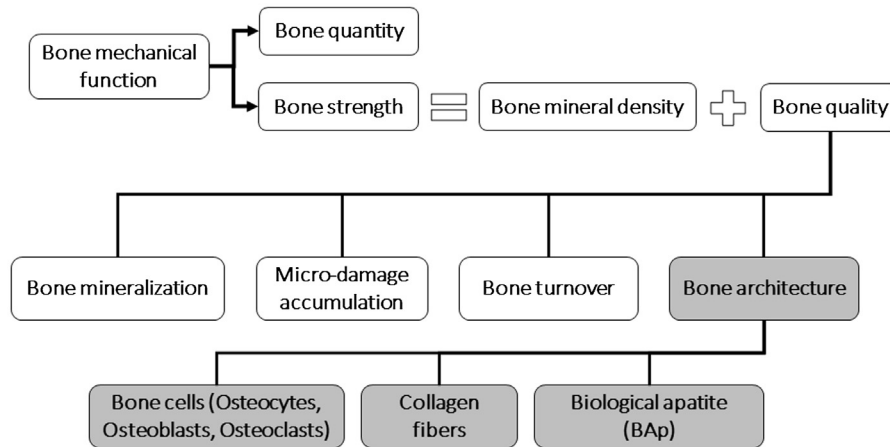


Fig. 1. The current concept of “bone quality.” Bone quality, which has been defined as “the sum of all characteristics of bone that influence the bone’s resistance to fracture” is completely independent of bone mineral density. The National Institutes of Health defines bone quality as comprising bone architecture, bone turnover, bone mineralization, and microdamage accumulation. Bone architecture mainly consists of biological apatite, collagen fibers, and bone cells such as osteocytes, osteoblasts, and osteoclasts.

in the *FKBP10* gene, which encodes FKBP65 protein, result in similar pathology as LH2 deficiency and diminished hydroxylation of telopeptide lysine residue in bone type I collagen [59,60]. These results support the concept that LH activity regulates lysine hydroxylation and subsequent cross-linking patterns in a tissue-specific manner.

2.3. Collagen cross-linking

Collagen cross-linking is one of the major determinants of bone quality and is associated with bone mechanical properties; therefore, it is considered an important predictor of bone fracture risk [44,61–63]. There are two distinct types of collagen cross-linking, enzymatic and non-enzymatic cross-linking. Non-enzymatic cross-linking, including advanced glycation end products (AGEs), are known to affect bone mechanical properties. Moreover, non-enzymatic cross-linking occurs spontaneously through glycation, not through collagen biosynthesis. Therefore, non-enzymatic cross-linking is not discussed in this article.

As discussed earlier, the activity of LHs determines the tissue-specific cross-linking type, while lysyl oxidase (LOX) controls the amount of enzymatic cross-linking. The importance of enzymatic collagen cross-linking in tissue development and maintenance has been examined using lathyrogens, such as aminoacetonitrile and beta-aminopropionitrile (BAPN), which irreversibly inhibit LOX activity [53,61,63]. In a previous animal study, 4 weeks of daily intraperitoneal injection of BAPN induced a 45% reduction of pyridinium cross-link content, resulting in a 26% reduction in the bending strength of rat femurs [61]. Further, it was recently reported that 3 weeks of dose-controlled intraperitoneal administration of BAPN inhibited collagen cross-linking and reduced bone strength, while no significant changes were observed in BMD [63]. These results clearly demonstrate the significance of enzymatic covalent cross-linking in bone mechanical properties, independent of BMD.

It has been elucidated that not only the expression of LOX itself, proteolytic cleavage and interaction with other extracellular molecules also regulate LOX activity. Periostin is a matricellular protein preferentially expressed in collagen-rich fibrous connective tissues, including bone, that coordinates the regulation of bone morphogenetic protein 1-mediated proteolytic cleavage of pro-LOX to active LOX [64]. Fibromodulin, a member of small leucine-rich proteins, is known to interact with collagen and affect collagen cross-linking, molecular packing, and fibril diameter [65]. Recently,

it has been reported that fibromodulin forms a complex with LOX and targets specific cross-linking sites of type I collagen [66]. These results suggest that tissue-specific expression of non-collagenous proteins may also participate in the tissue-specific pattern of collagen cross-linking.

