NATURAL FUNCTIONALLY-GRADED COMPOSITES IN HARD-TO-SOFT TISSUE (BONE-TENDON) JUNCTIONS

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Composite materials are often functionally engineered to imbue desired mechanical properties in materials for structural applications. Nature has long engaged in such composite engineering of biological organisms, which has evolved in both *flora* and *fauna* in response to specific mechanical demands. Incorporation of phenolic compounds (like lignin) in stiffening cell assemblies in plant basts, or of silica in plant leaves to resist chomping insect incursions, are good examples in the plant world. Skeletal bone in vertebrates is the classic example in the animal kingdom, a composite of flexible fibrous polymerized organic protein and platy-crystalline inorganic mineral that results in a mechanically strong, hard, tough tissue.

The musculo-skeletal system of vertebrates in fact comprises a variety of *both* hard and soft tissue types (bone, cartilage, tendon, ligament), generative cell types (osteoblasts, chondrocytes, tenocytes, fibroblasts, all of which can derive from multipotent mesenchymal stem cell precursors), and fibrous connective-tissue proteins (chiefly collagen, types I and II) that are susceptible to varying degrees of mineralization. In the case of bone, mineralization is extensive and forms a bi-continuous composite of mineral (chiefly partially-carbonated hydroxyapatite [$Ca_{10}(PO_4,CO_3)_6(OH)_2$] and precursors) and collagen (a triple α-helix polypeptide) that self-assembles into protein fibrils (mostly type I collagen). Bone continually remodels itself and also re-forms as a consequence of injury or around implanted prostheses (such as knee and hip prostheses). High-resolution analytical TEM reveals [1] a mineralization mechanism which entails initial creation, at the mitochondria of bone-forming cells (osteoblasts), of pre-packaged vesicles that fill with a calcium-phosphate hydrogel and thereafter migrate through the cell wall. The vesicle contents subsequently crystallize [2] in the extra-cellular space with the dissolution of the vesicle containment wall, shortly before self-assembling collagen is expressed from the osteoblasts, providing a “just-in-time” ready source of Ca and P for mineralization of collagen fibrils with close to (though not identical with) the Ca/P ratio of hydroxyapatite found in the mature bone composite.

The critical *connective junctions* between different tissue types in the musculo-skeletal system (bone, cartilage, tendon, muscle, ligament) involve several hard-tissue/soft-tissue interfaces, characterized by *gradients* in mineralization, cell type, cell morphology, and collagen self-assembly modes. For example, standard procedure for re-attachment of ruptured tendons—by surgically re-locating the tendon proximally to bone—re-establishes the important bone-tendon junction (*enthesis*) in a period of about one year. The process proceeds through growth, contiguous to the (fully mineralized) bone surface, of a *partially*-mineralized fibrocartilage layer (comprising collagen, expressed by chondrocyte cells, that self-assembles into principally Type II and Type X collagens). TEM [3] of ovine models shows that mineralization of this cartilaginous layer appears to occur *via* the identical mechanism established [1,2] for bone mineralization but initiated instead by chondrocyte cells. SEM [3] reveals that the cell-type in the remaining *un*mineralized cartilage portion gradually morphs into tenocytes, which form more elastic tendon fibers comprising, again, mostly Type I collagen (but also Types III, IV, V and IX self-assembly motifs). The resulting hard-tissue/soft-tissue enthesis junction is thus seen [3] to be a *multiply graded* interface involving three different cell types, several different collagen self-assembly motifs, and the *functional gradation* of a composite material paradigm spanning fully-hard tissue (bone) to fully-soft tissue (tendon).

[1] S. Boonrungsiman, E. Gentleman, R. Carzaniga, N.D. Evans, D.W. McComb, A. E. Porter and M.M. Stevens, PNAS 109 (2012) 141.
[2] V. Benezra, L. W. Hobbs and M. Spector, Biomaterials 23 (2001) 725; A. E. Porter, L. W. Hobbs, V. Benezra and M. Spector, Biomaterials 23 (2001) 921.
[3] L. W. Hobbs, H. Wang, W. M. Reese, B. M. Tomerline, T. Y. C. Lim, A. E. Porter, M. Walton and M. J. Cotton, Microscopy & Microanalysis 19 (2013) 182.