文章编号:1004-7220(2020)01-0016-09

·专家论坛·

Biomechanical Design in Osteogenic Engineering

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Abstract: The bone is a naturally occurring composite system comprising collagen matrix and hydroxyapatites capable of generating sufficient strength and toughness to support mechanical loads and resist fracture, respectively. The material strength depends largely on the elastic properties, whereas the toughness depends on not only the elastic, but also the plastic properties. Thus, both elastic and plastic properties must be considered in the analysis of bone biomechanics and the design of osteogenic materials. The bone is capable of optimizing its elastic and plastic properties by integrating stiff hydroxyapatites and ductile collagen fibrils into a hierarchically ordered architecture, an effective mechanism to support the bone strength and toughness. Such a mechanism can be used as a model for designing osteogenic materials.

中图分类号: R 318.01 文献标志码: A
DOI: 10.16156/j.1004-7220.2020.01.008

1 Introduction

Bone fracture and loss occur in injury and disease, followed by a lengthy period of recovery. An effective treatment strategy is to implant osteogenic materials to replace the lost bone and accelerate bone regeneration. For such a strategy, it is necessary to design and construct bone-mimicking materials with sufficient strength and toughness—two mechanical properties supporting mechanical loads and resisting fracture, respectively[1-4]. The material strength can be expressed as the maximum stress at yielding point (also known as yielding strength) in response to applied forces and is dependent primarily on the material elastic properties characterized by the reversible deformability[5]. The material toughness can be expressed as the total amount of strain energy dissipated during deformation in response to applied forces and is dependent on not only the elastic properties, but also the plastic properties characterized by the irreversible deformability[6-7]. Both material strength and toughness are interrelated, but are not necessarily congruent, depending on the involvement and levels of elastic properties (e.g., stiffness, extent of reversible deformation, and yielding strength) and plastic properties (e.g., extent of irreversible deformation)[8]. Often, it is difficult to acquire both maximum strength and toughness simultaneously in a single material system. Excessive enhancement of strength may increase the material brittleness and reduce the deformability in response to applied forces, dampening the capacity of strain energy dissipation and toughness. On the other hand, over-enhancement of toughness through modification of plasticity may negatively impact the material strength. Thus, strength and toughness must be compromised to achieve the needed strength to support mechanical loads and

收稿日期:2019-12-22

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toughness to resist fracture. The bone possesses such a capability, acquired by integrating stiff crystalline hydroxyapatites into the ductile collagen matrix and organizing the two components into a hierarchically ordered architecture. This architecture is capable of not only supporting heavy mechanical loads, but also resisting large fracture-inducing forces, representing the most elegant design strategy for biological load-bearing systems. Thus, the bone architecture can be used as a model to design materials for osteoge-nic engineering. This paper addresses the concepts and analyses of material strength and toughness; the mechanisms of material strengthening and toughening; and the principles and problems of design for osteogenic materials.

2 Mechanical properties of the bone and osteogenic materials

The functions of the bone, supporting mechanical loads and resisting fracture forces, depend on two mechanical properties—strength and toughness, which are related to the material elasticity and plasticity. These material properties are analyzed in this section for the bone and osteogenic materials. An important elastic property is the material stiffness—the ability of resisting reversible deformation in response to applied forces. Stiffness can be expressed by the Young's modulus for materials with linear elasticity and the incremental moduli of a stress-strain curve for materials with nonlinear elasticity[5]. Thus, it is necessary to establish a stress-strain curve in elasticity analyses.