Collagen cross-linking sites reportedly correlate with the molecular packing of collagen fibrils and subsequent nucleation of BAP [67]. Although the bio-mineralization process has been thought to be directed by acidic non-collagenous proteins, such as small integrin-binding ligand N-linked glycoprotein [68], it has been reported that type I collagen can initiate bio-mineralization in the absence of any other extracellular matrix molecules *in vitro* [69]. Currently, the significance and detailed mechanism of collagen cross-linking in the mineralization process remains unclear; however, it is possible that changes in collagen cross-linking may affect the inorganic phase of bone by controlling mineral nucleation.

Collagen cross-linking primarily stabilizes collagen molecules and has long been recognized as one of the major determinants of bone mechanical properties. A clinical study demonstrated a correlation between collagen cross-linking and bone fracture risk [62]. In addition, altered collagen cross-linking is frequently observed in aging and many systemic diseases [33,48,49] due to inappropriate post-translational modifications of collagen. It has been reported that impaired cross-linking in collagenous matrix affects osteoblast differentiation [70,71], indicating that collagen cross-linking also affects tissue turnover, which is another important determinant of bone quality. Therefore, the quantification and characterization of collagen cross-linking in bone may be useful for the diagnosis and understanding of bone status.

In summary, collagen cross-linking is an important determinant of bone quality by affecting bone fragility. Recent studies have partly clarified the regulatory mechanisms of tissue-specific cross-linking patterns of type I collagen. Notably, collagen characteristics of jaw bones could differ from those of long bones [72], although most of the accumulated knowledge regarding bone collagen is based on studies using long bones. Further, collagen cross-linking is not just an architectural component of bone; it most likely affects tissue turnover, micro-crack propagation, and mineral nucleation, all of which influence bone strength (Fig. 2). Detailed studies exploring the kinetics of tissue-specific post-translational modifications of type I collagen will aid in the diagnosis and understanding of bone status, including mechanical properties, in health and disease.

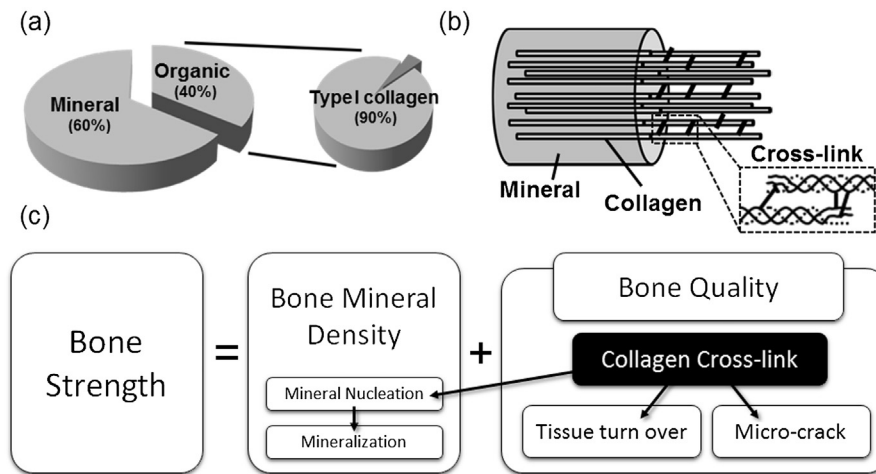


Fig. 2. Collagen as a template of bone. (a) Bone is a two-phase composite material composed of mineral and organic phases. Type I collagen is the major component of the organic phase. (b) Enzymatic cross-linking binds neighboring collagen molecules to form collagen fibrils, which is a template of bone. (c) Bone mineral density accounts for 60% of bone strength while bone quality accounts for the rest. Collagen cross-linking is not just a determinant of bone quality; it also affects tissue turnover, micro-crack propagation, and mineral nucleation, all of which influence bone strength.

