Enhancing strength in mineralized collagen

Enhancing strength in mineralized collagen

X-ray data reveal the role of prestress in hierarchical bio-composites at the nanoscale

By Fabio Nudelman[1] and Roland Kröger[2]

Living organisms build an assortment of mineralized tissues by combining biopolymers and minerals. Mineralization is fundamental to many biological functions, ranging from mechanical shock protection by shells, mastication by teeth, linear acceleration detection by otoconia in the inner ear, and body support by skeletons. Scientists have been investigating the material properties of these biominerals with the focus on the combination of organic and inorganic phases, and on the organization of microscopic building blocks across several length scales. Bone, which consists of nanocrystalline calcium phosphate in the form of hydroxyapatite embedded within collagen fibrils (*1*), is one of the most extensively studied biominerals. Fracture resistance of bones is generally attributed to the mineralized collagen fibril (*2*). On page XXX of this issue, Ping et al. (REF#) report that mineral growth inside collagen generates a fibril that is under tension, similar to prestressed concrete.

Bones have a hierarchical architecture where mineralized collagen fibrils are assembled into higher-order structures ranging from the sub-micron to the macroscopic scale (*3, 4*). The main advantage of this type of organization is two-fold: it provides many interfaces that serve as efficient crack-deviation, which enhances the toughness of bone; and it allows the formation of tissues with the mineralized collagen fibrils organized in different motifs, thereby imparting different mechanical properties (*3*). This hierarchical structure of bone is key for understanding both the mechanisms of bone formation and how its mechanical properties arise from its composition and the arrangement of its building blocks.

Using a combination of in-operando X-ray diffraction and Raman microscopy, Ping et al. used carbonate-based minerals to observe how mineral growth inside the collagen-generated compression on the fibrils, and how this stress is subsequently transferred from the fibrils to the mineral. This tension-transducing process leads to prestressed mineralized collagen fibrils. These are strengthened against external tensile pressures that, when organized into higher-order structures, generate the micro- and macroscopic stress as observed in bone (see the figure) (*5, 6*).

Ping et al. highlight prestressing as a widespread strategy to strengthen natural materials with load-bearing functions. A notable example is the trunk of a tree, which is under compression in the central region whereas the outer layers are under tension (*7*). This combination of forces helps the trunk dissipate stress when a load is applied and allows the tree to sustain bending forces without breaking. It is conceivable that the prestressed mineralized collagen fibrils affects the mechanical properties of bone in a similar way. An important difference between wood and bone, though, is that in the former, prestressing is generated not by reinforcing fibrils with minerals, but through the organization of cells in the interior of the tree and the orientation of the cellulose fibrils. This similarity in material properties--shared by vastly different biological systems--shows that different organisms can evolve similar strategies to achieve the prestressing of their structural tissues.

With regard to experimental techniques, Ping et al. provide a neat proof-of-principle demonstration for the in-operando use of advanced X-ray scattering for studying collagen mineralization. Small-angle X-ray scattering facilitates the determination of changes in the overall structure resulting from mineralization, whereas wide-angle X-ray scattering enables the characterization of the mineral at a much smaller crystal structure scale (*8*). By combining both x-ray scattering techniques, one may investigate molecular-level responses to mineralization not only in biomimetic systems, but also in real bones. Moreover, these measurements can be combined with high-resolution 3D X-ray imaging techniques to reveal the nanostructure, orientation, and organization of the hydroxyapatite crystals (*9, 10*). This would provide information on the relationship between the mechanical properties and bone structure at the nano- and micron scales.

The findings of Ping et al. raise several questions about collagen mineralization. Future studies may seek to address the mechanisms behind the molecular contraction and the dehydration of collagen, to explore the impact of size, shape, and orientation of the hydroxyapatite crystals, and to determine the degree of mineralization on the generation of compression forces inside the collagen. These factors are particularly interesting, given the complexity and multi-level organization of the hydroxyapatite crystals in the collagen fibrils, spanning both the intra- and extra-fibrillar spaces (*11*). Quantifying the contribution of prestressing to the overall mechanical properties of bone, and how it scales with the hierarchical organization of the fibrils will constitute an important step towards understanding how the properties of the tissue arise from its composition and structure across length scales. It will be exciting to determine if, and how, prestressing varies between bone tissues with different mechanical requirements and across different species.

This work draws attention to a broader perspective, namely the large variety of biominerals with load-bearing functions found in nature. Enamel and dentin, which compose the vertebrate tooth, are subject to forces during mastication. Shells have to be tough enough to provide protection without fracturing, and in some cases, must withstand significant deformations (*12*). This raises interesting questions regarding if prestresses at the sub-micron- and micron-scales constitute a mechanism to strengthen the mechanical properties of other mineralized tissues. Given the diversity of compositions, structures, and functions of biominerals, it is crucial to elucidate how prestressing is enabled in each case. Hence, using advanced correlative and *in situ* characterization methods, demonstrated in this work, constitutes a step-change in addressing these questions for our general understanding of biomineralization as well as the application of this knowledge in biomedicine, environmental protection, materials design and engineering.

REFERENCES AND NOTES

1.S. Weiner, W. Traub,. *The FASEB Journal* **6**, 879 (1992)
2.J. W. C. Dunlop, P. Fratzl, *Annual Review of Materials Research* **40**, 1-24 (2010).
3.S. Weiner, H. D. Wagner, *Annual Review of Materials Science* **28**, 271-298 (1998).
4.N. Reznikov et al., *Acta Biomater* **10**, 3815 (2014).
5.M. G. Ascenzi, *J Biomech* **32**, 935-942 (1999).
6.Y. N. Yeni, M. B. Schaffler, G. Gibson, D. P. Fyhrie, *Annals of Biomedical Engineering* **30**, 217-225 (2002).
7.R. Ennos, *Solid Biomechanics*. (Princeton University Press, Princeton, New Jersey, 2012).
8.Y. Xu *et al.*, *Nat Commun* **11**, 5068 (2020).
9.T. A. Grunewald *et al.*, *Sci Adv* **6**, eaba4171 (2020).
10.F. Schaff *et al.*, *Nature* **527**, 353-356 (2015).
11.N. Reznikov et al., *Science* **360**, (2018).
12.J. Ihli *et al.*, *Nat Commun* **12**, 5383 (2021).

[1]EaStCHEM School of Chemistry, The University of Edinburgh, Edinburgh, UK.
[2]Department of Physics, University of York, York, UK.
Email: fabio.nudelman@ed.ac.uk