Several biomechanical models can be used to test the mechanical properties of a material—stretching, compression, and 3-point beam bending under hydrated and dehydrated conditions. The 3-point beam bending model provides tensile and compressive information. Thus, this model is used in this paper to demonstrate the principles and procedures of mechanical analyses. In an experimental test, a specimen of the bone or an osteogenic material can be shaped into a rectangular beam with desired dimensions and set up for experimental assessment as shown in Fig. 1A. As the mechanical load of the bone is primarily borne in the axial direction, a simplified uniaxial mathematical model is used here. For stiffness test, two key parameters, the maximum stress and strain at the outer surface of the beam at each loading force, are required for the analysis. The maximum stress of a specimen beam at each loading force can be assessed based on the following equation[5]:

$$\sigma = 3FL/2bh^2 \quad (\mathrm{N/m^2 \text{ or Pascal in unit}}) \quad (1)$$

where σ is the maximum stress, F is loading force, L is beam loading span, b is beam width normal to the loading force, and $h$is beam thickness ($\sim 1/4$ of L) parallel to the loading force (Fig. 1). The maximum strain ε at the outer surface of the beam at each loading force is defined as[5]:

$$\varepsilon = c/R \quad (\text{dimensionless}) \quad (2)$$

with c the distance from the neutral surface to the outer surface of the beam and R the radius of curvature of the beam (Fig. 1). A stress-strain curve can be established based on stress and strain levels measured during a continuous force alteration process. For bone specimens and osteogenic materials, the initial segment of the stress-strain curve is usually linear, from which the Young's modulus can be calculated and used to represent the material stiffness. There often exists a short nonlinear segment of the stress-strain curve prior to material yielding. The stiffness of this nonlinear segment can be represented by incremental moduli measured at varying locations along the stress-strain curve in a simplified one-dimensional analysis. The strength of a specimen is defined as the maximum stress level at the yielding point, known as the yielding strength.

Also, strength can be defined as the maximum stress level at fracture, known as the ultimate strength. In this paper, "strength" represents the material yielding strength.

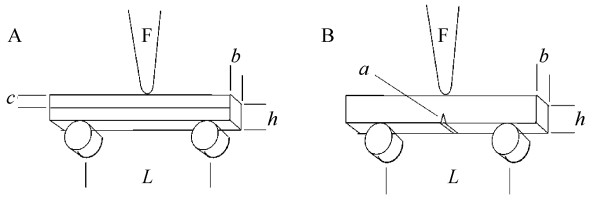

Fig.1 Experimental settings for assessing the stiffness and strength (A) and toughness (B) of bone and osteogenic material specimens by the 3-point beam-bending test. *F* is loading force, *L* is beam loading span, *b* is beam width normal to the loading force, *h* is beam thickness (~1/4 of *L*) parallel to the loading force, *c* is the distance from the neutral surface to the outer surface of the beam, and *a* is the crack depth.

The plasticity of a material is the ability of irreversible deformation in response to applied forces, representing a major mechanism of strain energy dissipation and a major factor contributing to the material toughness or the capacity of dissipating strain energy[4,6-8]. The material toughness is attributed to two components—the elastic and plastic components, and can be assessed based on the area of the force-displacement curve during a beam bending test[4,6]. The elastic component is the strain energy dissipated during the elastic or reversible deformation (represented by the elastic fraction of the force-displacement curve area); whereas the plastic component is the strain energy dissipated during the plastic or irreversible deformation (the plastic fraction of the force-displacement curve area) (Fig. 2). The elastic and plastic components are physically separated by the yielding point, at which reversible deformation ends and irreversible deformation begins. The 3-point beam-bending model described above can be used for toughness tests[3,6]. It is often required to make a small sharp crack at the center of the beam surface opposing to the surface of force application (Fig. 1). This crack serves as a pre-existing defect. Changes in the crack depth and their relationship to the toughness level during deformation are important measures for the assessment of the dynamic toughening process. The overall toughness of a material (K_J) can be expressed by the following equation[3,6-7]:

$$K_J = (JE')^{1/2} \quad (\mathrm{Pm}^{1/2} \text{ in unit}) \tag{3}$$

in which J is the rate of strain energy release with respect to crack extension and E' is $E/(1-\nu^2)$ with E the Young's modulus of the linear elastic portion of the force-displacement curve and ν the Poisson's ratio. $\mathrm{Pm}^{1/2}$ is the unit of toughness with P as Pascal and m as meter. J is defined as:

$$J = J_{el} + J_{pl} \tag{4}$$

with J_{el} the elastic component and J_{pl} the plastic component. J_{el} is defined as:

$$J_{el} = K^2/E' \tag{5}$$

with K the stress intensity factor for the elastic component calculated based on the following equation for the 3-point beam bending model[7,9]:

$$K = FL/bh^{3/2}[2.9(a/h)^{1/2} - 4.6(a/h)^{3/2} + 21.8(a/h)^{5/2} - 37.6(a/h)^{7/2} + 38.7(a/h)^{9/2}] \tag{6}$$

with a the crack depth (Fig.1).