3. Bone quality based on biological apatite (BAP)

3.1. Crystallographic texture and preferential orientation of BAP crystals and collagen molecules in bone

It is well recognized in the field of materials science that crystallographic texture and orientation – orderly arrangement of atoms, ions, and molecules – strongly affect the mechanical and/or functional properties of materials, including metals, ceramics, polymers, and combinations thereof. For example, BAP, a major inorganic component of bone, has a hexagonal crystal system that normally demonstrates greater anisotropy than materials with the cubic crystal system. In fact, BAP shows anisotropy in intrinsic mechanical properties such as Young's modulus. Nanoindentation studies of single hydroxyapatite crystals show that Young's modulus along the *c*-axis is greater than that along the *a*-axis [73]; this is attributed to the anisotropic arrangement of its constituent ionic atoms. BAP and collagen in bones are known to have unique crystallographic textures according to anatomical bone position [20], which have been clarified using diffractometer with X-rays, neutrons, and electrons [74]. Due to epitaxial crystallization of BAP on the collagen template through an *in vivo* self-assembly process [75], the crystallographic *c*-axis of BAP aligns almost parallel to the collagen fiber direction, forming an oriented nanocomposite, which makes bone stiff and tough in the extracellular matrix-oriented direction (Fig. 3). Hence, the degree of directionality of the BAP *c*-axis should be a determinant of bone mechanical property and its anisotropy.

Indeed, the three-dimensional BAP *c*-axis orientation distribution appears to correspond to *in vivo* distribution of principal stress. This is well explained using mandibular bone that is subject to complicated mechanical environments. The cortical bone portion apart from the tooth, such as corpus mandibulae, essentially exhibits a unidirectional preferential BAP *c*-axis orientation along the mesiodistal axis [20,74,76]. Conversely, the portion located beneath the tooth exhibits preferential BAP *c*-axis orientation along the biting axis (Fig. 4) [20]. In this case, the BAP orientation was analyzed utilizing microbeam X-ray diffraction (μ XRD), which mirrors the orientation of collagen fibers by co-alignment with them. It is clear that the direction of the preferential BAP *c*-axis orientation agrees with that of principal stress generated by muscles that sustain the mandible and are

involved in biting. In addition, long bone and parietal bone show uniaxial and planar orientation along the bone long axis and flat bone surface, respectively [20], which further supports the directional agreement between principal stress and preferential BAP *c*-axis orientation.

3.2. Contribution of the BAP *c*-axis orientation to bone mechanical property-validity as a bone quality parameter

To demonstrate the contribution of the BAP *c*-axis orientation to bone mechanical property, a bone regeneration model to analyze the recovery (change) of BAP orientation, as well as mechanical property, was prepared using rabbit ulna. The degree of BAP *c*-axis orientation as a candidate for bone quality parameter, BMD as a conventional and standard criterion for bone strength, and Young's modulus as an important parameter for mechanical property were analyzed by μ XRD, peripheral quantitative computed tomography, and nanoindentation, respectively [77]. The BAP orientation and Young's modulus were analyzed along the ulnar long axis in which the bone is principally loaded and the BAP *c*-axis preferentially orients.

In the ulnar regeneration process, BMD almost fully recovered by 12 weeks after osteotomy at which the degree of BAP orientation along the ulnar long axis was quite low compared to the intact value. In the following 12 weeks, the BAP orientation significantly restored to the intact level. Young's modulus at postoperative 12 weeks showed approximately 60% recovery despite full recovery in BMD [21]. These results clearly suggest that BMD alone is insufficient for determining bone strength. Many studies have indicated the limited capability of BMD alone for the determination of bone mechanical properties [78–80]. Correlation and multiple regression analyses revealed that the degree of BAP *c*-axis orientation had a more significant contribution to Young's modulus than BMD [21]. This finding explains one of the reasons for the reported dissociation between BMD and bone strength; that is, bone strength improved without increased bone density in the later phase of bone regeneration [81,82]. Because of the intrinsic mechanical anisotropy of BAP and collagen, their directional arrangement generates anisotropy in bone mechanical property [21]. This mechanical anisotropy increases as the BAP/collagen orientation becomes prominent. Therefore, Young's modulus parallel to the BAP *c*-axis/collagen

