Bone Ultrastructure – Collagen-reinforced Mineral Matrix or Interpenetrating Network of Hydroxyapatite Crystals and Collagen Molecules?

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ABSTRACT
We develop and verify three different continuum-micromechanics representations of the collagen-mineral interaction in the elasticity of mineralized tissues: (i) mineral foam matrix with collagen inclusions [5], (ii) interpenetrating network of hydroxyapatite crystals and collagen molecules, (iii) composite of fibrils (collagen-hydroxyapatite network) embedded in a collagen-free extrafibrillar mineral foam matrix. The most advanced concept (iii), with the best predictive capabilities, integrates the two others into a consistent whole. There, the collagen is clearly represented as the governing element in inducing tissues’ the anisotropy, by (i) the anisotropy of molecular collagen itself, (ii) the anisotropy of the fibrils, and (iii) the oriented morphology of the cylindrical fibrils in the isotropic extrafibrillar space.

KEY WORDS
bone, hydroxyapatite, collagen, crystal foam, fibril, homogenization, continuum micromechanics

1 Introduction
The organization of the elementary components within the ultrastructure of mineralized tissues (bone and mineralized tendons) has provoked some controversy; especially with regards to its impact on the mechanical properties of the ultrastructure. Herein, we aim at shedding some light on the issue, by developing and verifying three different continuum-micromechanics representations [11] of the collagen-mineral interaction in the elasticity of mineralized tissues: (i) mineral foam matrix with collagen inclusions, (ii) interpenetrating network of hydroxyapatite crystals and collagen molecules, (iii) composite of fibrils (collagen-hydroxyapatite network) embedded in a collagen-free extrafibrillar mineral foam matrix (Figure 1).

2 Micromechanical representation
The extrafibrillar space (Figure 1a) consists of highly disordered hydroxyapatite crystals (HA) of several nanometers characteristic length in a liquid environment (water with non-collagenous organic matter, uw). The isotropic stiffness of the extrafibrillar space, \( C_{uw} = 3k_{uw}K + 2\mu_{uw}J \), is estimated from the tissue-independent stiffness properties \( C_{uw} \) and \( C_{HA} \), Table 1, and tissue-specific volume fractions \( f_{HA} \) and \( f_{uw} \). by means of a self-consistent scheme reflecting the disorder of crystals [4],

\[
\sum \bar{f}_r (\mathbf{c}_r - \mathbf{C}_{cph}) : \left[ \mathbf{I} + \mathbf{P}_r^{SCS} : (\mathbf{c}_r - \mathbf{C}_{cph}) \right]^{-1} = 0 \quad r \in [HA, uw], \quad \bar{f}_{HA} + \bar{f}_{uw} = 1 \quad (1)
\]

The \( \mathbf{P} \)-tensors are given in [4].

The stiffness of the fibrils (Figure 1b) is also estimated by a self-consistent scheme, reflecting a spaceframe of disordered crystals with oriented collagen molecule ‘ropes’ inbetween [4],

\[
\mathbf{C}_{fib}^{SCS} = \sum \bar{f}_s \mathbf{c}_s : \left[ \mathbf{I} + \mathbf{P}_s^{SCS} : (\mathbf{c}_s - \mathbf{C}_{fib}^{SCS}) \right]^{-1} : \left[ \sum \bar{f}_s \left( \mathbf{I} + \mathbf{P}_s^{SCS} : (\mathbf{c}_s - \mathbf{C}_{fib}^{SCS}) \right) \right]^{-1}
\quad r, s, \in [HA, col, uw], \quad \bar{f}_{HA} + \bar{f}_{col} + \bar{f}_{uw} = 1 \quad (2)
\]

In Eqn.(2), the estimation is again based on tissue-independent stiffness properties, \( C_{col} \), and \( C_{HA} \), Table 1, as well as tissue-specific volume fractions, \( f_{HA} \), \( f_{uw} \), and \( f_{col} \). Eqn.(1) and (2) are solved iteratively for \( C_{cph} \) and \( C_{fib} \), respectively. The expressions for the \( \mathbf{P} \)-tensors are given in [4].

Within the ultrastructure (Figure 1c), the fibrils act as cylindrical inclusions (with \( C_{fib} = C_{fib}^{SCS} \) from the preceding homogenization step) in an extrafibrillar crystal-foam matrix (with \( C_{cph} = C_{cph}^{SCS} \) from the preceding homogenization step). The estimated ultrastructural stiffness reads as [4]

\[
C_{ultra}^{MT} = \left\{ (1 - f_{fib})C_{cph} + f_{fib}C_{fib} : \left[ \mathbf{I} + \mathbf{P}_{fib}^{s} : (\mathbf{c}_{fib} - \mathbf{c}_{cph}) \right]^{-1} \right\} : \left\{ (1 - f_{fib})\mathbf{I} + f_{fib} \left[ \mathbf{I} + \mathbf{P}_{fib}^{c} : (\mathbf{c}_{fib} - \mathbf{c}_{cph}) \right]^{-1} \right\}^{-1} \quad (3)
\]