The plastic strain energy release rate J_{pl} is generated during plastic deformation and can be assessed by using the following equation[3,6]:

$$J_{pl} = 2A_{pl}/b(h-a) \tag{7}$$

in which A_{pl} is the area of the plastic portion under the force-displacement curve. The overall toughness of a material can be assessed by integrating eq. 4–7 into eq. 3. Often, toughness is expressed as a function of crack extension. Such a curve is defined as a crack growth resistance curve or *R*-curve. This curve represents the progression of toughness changes during crack extension and the ability of resisting crack extension. An elasto-plastic material is often characterized by the presenceof a non-linear rising *R*-curve because of

increasing plasticity along with crack extension during fracture.

There are two additional parameters used for expressing the mechanical properties of materials—hardness and ductility. Hardness is defined as the relative penetration depth of an object or indenter into a material at the surface in response to applied forces[4]. The material hardness is usually related to stiffness. Ductility is defined as the maximal strain of a material at fracture in response to applied forces[4]. Ductility can be elastic with reversible deformation or plastic with irreversible deformation. Elastic ductility can be represented by stiffness with an inverse relationship. Plastic ductility is related to plasticity. As such, these parameters will not be discussed further.

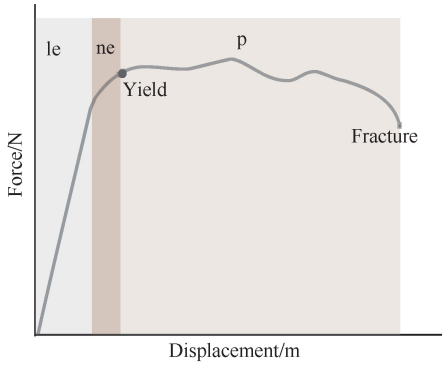

Fig.2 A force-displacement curve from a beam bending test as shown in Fig. 1 A. le represents the linear elastic region; ne the nonlinear elastic region; and p the plastic region. The area under the force-displacement curve in each region represents strain energy dissipated by deformation in response to applied forces. The yielding point separates the elasticfrom the plastic region.

3 Mechanisms of material strengthening and toughening

Strength and toughness are two mechanical properties essential to the two functions of the bone-supporting mechanical loads and resisting fracture, respectively. A major goal of biomechanical analyses in osteogenic engineering is to study and understand the mechanisms of material strengthening and toughening, providing a foundation for the design of osteogenic materials. The material yielding strength is dependent on the elastic properties determined by the material composition and atomic/molecular arrangement, and crystalline materials are stiffer and stronger than amorphous materials. In the bone, the presence of hierarchically organized crystalline hydroxyapatites is the basis for the strength that supports the mechanical loads. The toughness of the bone evolvesthrough more complicated mechanisms, involving elastic and plastic components[4,6-7]. The elastic component is the strain energy dissipated through reversible elastic deformation in response to applied forces (represented by the area under the elastic segment of the force-displacement curve), whereas the plastic component is the strain energy dissipated through irreversible plastic deformation following material yielding (represented by the area under the plastic segment of the force-displacement curve) (Fig.2). In the bone, the plastic component is the primary contributor to the material toughness[4,6-7].

