Self-Assembled Collagen-Apatite Matrix Exhibits Bone-like Hierarchy

Mimicking bone at all relevant length scales from nano to macro has remained a significant challenge and presents an important frontier in the field of materials science and regenerative medicine. Previous attempts to prepare bone-like materials could replicate the predominant coalignment of the organic and mineral phase only up to the fibrillar level (~100–300 nm) and with a very low collagen concentration (less than 3 mg/L), preventing the formation of the characteristic three-dimensional (3D) architecture that is typical of the bone tissue. Now, M.-M.G. Guille and N. Nassif of Collège de France (University P and M Curie, EPHE); F. Gobeaux of Collège de France and the Université Paris Sud; J. Seto of Max Planck Institute, Potsdam; P. Panine of the European Synchrotron Radiation Facility of Grenoble, and their colleagues have prepared a collagen-apatite matrix featuring both the organization of collagen fibrils in 3D and the coalignment of the hydroxyapatite crystals within the matrix from the nano- to millimeter scale.

As reported in the June 8 issue of *Chemistry of Materials* (DOI: 10.1021/cm903594n; p. 3307), the research team coprecipitated calcium phosphate salts and pure collagen monomers (300 mg/L) based on a novel process for biomimetic hydroxyapatite precipitation (the latter of which was published in the May 18 online edition of *Chemistry of Materials*, DOI: 10.1021/cm903596q). They then performed a series of analyses aimed at characterizing the generated material (see Figure 1). In particular, x-ray diffraction measurements revealed a heterogeneous organization with the coexistence of aligned and isotropic scattering patterns, as found in natural bone tissue. Two-dimensional small-angle x-ray scattering pattern confirmed the 67-nm axial periodicity characteristic of collagen fibrils, while wide-angle x-ray diffraction generated patterns that were very similar to those featured by demineralized fish bone.

Transmission electron microscopy measurements of ultrathin sections showed that the crystallites were indeed located within the collagen fibrils, and without the presence of the collagen fibrils, the hydroxyapatite crystals did not organize in parallel arrays. Scanning electron microscopy confirmed the role of the acidic polymer polyaspartate in mimicking the role of specific soluble proteins

Furthermore, polarized light microscopy data confirmed that the collagen-apatite-matrix displays the long range organization typical of bone and showed the same

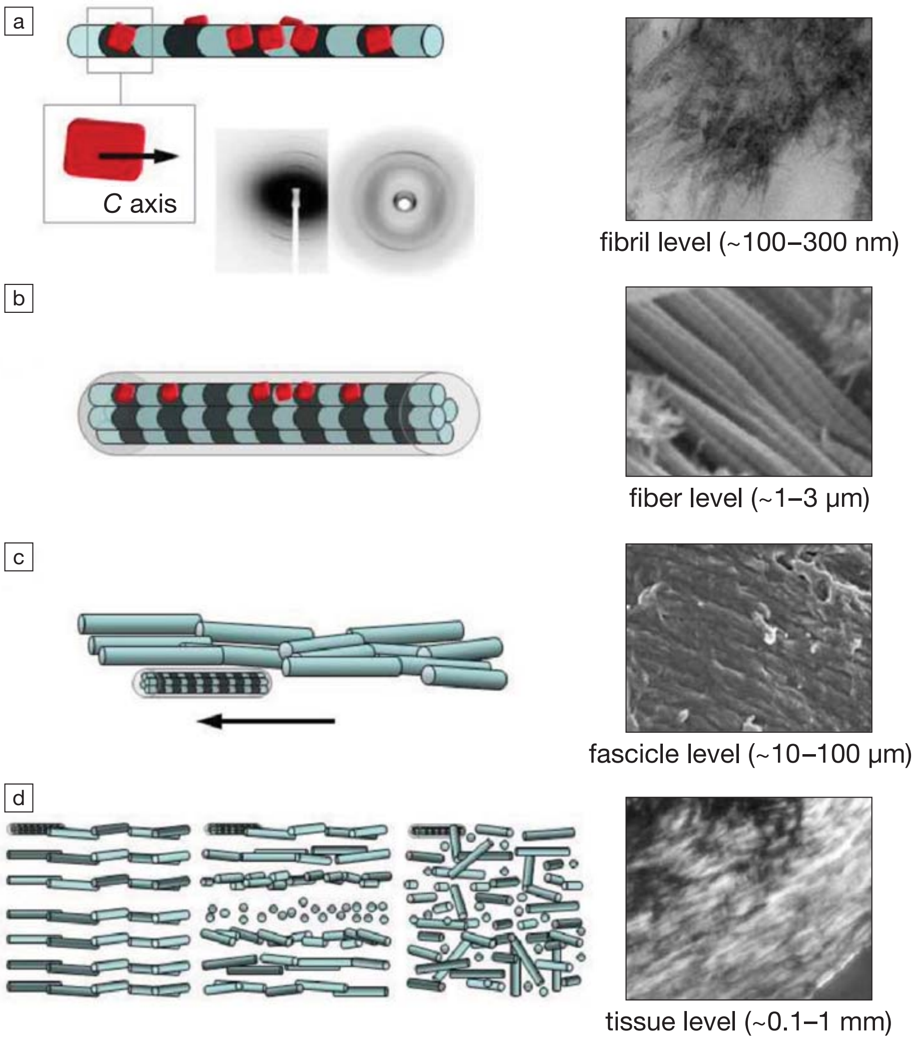

Figure 1. Schematic representation of different levels of organization found in the collagen-apatite. (a) The "mineralized collagen fibril" level (~100–300 nm) is demonstrated by transmission electron microscopy, small-angle x-ray scattering, and wide-angle x-ray diffraction. The c-axis of the hydroxyapatite crystallites is regularly aligned along the collagen fibrils. (b) The "fibril array" level (~1–3 μm) is illustrated by scanning electron microscopy. Fibers result from the parallel staggered packing of collagen fibrils. (c) The fibers are oriented along a preferential direction (~10–100 μm). (d) The "fibril array patterns" level (~0.1–1 mm) is illustrated by polarized light microscopy. The matrix is characterized by a spatial coexistence of different domains, that is, isotropic, aligned (nematic), and twisted (cholesteric). Reproduced with permission from *Chemistry of Materials* **22** (11) (2010) 3307; DOI: 10.1021/cm903594n. © 2010 American Chemical Society.

fibril array patterns. Further confirmation of the coalignment of the collagen and the apatite in the material is based on mechanical characterization, which revealed a difference between the Young's moduli of the transverse and longitudinal sections (6.5 GPa and 4.3 GPa, respectively).

The large set of data extracted from the comprehensive analysis confirmed that the mineralized matrix material has indeed all structural and mechanical properties of a hierarchical organized bone tissue, although its stiffness and mineral content is still lower.

According to the researchers, this research presents a major breakthrough of generating synthetic bone tissue that may eventually be used as bone replacement material, scaffolding structures for tissue engineering, or as a model system to advance the understanding of the formation, structure, and properties of materials in biology.

Other members of the research team include E. Belamie, P. Davidson, G. Mosser, and P. Fratzl.

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