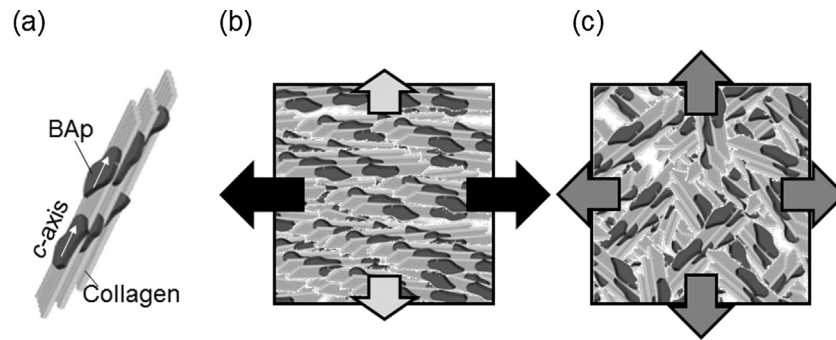


Fig. 3. Relationship between extracellular matrix orientation and anisotropy in bone mechanical property. (a) A schematic illustration of bone microstructure as a composite of biological apatite (BAP) and collagen. The BAP c-axis is almost parallel to the collagen fiber direction. (b) A schematic illustration of unidirectionally oriented BAP/collagen microstructure and resultant anisotropic mechanical properties. In this situation, the preferentially oriented direction (horizontal) shows higher mechanical properties. (c) A schematic illustration of randomly oriented BAP/collagen microstructures and isotropic mechanical properties. Arrows in (b) and (c) represent parameters for mechanical properties such as Young's modulus and toughness.

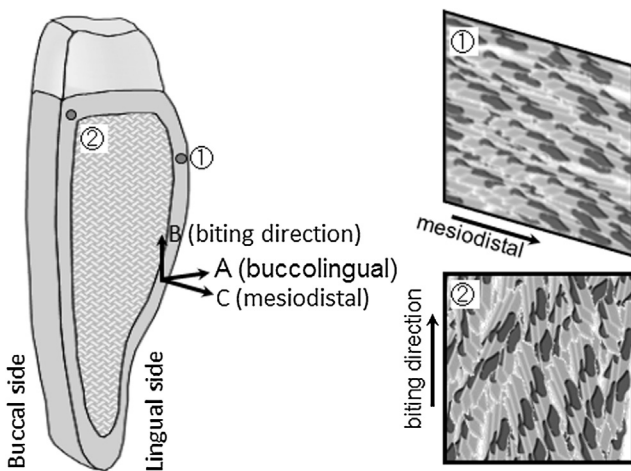
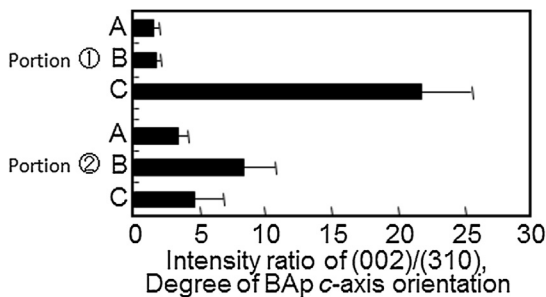


Fig. 4. Distribution of the biological apatite (BAP) c-axis/collagen orientation in the mandible. The cortical bone portion apart from the tooth (①) essentially exhibits a unidirectional orientation along the mesiodistal axis. The portion located beneath the tooth (②) exhibits orientation along the biting direction [20].

orientation direction may increase without a significant increase in Bap density (BMD).

The degree of BAP c-axis orientation significantly and positively correlated with the mechanical parameter of bone not only in regenerated bone, but also in other bones such as intact and pathological bones [83,84]. Interestingly, this correlation was also seen in tooth dentin [85]. Thus, the BAP c-axis orientation is a potent parameter for bone quality and may be useful in evaluating and predicting mechanical properties of various types and conditions of biological hard tissues. However, μ XRD analysis introduced here is invasive. Less invasive or noninvasive analytical

methods should be established for clinical evaluation of the BAP c-axis orientation. One possible candidate is to analyze a speed of ultrasound that has been demonstrated to be associated with the degree of BAP orientation [86].