There are two mechanisms that toughen materials—intrinsic and extrinsic toughening, defined based on the location of irreversible deformations in reference to a pre-fracture crack[4,6-7,10] (Fig.3). Intrinsic toughening involves processes of molecular and larger particle dislocation, sliding, and cleavage in front of the crack leading edge; whereas extrinsic toughening involves processes of micro-crack and larger crack formation, crack deflection, and across-crack bridge formation behind the crack leading edge (Fig.3)[4,6-7,11]. Regardless of the intrinsic or extrinsic mechanisms, material cleavage, crack formation, and crack deflection occur primarily in the stiff crystalline hydroxyapatite compartment;

molecular and particle dislocation and sliding may occur in both hydroxyapatite and collagen matrix compartments; whereas the across-crack bridges are built by ductile collagen fibrils. All these intrinsic and extrinsic mechanisms contribute to the dissipation of strain energy during material deformation, preventing stress concentration and material fracture. The bone is a system with optimized elastic and plastic components, providing sufficient strength to support mechanical loads and adequate toughness to dissipate strain energyand resist fracture[4,6-7].

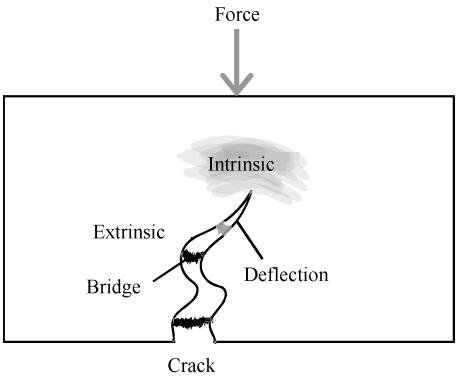

Fig. 3 Schematic demonstration of intrinsic and extrinsic toughening mechanisms.

It is important to note that the material toughness should be analyzed by taking into consideration multiple material properties. In elastic materials with little plasticity, the toughness is related to the material stiffness and strength. Increasing or decreasing stiffness to a certain level may lower toughness as the extent of deformation or force is reduced at the fracture point, respectively, thus reducing the area under the force-displacement curve anddampening strain energy dissipation[8] (Fig.4). In the case of strength elevation with a constant stiffness level, the extent of deformation must be increased, thereby enhancing the material toughness. Cases with other combinations of stiffness and strength can be analyzed in a similar way based on the force-displacement curve shown in Fig. 4. In elastoplastic materials, the interplay of the material stiffness, strength, and plasticity determines the level of toughness. For a given stiffness and strength level, increasing the material plasticity enhances the toughness. Often, altering the material stiffness or strength causes changes in plasticity and toughness, depending on the composition and molecular arrangement of a material. The outcomes of toughness and its relationship with stiffness, strength, and plasticity must be analyzed for each type of material by taking all elastic and plastic properties into account based on the total amount of strain energy dissipated during deformation in response to applied forces or the area of the force-displacement or stress-strain curve.

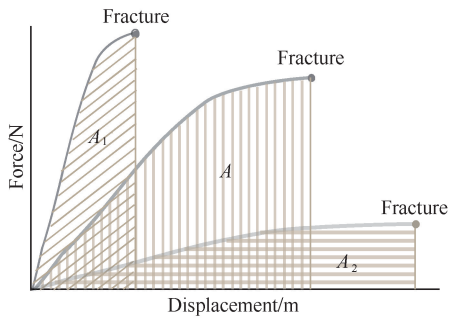

Fig.4 Schematic demonstration of changes in the area under the force-displacement curve (representing the amount of strain energy dissipated during deformation) in responseto variations in the stiffness and strength of materials. An increase or decrease in stiffness and strength to certain levels reduce the area under the force-displacementcurve (A_1 and A_2, respectively) in reference to that (A) at the median levels of stiffness and strength.

4 Bone mineralization and development

The formation of the bone is a process of controlled calcium phosphate (CaP) deposition into collagen fibrils and CaP transition to crystal hydroxyapatites, establishing a hierarchically ordered, hydroxyapatite-collagen-interweaved architecture. This process is regulated by multiple factors, including selected motifs of collagen[12], collagen fibril density[13], non-collagenous

