Finite element 3D modeling of mechanical behavior of mineralized collagen microfibrils

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ABSTRACT

**Purpose:** The aim of this work is to develop a 3D finite elements model to study the nanomechanical behavior of mineralized collagen microfibrils, which consists of three phases, (i) collagen phase formed by five tropocollagen (TC) molecules linked together with cross-links, (ii) a mineral phase (Hydroxyapatite), and (iii) impure mineral phase, and to investigate the important role of individual properties of every constituent.

**Methods:** The mechanical and geometric properties (TC molecule diameter) of both tropocollagen and mineral were taken into consideration as well as cross-links, which was represented by spring elements with adjusted properties based on experimental data. In this paper an equivalent homogenized model was developed to assess the whole microfibril mechanical properties (Young’s modulus and Poisson’s ratio) under varying mechanical properties of each phase.

**Results:** In this study, both equivalent Young’s modulus and Poisson’s ratio, which were expressed as functions of Young’s modulus of each phase, were obtained under tensile load with symmetric and periodic boundary conditions.

**Key words:** Cross-links, Finite elements, Mechanical properties, Mineral, Mineralized collagen microfibril, TC molecules

INTRODUCTION

Hierarchic structures in bio-composite such as bone tissue have many scales or levels, specific interactions between these levels, and highly complex architecture in order to achieve its biological and mechanical functions (1). The complexity and heterogeneity of bone tissue requires multiscale modeling to understand its mechanical behavior and its remodeling mechanism (2). In long bones, such as the femur, three parts are distinguished, from the center outwards: the marrow, spongy bone, and cortical bone. Human cortical bone structure consists of six structural scale levels which are the (macroscopic) cortical bone, osteonal, lamellar, fibrous, fibril, and microfibril (Fig. 1). In fact, microscopic analysis reveals a complex architecture that can be described as hollow cylinders juxtaposed next to each other and sealed by a matrix. The cylinders are called osteon, the holes are denominated Haversian canals, and the matrix is the interstitial system. Further analysis reveals that osteons are in fact an assembly of cylindrical strips embedded in each other and each lamella is composed of a network of collagen fibers with a helical orientation and inserted into hydroxyapatite crystals. The orientation of collagen fibers may be different between two consecutive lamellae. Each fiber is comprised of a set of fibrils. Each fibril is in turn composed of microfibrils. Finally, each microfibril is a helical arrangement of five tropocollagen (TC) molecules (3).

On an ultrastructure level, mineral and collagen are arranged into higher hierarchic levels to form microfibrils, fibrils, and fibers (4-6). The presence of sub-structures in collagen fibrils has been debated for years. Recent studies suggest the presence of microfibrils in fibrils, experimental works prove that all collagen-based tissues are organized into hierarchic structures, where the lowest hierarchic level consists of triple helical collagen molecules (7-9) and the multiscale structure was defined as triple helical collagen molecules - microfibrils - fibrils - fibers. A longitudinal microfibrillar structure with a width of 4 to 8 nm was visualized in both hydrated (10) and dehydrated (11) form. Three-dimensional image reconstructions of 36 nm-diameter corneal collagen fibrils also showed a 4 nm repeat in a transverse section, which was related to the microfibrillar structure (12). Using x-ray diffraction culminating in an electron density map, (8) suggested the presence of right-handed super twisted microfibrillar structures in collagen fibrils.

Experimentally, single tropocollagen molecules and mechanical properties of fibrils of mineralized collagen studies have been performed by several Authors (13-15). Other studies on the hydrated collagen microfibril in the small strain regime based on x-ray diffraction (16, 17) and atomic force microscopy (AFM) (9-15) were also achieved to investigate the stress-strain relationship and the elastic properties of microfibrils. Furthermore, the effect of collagen-mineral deformation processes (18) was studied to estimate the mechanical properties of mineralized collagen fibrils and bone tissue. Recently, an atomistic model of the
collagen microfibril dynamic has been developed (5). This model, in fact, interprets in full detail mechanical behavior at the microfibril level. However, it requires extremely high computational power because of its size. In spite of all these efforts made on this scale, a 3D finite element (3D FE) model which can represent the ultrastructure of mineralized collagen that takes into consideration the TC, mineral phases as well as the cross-links are still absent.