3.3. Usefulness of the BAP c-axis orientation in bone assessment, bone medication, and implantology

Because the BAP c-axis orientation determines bone mechanical properties, this parameter is beginning to be applied to bone assessment, bone medication, and optimization of dental and orthopedic implants. The degree of bone regeneration [21,87] and the pathological conditions of diseased bone including some types of osteoporosis [88–90], osteoarthritis [91], osteopetrosis [92], and cancer metastasis [83,84] were reported to be assessed using the degree of BAP orientation. For example, in the primary type osteoporosis induced by estrogen deficiency, the degree of BAP c-axis orientation both in the cortical and trabecular bone of lumbar vertebrae increases along the craniocaudal axis in which principal stress is applied [88]. In this situation, the bone appear to increase the degree of BAP orientation to enhance mechanical anisotropy along the loaded direction to bear the increased stress due to osteoporotic bone loss. For bone medication, the effects of anti-osteoporotic agents such as alfacalcidol, risedronate, alendronate, minodronate, and teriparatide on the BAP orientation have been investigated [88,93–95], revealing that some agents can prevent the excessively increased BAP orientation which might be a risk for weakness against abnormally-directed load.

Recently, implants have been designed to induce bone tissue with optimal BAP/collagen orientation. Grooved structures at a certain angle on implant surface were confirmed to successfully induce preferential BAP/collagen orientation [22,96] which continuously changed from inside the groove to the host bone through the bone tissue inside the groove (Fig. 5). These results suggest that the optimally oriented grooves transmit the load to the bone tissues surrounding implants through the grooves, which is evidenced by increased osteocyte number and highly aligned osteocytes along the principal stress direction in the grooves. With the use of metallic implants, the stress shielding phenomenon due to the large difference in Young's moduli between bone and implant materials is a great problem [97]. Stress shielding induces significant bone loss and disruption of bone quality [98,99], leading to an increased fracture risk. An optimally oriented groove structure on the implant surface is a promising method to overcome the stress shielding phenomenon and achieve bone tissue with appropriate bone quality. In 2017, a dental implant with

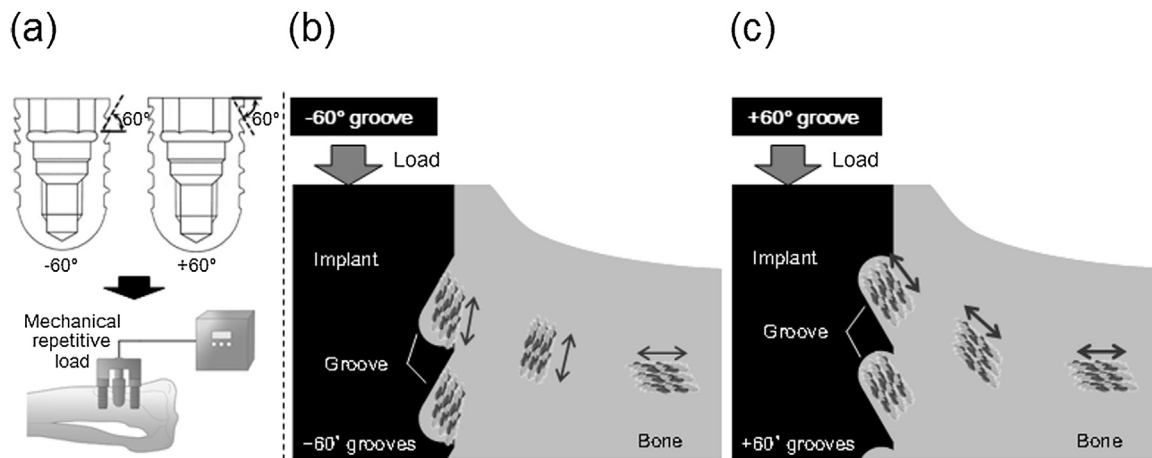


Fig. 5. Effect of optimally oriented grooves on the implant surface upon the bone microstructure of biological apatite (BAP)/collagen orientation. (a) Implant designs and implantation in rabbit tibia with application of repetitive load. (b, c) Schematic illustrations of the preferentially oriented BAP/collagen with groove angles of -60° and $+60^\circ$. Only with the $+60^\circ$ groove, the direction of the BAP/collagen fiber orientation continuously change from inside of the groove to the host tibia bone through the bone tissue inside the groove. This figure is quoted from Ref. [22], which is licensed under CC BY-NC-ND.

an oriented groove structure on its neck will be commercially available in Japan.