proteins[14-16], acid amino acids[17], prenucleation clusters[18], and the level of hydration[19]. The ductile collagen compartment is built from collagen peptides[20-21]. Each collagen peptide is capped by N-telopeptide at the N terminus and C-telopeptide at the C terminus. Three collagen peptides are self-assembled into a helical rope-like structure known as tropocollagen with diameter of ~1.5 nm and length of ~300 nm. The tropocollagen helices are self-assembled in a staggered manner with a sequential axial-position shift of ~67 nm, forming collagen fibrils of 50 to several hundred nm in diameter (Fig. 5). The C-telopeptide end of a collagen helix is separated by a gap space of ~36 nm from the adjacent N-telopeptide end of the next collagen helix. Such collagen helix dimensions and organization allow a laterally repeated alignment of every five tropocollagen helices, giving a banded pattern to collagen fibrils (Fig. 5)[20-21]. Hundreds to thousands of collagen fibrils are organized in to a collagen bundle, a basic matrix structure present in the bone and other organs.

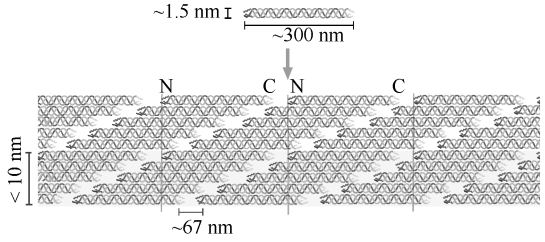

Fig. 5 Formation of collagen fibrils by self-assembly. Tropocollagen helices are assembled into sub-fibril structures, which develop into collagen fibrils. Note that the pattern of tropocollagen helix alignment in the shaded region is identical to that in the unshaded region. N: N-telopeptide. C: C-telopeptide.

Collagen mineralization in the bone begins with CaP deposition to the gap space between the N- and C-telopeptide ends of tropocollagen helices[22] (Fig.6). It has been thought that amorphous CaP (ACP) is the first phase that deposits to the gap space[23]. The ACP phase can transform to octacalcium phosphate (OCP), an intermediate metastable crystalline phase of CaP, which can further transform to the final stable crystalline phase hydroxyapatite (HAP)[24]. CaP phase transition occurs spontaneously based on the rule of energy minimization. ACP is the most unstable phase requiring the highest amount of maintenance energy, whereas HAP is the most stable phase requiring the lowest amount of maintenance energy. The HAP phase is present in the form of nanoplatelets within the spaces between tropocollagen helices[24-26]. OCP is an important metastable phase present in the premature bone, serving as a precursor for HAP development and playing a critical role in osteogenesis[27].

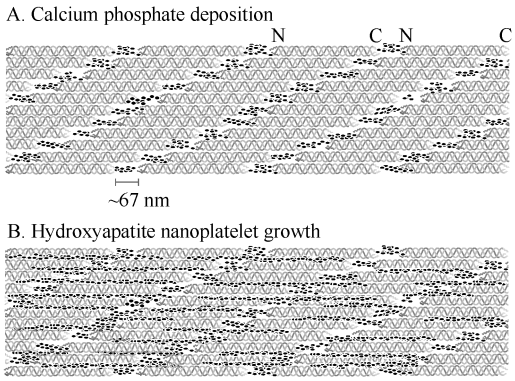

Fig.6 Bone mineralization. (A) Deposition of calcium phosphate (CaP) clusters (likely amorphous CaP or ACP) to the gap spaces between tropocollagen helices during the initial stage of bone formation. (B) Growth of crystalline CaP nanoplatelets (octacalcium phosphate at first, followed by hydroxyapatite) from CaP clusters. Dark dots represents calcium phosphate structures.

The mechanisms of initial ACP deposition and ACP transition to crystalline CaPs have not been well understood. The ACP phase may start from Posner clusters, ~1 nm-sized CaP structures with ~8 calcium ions[23]. The Posner clusters serve as nuclei for ACP deposition. ACP