In the current work, as an attempt to be closer to reality, a 3D FE model is proposed to study the mechanical behavior of mineralized collagen microfibril considering these aspects: (i) the dimensions and composition of the constituents (mineral, TC molecules, and cross-links) (ii) studying the effect of elastic mechanical properties of constituents on the whole microfibril structure. The proposed 3D FE model confers the ability to study bone mechanical properties on a nanoscopic scale and enable simple parametric studies to investigate the effect of mechanical and geometric parameters (mineral density, cross-links, elastic properties of bone component,…etc) related to bone quality.

MATERIALS AND METHODS

In this section we discuss: (i) the structure of the microfibril and the mechanical behavior of these elementary constituents, (ii) selection and development of the 3D FE model.

The microfibril is a helical assembly of five TC molecules (order five rotational symmetry), which are offset against one another with apparent periodicity of 67 nm (Fig. 2). This periodic length is denoted by the letter D and it is used as a primary reference scale to describe the structural levels. The helical length of a collagen molecule is $4.34 \times D \approx 291$ nm and the discrete gap (hole zone) is $0.66 \times D \approx 44$ nm ($\approx 35$ nm in some other references) between two consecutive TC molecules type 1 in a strand. These gaps in bone are the nucleation sites for hydroxyapatite crystals (the mineral component of bone tissue) to be deposited (19, 20). Those five molecules create a cylindrical formation with a diameter 3.5 to 4 nm and its length is unknown. The orientation and axial arrangement of TC molecules in the microfibril have been deducted from an electron-microscopic observation showing transverse striations with a period D. The
origin of this streaking was performed by a gradation in the arrangement of elements that are staggered tropocollagen (TC) with themselves at an interval D. The strength and stability during maturation of the microfibrils are achieved by the development of intermolecular cross-links (21, 22).

A microfibril is composed of the TC molecule type I linked together by cross-links and mineral phases:

**Tropocollagen molecules:** At the lowest hierarchic level, bone structure is composed of TC molecules which can be viewed as a rod approximately 300 nm in length and 1.5 nm in diameter, made up of three polypeptide strands, each of which is a left-handed helix (23). The arrangement of the TC molecules comes from the strong chemical bonds (cross-linking) that form between adjacent collagen molecules throughout the collagen bundles (4, 6). The force-strain curve during tensile load of a single TC molecule reported in (4) shows an initial linear elastic regime followed by the onset of nonlinear, stiffening behavior at larger strains beyond approximately 30% to 35% strain. It has been suggested by (14) that regime I is characterized by uncoiling of the TC molecule and regime II is associated with a larger modulus because of stretching of covalent bonds. In this work the tensile test is performed at small strain (ε < 2%), that is why the TC molecules are considered with linear elastic behavior.

**Mineral:** The mineral phase is almost entirely composed of impure hydroxyapatite crystals Ca₁₀(PO₄)₆(OH)₂. Those crystals are plate-like in shape. The size of the mineral plates varies among different kinds of bones, different animals, and even different measurement techniques, e.g., transmission electron microscopy (TEM) and small-angle x-ray scattering (SAXS) (24). A wide range of mineral plate dimensions has been reported in the literature: 15 to 150 nm in length, 10 to 80 nm in width, and 2 to 7 nm in thickness, while the distance between the neighboring plates is of the same order of magnitude as the thickness (25). Hydroxyapatite mineral is stiff and extremely fragile and exhibits elastic isotropic behavior (26-28). The mechanical properties of hydroxyapatite and TC molecules crystals reported in the literature are grouped in Table I.

**Cross-links:** Binding two TC molecules. The microfibril structure is stabilized by means of intermolecular cross-links, formed between telopeptides and adjacent triple helical chains by means of lysine–lysine covalent bonds. Recently, Uzel et al. (6) proposed a rheologic model of cross-links behavior with three regimes: (i) elastic regime, (ii) delayed response because of the unraveling of the telopeptide, and (iii) the friction representing the intermolecular slippage. As this work focuses on the small deformation of microfibrils, only the first regime (elastic) was taken into consideration. In order to represent the FE behavior of a cross-link, linear spring elements of tensile stiffness K_{cr} (34) were used:

\[
K_{cr} = 17 \text{kcal/mol/A}^2 = 1181.143 \text{e-11N/nm}
\]

(1 kcal/mol/A ≈ 69.48 pN)

**Geometry FE model:** A repetitive portion of mineralized collagen microfibril was selected; it is cylinder-shaped with a diameter of 4 nm. Some studies suggest that collagen microfibrils have a quasi-hexagonal structure (35). Smith et al. (36) consider that each microfibril consists of exactly five molecules in a generic circular cross-section. In this work, the smith model was adopted.