3.4. Artificial induction and control of the BAP orientation

To achieve control of bone mechanical properties or enhancement of bone mechanical properties during bone regeneration, artificial induction of the BAP orientation is effective. The artificial induction of the BAP orientation has been attempted on the basis of two strategies, with and without stress loading: forcibly imposing mechanical stimuli to bones to facilitate an adaptive response to change the degree of BAP orientation [100] as mentioned above, and usage of anisotropically patterned substrates to align bone forming osteoblasts to secrete oriented extracellular matrix. Here, the latter strategy is introduced.

Cell alignment caused by surface patterning is well-known as contact guidance. Recent investigations revealed that osteoblast alignment is very important for the production of oriented BAP/collagen structure. Osteoblast alignment can be achieved using metallic substrates with uni-directional periodic steps induced by plastic deformation [101,102] or laser-induced periodic surface structures (LIPSS) treatment [103], or collagen substrates with anisotropic molecular arrangement [104]. Osteoblast-produced collagen fibers and *c*-axis of precipitated BAP crystals show preferential orientation along the cell-elongated direction. Importantly, the degree of preferential BAP *c*-axis orientation positively correlates with the degree of osteoblast alignment; that is, highly aligned osteoblasts produce highly oriented extracellular matrix. Osteoblast alignment determines extracellular matrix orientation; thus, control of cell alignment is a potent method to achieve the bone-mimetic anisotropic BAP/collagen composite microstructure. This methodology might be beneficial to induce oriented bone structures and related favorable mechanical properties in the early phase of bone regeneration in which a randomly organized woven bone with insufficient strength is predominantly formed.

In summary, the preferential BAP/collagen orientation is responsible for anisotropic mechanical properties of bone, therefore, considering this in bone evaluation, bone therapy, implant development, drug discovery, and so on, is crucially important for bone that functions in anisotropic stress field.

4. Bone quality based on osteocytes

4.1. Osteocytes in bone tissue

Osteocytes, which differentiate from osteoblasts on the bone surface, reside in osteocyte lacunae in bone matrix and comprise 90–95% of all adult bone cells [105]. Osteocytes have been thought as enigmatic cells since their function has remained unclear for a long time due to the difficulty of establishing osteocyte cultures *in vitro*. From this view point, histological analyses have been mainly performed to speculate osteocyte function. However, osteocyte function has been incrementally clarified since the isolation of avian osteocytes and osteocyte-like cells (MLO-Y4; derived from mouse long bones) in 1992 [106] and 1997 [107], respectively. Furthermore, molecular and transgenic technologies have been radically developed. Hence, a greater number of investigations on osteocyte function using these novel approaches are performed. However, MLO-Y4 expresses undetectable levels of osteocyte specific genes such as *dentin matrix protein 1 (Dmp1)* and *Sost* [108], although osteocytes highly express these genes in three-dimensional environments. Thus, understanding the original functions of osteocytes remains challenging even when using MLO-Y4 cells.

An expression pattern of specific markers is dramatically changed when osteoblasts differentiate into osteocytes. Osteoblasts abundantly express core-binding factor alpha 1 (Cbfa1)/Runt-related transcription factor 2 (Runx2) [109], osterix [110], alkaline phosphatase [111], and type I collagen [112], whereas osteocytes abundantly express phosphate-regulating neutral endopeptidase on the phosphatase-regulating neutral endopeptidase on the chromosome X (PHEX) [113], matrix extracellular phosphoglycoprotein (MEPE) [114], E11 (podoplanin) [115], DMP1 [116], sclerostin [117] and fibroblast growth factor 23 (FGF23) [118]. Alteration in these cell-specific markers during differentiation is one of characteristics of osteocytes. The expression of osteocyte-specific proteins is correlated with the regulation of cell apoptosis, viability, autophagy, cell signaling and mechanical sensing [119,120].

The formation of dendrite processes is another characteristics of osteocytes during osteoblast embedment into bone matrix. Dendrite processes contact the processes of adjacent osteocytes, osteoblasts, and bone lining cells on the bone surface through gap junctions [121,122]. Gap junctions, which are formed by connexins

(Cx), are transmembrane hemichannels that allow molecules with molecular weights approximately <1 kDa such as small metabolites, ions, and intracellular signaling molecules to transit through them. Ubiquitously expressed Cx43 has been identified in primary osteocytes *in vivo* [123] and MLO-Y4 cells [107]. Cx45 is also expressed in MLO-Y4 cells [124]. Gap junctions including Cx are associated with cell proliferation, differentiation, apoptosis, autophagy, cell signaling and mechanical sensing as well as osteocyte specific proteins, as mentioned above [121,122].