can transform into OCP within a short period *in vitro*, ranging from 20 min to hours depending on experimental conditions such as temperature and pH[23]. Increasing temperature at neutral pH promotes ACP-to-OCP transition. However, the ACP-to-OCP transition time *in vivo* remains to be assessed during bone development and regeneration. ACP-to-OCP transition may involve two mechanisms: dissolution followed by reprecipitation and Posner's cluster-mediated transformation[24]. For the first mechanism, established ACP clusters are dissolved into spherules, followed by CaP reprecipitation onto spherules to establish OCP structures[23]. For the second mechanism, crystalline Posner's clusters may serve as nuclei that initiate direct OCP formation[23,28-29]. For the transformation of OCP to HAP, as the atomic structure of OCP is similar to that of HAP, HAP may directly grow from the surface of an OCP structure, a process known as epitaxial growth[24]. A preferred surface for growth is (100), a crystal face perpendicular to the *a* axis, but parallel to the *b* and *c* axes of an OCP unit cell[24,30]. The dissolution and reprecipitation processes may also occur in OCP-HAP transition[24]. However, all these CaP transformation mechanisms need further conformations.

CaP deposition to collagen fibrils has been considered a process regulated by non-collagenous proteins and selected amino acids. Non-collagenous proteins include osteonectin[31], bone sialoprotein[14], phosphophoryn[32-33], biglycan[34-35], decorin[36], tyrosine-rich acidic matrix protein[37], and dentin matrix protein 1[38]. These proteins constitute up to ~10% of the extracellular matrix compartment of the bone. Osteonectin, also known as secreted protein acidic and rich in cysteine, is a glycoprotein expressed and secreted from osteoblasts. This protein is involved in the regulation of collagen fibril formation, osteoblast differentiation, and osteoclast activity, contributing to bone development[31]. Bone sialoprotein is an acidic protein present in the bone and dentin, playing a role in regulating the development of the cortical bone[14]. Phosphophoryn can bind calcium ions and interact with the collagen matrix, initiating CaP deposition and bone mineralization[32-33]. Biglycan is a proteoglycan comprising a core protein and glycosaminoglycan chains (chondroitin sulfates and dermatan sulfates)[34-35] and can interact with bone morphogenetic protein 2 to stimulate osteoblast differentiation and bone formation[35]. Decorin is a proteoglycan with structure similar to that of biglycan and plays a role in regulating collagen fibril formation and mineralization[36]. Tyrosine-rich acidic matrix protein regulates the formation of collagen fibrils[37]. Dentin matrix protein 1 can bind to collagen fibrils to promote CaP deposition and bone mineralization[38]. Charged amino acids, such as aspartic acid, glutamic acid, phosphoserine, arginine, and lysine, have also been shown to mediate collagen mineralization[17]. Overall, although numerous non-collagenous proteins and amino acids have been identified as regulators for collagen fibril formation and mineralization, it remains poorly understood how these factorscoordinate in the regulation of bone development and regeneration.

5 Design of osteogenic materials

Bone loss occurs in injury and disease. A common treatment strategy is to implant bone-substitute materials to replace the lost bone and induce bone regeneration. Osteogenic mineralization is an essential process of bone regeneration, providing the foundation of mechanical strength and toughness, two essential properties for bearing mechanical loads and resisting fracture forces, respectively. A fundamental question is how to design materials that provide the needed strength and toughness. This is a question difficult

to address as strength and toughness are controlled by distinct material compositions, structures, elasticity, and plasticity. The strength of a material is dependent on its composition, molecular arrangement, and elastic properties. Crystallization and stiffening are two processes that augment the strength of a material. An example is hydroxyapatite, a phase of crystallized calcium phosphate, which is stiffer and stronger than amorphous calcium phosphate, providing strength necessary for bearing mechanical loads. However, hydroxyapatite itself is inadequate to serve as a bone-substituting material as it is too brittle (liable to shattering in response to applied forces) and is not tough enough to resist fracture forces[8].

In contrast, the toughness of a material is dependent on not only the material composition, molecular arrangement, and elasticity, but also plasticity. The complexity of toughness analyses can be seen in the section of "Mechanical properties of the bone and osteogenic materials" above. The relationship between toughness and strength is not always "proportional to each other". Increasing strength may not necessarily enhance toughness, especially, when the extent of deformation is reduced and the material becomes more brittle, and increasing toughness may compromise strength. The relationship of toughness with strength and other material properties, such as stiffness, reversible deformability, and plasticity, can be assessed by a simple analysis of the area under the force-displacement curve, which represents the amount of strain energy dissipated during deformation and the level of toughness.