where \( \mathbf{P}_{fib}^{c} \) is found in [4].
3 Model validation

The validation of the different concepts is based on two independent sets of experiments. The first experimental set relates to ‘universal’ (tissue and location-independent) phase stiffness values (Table 1): Tests with an ultrasonic interferometer coupled with a solid media pressure apparatus [7, 3] reveal the isotropic elastic properties of hydroxyapatite powder, which, in view of the largely disordered arrangement of minerals [2, 5], are sufficient for the characterization of the mineral phase [6]. Finally, given the absence of direct measurements of (molecular) collagen, the elastic properties of (molecular) collagen are approximated by those of dry rat tail tendon, a tissue consisting almost exclusively of collagen. By means of Brillouin light scattering, Cusack and Miller [1] have determined the respective five independent elastic constants of a transversely isotropic material (Table 1). We assign the standard bulk modulus of water to the phase comprising ultrastructural water and non-collagenous organic matter. In case of dry tissues, we adopt $c_{uuw} = 0$.

The second, independent experimental set refers to a large number of tissue-specific phase volume fractions and corresponding (ultrastructural) stiffness values. The volume fractions can be determined from weighting experiments on wet, dried, and demineralized tissues [10]. The ultrastructural stiffnesses are determined from ultrasonic tests in the MHz frequency range [8].

The developed micromechanical models show remarkable predictive capabilities. Still, there are significant differences in the performance of these three different micromechanical concepts, related to the sophistication with which the ultrastructure of bone is modelled. Consideration of the fibrillar organization of bone ultrastructure improves over simpler concepts like an interpenetrating network of mineral crystals and collagen molecules, which in turn is superior to a crystal-foam representation with collagen inclusions.

4 Conclusion

In fact, the most advanced concept (iii) shows the best predictive capabilities, Figure 2. It integrates the two others to a consistent whole: The fibrils are regarded as interpenetrating network of collagen molecules and the minority of the mineral crystals present in the tissues. At a higher observation scale, the fibrils function as templates or reinforcement in an extrafibrillar crystal foam-type matrix, hosting the majority of the minerals present in the bone ultrastructure. The reinforcement function corresponds to low-mineralized tissues (such as deer antler) where the extrafibrillar mineral foam is softer than the fibrils, whereas the template function corresponds to high-mineralized tissues (such as cow tibia) where the extrafibrillar mineral foam is stiffer than the fibrils. The collagen is clearly represented as the governing element in inducing tissues’ the anisotropy, by (i) the anisotropy of molecular collagen itself, (ii) the anisotropy of the fibrils, and (iii) the oriented morphology of the cylindrical fibrils in the isotropic extrafibrillar space.

References


\[ V_{ef} = V_{HA} + V_{uw} \]
\[ 1 = f_{HA} + f_{uw} \]

\[ V_{fib} = V_{HA} + V_{col} + V_{uw} \]
\[ 1 = f_{HA} + f_{col} + f_{uw} \]

\[ \ell_{ef} = 100 - 500 \text{ nm} \]

\[ \ell_{fib} = 100 - 500 \text{ nm} \]

\[ \ell_{u} = 5 - 10 \mu\text{m} \]

Figure 1. Concept III: Consideration of fibrils as morphological unit
Table 1. ‘Universal’ (tissue and location-independent) isotropic (or transversely isotropic) phase stiffness values

<table>
<thead>
<tr>
<th>phase</th>
<th>bulk modulus $k$ [GPa]</th>
<th>shear modulus $\mu$ [GPa]</th>
<th>experimental source</th>
</tr>
</thead>
<tbody>
<tr>
<td>hydroxyapatite</td>
<td>$k_{HA} = 82.6$</td>
<td>$\mu_{HA} = 44.9$</td>
<td>[7]</td>
</tr>
<tr>
<td>ultrastructural water and non-collageneous</td>
<td>$k_{uw} = 2.3$</td>
<td>$\mu_{uw} = 0$</td>
<td></td>
</tr>
<tr>
<td>ultrastructural water and non-collageneous</td>
<td>$c_{ijkl}$ [GPa]</td>
<td>$c_{ijkl}$ [GPa]</td>
<td></td>
</tr>
<tr>
<td>collagen</td>
<td>$c_{col,3333} = 17.9$</td>
<td>$c_{col,1133} = 7.1$</td>
<td>[1]</td>
</tr>
<tr>
<td>collagen</td>
<td>$c_{col,1111} = 11.7$</td>
<td>$c_{col,1122} = 5.1$</td>
<td></td>
</tr>
<tr>
<td>collagen</td>
<td></td>
<td>$c_{col,1313} = 3.3$</td>
<td></td>
</tr>
</tbody>
</table>

Figure 2. Consideration of the fibrillar organization of bone ultrastructure (concept III): Comparison between stiffness predictions of micromechanical model and experimental stiffness values: (a) radial normal stiffness values, $C_{ultra,1111}^{MTIII}$ versus $C_{1111}^{exp}$, (b) axial normal stiffness values $C_{ultra,3333}^{MTIII}$ versus $C_{3333}^{exp}$, based on experimental values from the literature: L79...[10], L83...[8], L94...[9]