Figure 3 illustrates the unfolding in plane of a chosen repetitive portion with mention of the dimensions and provision of each phase.

The proposed model is a periodic repetitive portion

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**TABLE I - MECHANICAL PROPERTIES OF PHASES (TROPOCOLLAGEN MOLECULES AND HYDROXYAPATITE CRYSTALS)**

| Phases                  | Young's modulus (GPa) | Poisson's ratio | Source                          | Source                        |
|-------------------------|-----------------------|----------------|---------------------------------|--------------------------------|
| Tropocollagen molecules | 2.7                   | 0.27           | Sansalone et al 2007 (29)       |                                |
|                         | 2.4                   | --             | Vesentini et al 2005 (30)       |                                |
|                         | 0.35-12               | --             | Sun et al 2002 (13)             |                                |
|                         | 1.5                   | 0.38           | Wagner and Weiner 1998 (31)     |                                |
|                         | 2.8-3                 | --             | Sasaki and Odajima 1996 (16)    |                                |
| Hydroxyapatite crystals | 114                   | 0.30           | Wagner and Weiner 1998 (31)     |                                |
|                         | 150                   | 0.27           | Cowin 1989 (32)                 |                                |
|                         | 170                   | 0.33           | Currey 1969 (33)                |                                |
with specific geometry properties given in Table II.

A three-dimensional finite element model of mineralized collagen microfibril with symmetric and periodic boundary conditions is considered here, with an array of five TC molecules cross-linked together using springs. The whole model is put into a mineral matrix. This study focuses on the elastic behavior of the mineralized collagen microfibril in the small strain regime ($\varepsilon < 2\%$). Plasticity and rupture in both phases (TC and mineral) and relative sliding on the interface between the two phases was not considered here. The bottom surface of the microfibril was encastred and a uniaxial force ($F$) along the axis of the collagen molecules was applied to the top surface of the microfibril. Deformation and microfibril elongation was computed by the FE model. Each collagen domain represents a collagen triple helix in a wet environment described as homogeneous elastic phase considering that a single collagen molecule would not exhibit shear deformation. Each collagen domain then deforms only under tension, similar to the situation present in bead models of collagen (4).

Both the pure and impure portions of mineral have been assumed to be in homogeneous phase for these reasons: (i) the aim of this study was to propose a realistic 3D FE model of mineralized microfibril. This model can be enhanced in future works by including more details and refinements, (ii) to simplify the design of our FE model and (iii) because of lack of information dealing with the mechanical properties of the immature phase located between the TC molecules in a transversal direction. In the FE model, the same mechanical properties are introduced for both phases.

The mineralized collagen microfibril composed of the three phases is considered an elastic material and the constitutive law used is given by:

$$
\sigma_{ij} = C_{ijkl} \varepsilon_{kl}
$$

[1]

where $\sigma_{ij}$ is the Cauchy stress component, $\varepsilon_{kl}$ the linear strains, and $C_{ijkl}$ are the components of elasticity tensor.

The proposed FE model was coupled to an optimization algorithm based on minimizing to this FE model to minimize the spring back without having recourse to an experimental database. The inverse identification algorithm developed consists of minimizing the objective function, which is the difference between the predicted force-displacement curves obtained respectively by the equivalent (monophase) model, and the 3D simulation using the multiphase model. This method is called inverse identification and is used to calculate equivalent proprieties.

RESULTS

Computational results

As mentioned above, from the previous literature, both the mechanical and geometric properties of collagen and mineral do not have fixed values because of the nature of the alive human bone tissue. In fact, these variations have a significant influence on the properties of the collagen mineralized microfibril on the nano-scale and the cortical bone on the macro-scale. A parametric study was performed in order to investigate the influence of both geometric (TC molecule diameter) and mechanical parameters on the mechanical behavior of the microfibril.