In the design of osteogenic materials, an ideal strategy is to optimize the levels of material strength and toughness, providing adequate capacities of bearing mechanical loads and resisting fracture forces. Nature has provided a model for such a strategy—integrating brittle crystalline apatites into a ductile bio-matrix. The mammalian bone and tooth are examples of such composite materials. These materials can be used as models for designing osteogenic materials. The bone consists of several fundamental components—hydroxyapatites, collagen matrix, and non-collagenous proteins. Hydroxyapatites are being utilized during evolution for bone construction because of their availability in nature, the property of self-crystallization, and the capability of inducing and controlling the morphogenesis of collagenmatrix to establish a hierarchically ordered hydroxyapatite-collagen interweaved architecture during bone development[24]. The collagen matrix is responsible for the ductility of the bone, whereas the hydroxyapatite compartment provides the strength. Both collagen and hydroxyapatites work together to generate the bone toughness by hydroxyapatite dislocation, cleavage, and shattering within the ductile collagen matrix and across-crack collagen fibril bridging in response to fracture forces, major plastic processes dissipating strain energy during bone fracture. Taken together, appropriate arrangement of hydroxyapatites and collagen matrix is critical to the establish-ment of sufficient strength and toughness of the bone.

In the mammalian bone, hydroxyapatite nanoplatelets are integrated into the gaps between tropocollagen helices at the nm-level[22]. A dense network of hydroxyapatite nanoplatelets can be found in each collagen fibril, establishing mineralized collagen fibrillar systems at the 100-nm-level. The mineralized collagen fibrils are organized into ordered collagen bundles at the μm-level. The collagen bundles are organized into osteons at the mm-level. The osteons are organizedinto the bone at the tissue level. Such a hierarchically ordered composite architecture is capable of bearing heavy mechanical loads and

resisting large fracture forces. In particular, the ordered alignment of hydroxyapatite nanoplatelets in collagen fibrils is one of the most effective design for resisting fracture forces and supporting mechanical loads[22,39]. Such a hydroxyapatite-collagen-integrated architecture can serve as a model for the design of osteogenic materials.

Several types of hydroxyapatite-collagen-integrated composite material have been constructed *in vitro*[12-13,19,40-41]. Type I tropocollagen can self-assemble into collagen fibrils that attract calcium and phosphate ions to initiate CaP deposition and crystallization, establishing hydroxyapatite-collagen composite structures[13]. The density of collagen fibrils is a critical factor controlling the process of collagen mineralization[13]. Other experimental conditions, such as the Ca/P ratio, pH, temperature, and level of hydration, also influence the process and rate of collagen mineralization. Various non-collagenous proteins may be used to promote collagen fibril formation and mineralization. For each design model, the level of material toughness can be improved by enhancing the material plasticity at a selected level of strength. However, it remains difficult to design and construct a truly bone-compatible and bone-mimicking composite material with the needed strength and toughness for bone replacement. A major challenge is to establish ordered crystalline CaP nanoplatelets within the gaps of tropocollagen helices at an adequate level of density and organize mineralized collagen fibrils into a hierarchically ordered composite architecture with sufficientstrength and toughness.

6 Conclusions

The hierarchically ordered architecture of collagenmatrix and hydroxyapatites in the bone is an ideal model for the design and construction of materials for osteogenic engineering. Strength and toughness are two mechanical properties that determine the functions of the bone and osteogenic materials. These properties must be controlled to support sufficient mechanical loads and resist fracture forces. The physiological levels of strength and toughness of the bone should be used as standards for the design and construction of osteogenic materials. Such a goal can be achieved by integrating crystalline calcium phosphate nanoplatelets and collagen fibrils into a hierarchically ordered composite structure.

Acknowledgements: This paper is dedicated to Dr. Y. C. Bert Fung, the man who has established the foundation of Biomechanics and set the standards of education and research in this discipline. Today, biomechanics education and research programs have been established in almost all major higher education institutions. The impact of Biomechanics will be extant for generations to come. The concepts presented in this article have been developed through US NSF-funded investigations.

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