Recently, the NIH defined bone quality as comprising bone architecture, bone turnover, bone mineralization and micro-damage accumulation [19]. In our study using dental implants, mechanical repetitive loading improved bone architecture by upregulation of osteocyte number and dendrite processes with preferential alignment of BAp *c*-axis/collagen fibers [22,125]. Moreover, we also demonstrated that preferential alignment of osteocytes is in accordance with the principal direction of mechanical load in an animal model using hip implants [96]. Therefore, not only BAp *c*-axis and collagen fibers, but also osteocytes are thought to play important roles in controlling the current concept of “bone quality”.

4.2. Mechanosensation in osteocytes

Bone tissue constitutively receives dynamic and/or static load such as gravity, daily movements and various exercises. Appropriate mechanical load increases bone mass, whereas mechanical unload induces bone loss [126]. This indicates that bone tissue is highly mechanosensitive [127]. However, it is unclear which bone cells have this ability and how these cells sense whole bone loads must be distinguished.

Several mechanisms by which bone tissue reacts in response to mechanical stresses have been revealed. Mechanical loads are mainly converted into mechanical stimuli such as fluid shear stress, hydrostatic pressure, and direct cellular deformation. Applied mechanical loads are received as mechanical strain in bone tissue. It is thought that 1000–3000 microstrain in bone matrix increases bone mass, whereas 100–300 microstrain decreases bone mass according to the mechanostat theory [8,128]. Mechanical strain induces matrix deformation surrounding osteocytes and dendrite processes. Matrix deformation then creates fluid shear stress surrounding the dendrite processes of osteocytes. Finally, osteocytes sense the fluid shear stress and promote signaling molecules [129,130]. From this viewpoint, osteocytes, not osteoblasts and osteoclasts, are the most relevant cells that respond to mechanical stimuli.

Cx43 has been thought to be one of the key regulator molecules in osteocyte responses to mechanical stimuli. Cx43-related hemichannels are normally closed under physiological conditions. These hemichannels in osteocytes release anabolic factors such as prostaglandin E2 and adenosine triphosphate (ATP) in response to mechanical loads *in vitro* [131,132]. Moreover, AKT kinase activation play a crucial role in opening Cx43-related hemichannels under mechanical loaded conditions [133]. Hence, Cx43 has been thought to be requisite for sensing mechanical loads. However, interestingly, some recent studies have shown that deletion of Cx43 in osteocytes increased bone mass in response to mechanical loads [134,135]. These experimental results contradict those of previous studies, indicating that the role of Cx43 in osteocytes under loaded conditions must be further elucidated.

Morphological and numerical alterations in osteocytes occur in response to mechanical stimuli. Morphological differences between fibula and calvarial osteocytes have been demonstrated; because fibula osteocytes are more elongated whereas calvarial osteocytes are more spherical. These morphological changes are believed to be caused by the type of mechanical loading because preferential load in the fibula and calvaria are unidirectional and

bidirectional, respectively [136]. Moreover, it has been demonstrated that spheroid MLO-Y4 cells, which are partially adherent or suspended, had stiffness <1 kPa, whereas flat MLO-Y4 cells, which are adherent, has stiffness >1 kPa [137]. These phenomena occurred via osteocyte deformation under fluid flow [138]. The authors concluded that spheroid osteocytes are more mechanosensitive than flat osteocytes [137]. Recently, we have demonstrated that osteocytes become more spherical with increased dendrite processes under loaded conditions. Additionally, the number of osteocytes around dental implants significantly increased under loaded conditions, strongly suggesting that mechanical stimuli via dental implants change osteocyte shape and development of osteocyte network [125]. Hence, morphological alterations of osteocytes in response to mechanical stimuli may play an important role in controlling bone quality.

We have demonstrated that preferential orientation of osteocytes within grooves of hip implants occurred along the principal direction of mechanical load in beagle dogs [96]. However, osteocytes can access perilacunar calcium (osteocytic osteolysis) during periods of calcium depletion and synthesize new matrix upon calcium repletion [139], which suggest that determination of preferential alignment and osteocyte shape are not always dependent on mechanical stimuli. Preferential cell alignment of osteoblasts (see Section 3-4) and osteocytes may be key factors regulating bone quality.