Figure 5 depicts that the equivalent Young's modulus of the microfibril is increased with increasing of the
Young’s modulus of the mineral. Figure 6 shows the effect of cross-links number on equivalent Young’s modulus. A non linear curve is obtained. It is composed of two parts: (i) from \( N = 1 \) to \( N = 20 \), the cross-links number has an effect and (ii) a constant value “plateau value". It also shows that when the quantity of collagen increases, the microfibrils ductility increases.

Figure 7 shows that if the Young’s modulus of the mineral increases, the equivalent Poisson’s ratio decreases due to the hardness of the mineral material i.e. as its Young’s modulus increases the hardness of the collagen microfibril increases, and its ductility decreases. On other side as the Young’s modulus of collagen (soft and elastic material) increases, the ductility of whole structure increases, which can be expressed by the increase of Poisson’s ratio as shown in Fig. 8.

Figure 9 shows that Von Mises stress increase when the \( E_m \) increases; it means that the microfibril becomes more rigid and resistant.

DISCUSSION

Bone is a composite material, where the nanoscale characteristics of individual constituents are important to the overall quality of bone and its mechanical properties (37). On the nanoscale, it is important to determine the influence of the structural properties of individual TC molecules and the single mineralized crystallites within the bone matrix. The physical and mechanical properties of each constituent, such as mineral crystal size and orientation, collagen diameter and orientation, and hardness and elastic modulus of nanoscale structures are all characteristics that contribute to the fragility and strength of mineralized collagen microfibrils.

The collagen network in bone provides resistance against fractures and may be susceptible to change with ageing and disease (38). For example, in osteogenesis imperfecta, a disease characterized by decreased material properties and bone fragility, some mutations in the amino-acid sequence of type I collagen can lead to the formation of branched fibers responsible for brittle bone and abnormal mineralization (39). The major role of collagen, as it is known, is to give bone its ductile properties, so understanding how collagen microfibrils are oriented as well as being familiar with its geometry is important to study bone quality (40, 41). The orientation of the collagen within the bone matrix is important to bone stiff-
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Figure 6 shows that the number of cross-links has most influence on the increase in the equivalent Young’s modulus. If the number of cross-links increase the bone material will be more stiff. However, when N>20 Young’s modulus does not depend on the number of cross-links and it remains at constant “plateau value”; the same observation has been also found by J. Buehler (4) in his molecular multi-scale study of collagen fibrils, where it is found that the yield and fracture stress depend on the cross-link density $\beta$ only when $\beta<25$ and it is interpreted as the plateau value. This can be explained by a change in the molecular deformation mechanism from predominantly shear (for $\beta<25$) to molecular fracture (for $\beta>25$).

Thus, a 3D multiscale study is necessary since although the 2D model reflects the mechanical properties of the phases, it does not allow us to present microfibrils with their specific arrangements as well as the orientation of their TC molecules. Our proposed model combines the two geometric and mechanical features of each phase. The results found confirm the functionality of this 3D finite element model and justify the results found by the above theoretical and experimental studies. The individual properties of the collagen fibers and mineral crystals cannot fully account for the mechanical behavior of the bone matrix. Rather, it is the interaction and orientation of the constituents that could influence bone quality (53). This condition is satisfied by the model being proposed which takes into account the effect of cross-links whose
importance for the maturity of bone’s mechanical properties remains unclear (54, 55). The manner in which the TC molecules are linked can have a profound effect on the strength, stiffness, and fragility of the tissue.

In this paper we study the mechanical behavior of microfibrils for the first time using the 3D FE model and the inverse identification method. The results found in this study seems logical and consistent; they prove the functionality and reliability of this model which can also be used to investigate many other phenomena and the role of the same parameters linked to the mechanical and biological behavior of bone in this scale. This work also allows us to understand this nanoscale better and study the upper level scale which is the collagen fibril with the same methods by using the results found in this scale. However, before proceeding to the next level to reach one’s cortical bone it is necessary to improve these results by studying the effects related to the mechanical characteristic of constituents. With the model being proposed, we try to model microfibrils and touch on the reality, but this model is still limited owing to the specific arrangement of collagen molecules, the orientation of mineral crystals, the complicated distribution of each phase, and the manner of modeling the cross-links.

The current work studied microfibril mechanical behavior under tensile and compression tests. During tension/compression, the shape of the model is not important since the reaction depends only on the area of the section. The shape plays a role during bending and torsion tests; if the quasi–hexagonal structure of microfibrils is admitted, this model cannot be used for torsion and bending.

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