More recently, some studies have suggested that the primary cilium is also a mechanosensor in osteocytes. The primary cilium is a single, immotile organelle that extends from the cell surface of nearly every mammalian cell including osteocytes [140,141]. It has been reported that osteocytes respond to fluid flow with cilia-dependent increases in cyclooxygenase-2 gene (COX-2) expression and prostaglandin E2 production [142]. Moreover, conditional deletion of polycystic kidney disease 1 (Pkd1), which is linked to the primary cilium, impaired skeletal mechanosensing in mouse osteoblasts and osteocytes [143,144]. Therefore, the primary cilium in osteocytes may also play a role as a mechanosensor. However, further studies are required to clarify the direct relationship between the primary cilium in osteocytes and mechanical loading.

4.3. Osteocytes improve bone quality through drug therapies

Osteoporosis is defined as a disease characterized by low bone mass and microarchitectural deterioration of bone tissue, leading to enhanced bone fragility and a consequent increase in fracture risk [145]. It is mainly treated by several types of drugs such as bisphosphonates, monoclonal antibody against receptor activator of nuclear factor-kappa B ligand, parathyroid hormone (PTH), vitamin D3, calcium drugs, selective estrogen receptor modulators, and female hormone drugs. For instance, bisphosphonates suppress osteoclast activity by inhibiting key enzymes of the intracellular mevalonate pathway, which increases bone mass [146]. Moreover, intermittent PTH administration downregulates the production of sclerostin in osteocytes, which also increase bone mass [147]. These drugs both affect bone quantity, albeit through different modes of action. PTH therapy rapidly upregulates bone-formation markers, followed by upregulation of bone-resorption markers [148]. Hence, intermittent PTH administration improves not only bone quantity, but also bone quality by controlling bone cells such as osteocytes, osteoblasts, and osteoclasts in a balanced manner. Conversely, bisphosphonate therapy strongly inhibits the function of osteoclasts. Thus, prolonged use of bisphosphonates may lead to unrepaired microdamage of bone tissue, resulting in accumulated microdamage in bone and fracture risks of femoral heads. Indeed, fracture of femoral heads is an adverse effect of bisphosphonate

therapies [149]. Therefore, bisphosphonate therapies may enhance bone quantity without improving bone quality.

In summary, osteocytes are predominant mechanosensors in bone cells. However, the mechanisms by which osteocytes in jaw bone sense mechanical stimuli remain unclear, since osteocytes in jaw bone may differ from those in long bones. Further studies to investigate the effect of mechanical stimuli on osteocytes *in vitro* and *in vivo* are required to clarify osteocyte-associated bone quality as well as BAp and collagen fibers.

5. Conclusion and future direction

Since the NIH consensus was published in 2000 [16], various types of bone quality parameters were proposed. Of these, recent investigations have revealed promising ones that significantly reflect mechanical properties of bone. It might be productive that such potent bone quality parameters are utilized into dental and medical fields for bone diagnosis and therapy, additional to BMD. For clinical use, however, the mechanisms underlying the control of each bone quality parameter and interaction among them need to be understood. As discussed in this article, jaw bone has unique characteristics in terms of collagen, BAp orientation and osteocytes. This is most likely due to the different developmental origin (neural crest-derived) and unique mechanical loading circumstances of the jaw bones in comparison with other skeletal bones. Therefore, it is of great importance to accumulate scientific evidences that support the clinical diagnosis of the jaw bone status [150–152]. Additional research is currently underway to clarify the mechanisms including genetic, molecular, cellular, and tissue-related events and the understanding is becoming deepened. The relationships between BAp/collagen orientation and other bone quality parameters introduced in this review – collagen chemistry and osteocytes – are now becoming clarified. A negative relationship between the amount of AGE cross-links and the degree of BAp orientation is also suggested [85]. Moreover, synchronous alteration of anisotropic features in osteocyte network and BAp/collagen arrangement was reported [83,84,153,154]. Future studies are expected to provide insight into the potential applications of bone quality parameters for clinical use in prosthodontic dentistry as well as orthopedic fields.

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