

# On the Horizon From the ORS

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## Rocks in a Bag

Bone is a fabric of organic material reinforced and stiffened by hydroxyapatite mineral crystals attached and interdigitated with proteins.<sup>1</sup> When mineralized regions grow large enough, however, they could interfere with each other, conceivably in a manner analogous to rocks in a bag. At the 53rd Meeting of the Orthopaedic Research Society, a presentation by K. Tai, working with F. Ulm and Christine Ortiz from The Massachusetts Institute of Technology,<sup>2</sup> showed fairly conclusively that “rocks in a bag” could be a better model for bone than one might think.

Dr. Ortiz runs a laboratory where nanoindentation testing of bone is performed. In nanoindentation experiments, a diamond-tipped indenter is forced into a highly polished bone surface. The resulting force-displacement results can be used to calculate the stiffness and failure properties of the tissue. When the indentations are completed, atomic force microscopy (AFM) can be used to image the resulting indentations in the polished surface. It is when the nanometer resolution AFM images are examined that the story begins to become interesting.

The nanoindenter tips are smooth, three-sided pyramids. Therefore, when the indentation is imaged by AFM, one expects to see a smooth, pyramidal dent in the surface. Generally, this is what is seen. However, at lengths smaller than a micrometer, the surface of the polished and indented surfaces is “cobble” with a particulate structure  $<1 \mu\text{m}$  in diameter (about  $0.25 \mu\text{m}$ ) but much larger than the ordinarily observed hydroxyapatite crystal (tens of nanometers in length, but only 1 to 5 nm in thickness).

The “cobbles” can be idealized as interacting particles with an interparticulate friction coefficient. Tai et al<sup>2</sup> measured the friction coefficient by preparing deorganified bone powder and measuring the “angle of repose” of the powder in vacuo. The angle of repose is simply the angle that the side of the pile of powder has in one gravity. Angle of repose is the key input for the Mohr-Coulomb strength theory, a simple strength theory widely used for soils and rock. Essentially, using the angle of repose and the soils failure theory, Tai et al<sup>2</sup> were able to predict accurately the original indentation test results using nonlinear finite element analysis.

The concept of rocks in a bag explains much of bone tissue mechanics that otherwise is fairly obscure. For example, the Mohr-Coulomb theory predicts different tensile and compressive strengths for a material, just as is observed for actual bone tissue. In addition, soils in compression tend to fail along slip lines, whereas soils in tension tend to separate into individual grains. This difference in failure modes is sufficiently like the difference between linear bone microcracks caused by compression<sup>3</sup> and the microcomminuted (diffuse) damage<sup>4</sup> caused by tension<sup>5,6</sup> that Tai et al<sup>2</sup> seem to have presented a key new observation of the mechanical organization of bone at the ultrastructural level.

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## The Osteocytes Speak, the Kidney Responds

It has long been recognized that the kidney controls bone structure by vitamin D- and parathyroid hormone-mediated regulation of calcium and phosphorus availability. Recent characterization of the role of fibroblast

growth factor 23 (FGF-23) has begun to define an interrelated pathway that links kidney activity with osteocyte function.<sup>7</sup> Unlike other members of the FGF family that act as paracrine factors, FGF-23 is a hormone secreted by osteocytes. FGF-23 enters the circulation and inhibits phosphate resorption and production of 1,25-dihydroxyvitamin D<sub>3</sub> in the kidney.<sup>8</sup>

Two recent studies<sup>9,10</sup> describe the characterization of mutations in dentin matrix protein 1 (DMP-1) that gives rise to autosomal recessive forms of hypophosphatemic rickets (ARHR). These studies provide additional critical information regarding this interrelated pathway linking kidney activity with osteocyte function. DMP-1 is a member of the small integrin-binding ligand, N-linked glycoprotein (SIBLING) family of non-collagenous proteins that includes osteopontin, bone sialoprotein, matrix extracellular phosphoprotein, and dentin sialoprotein. DMP-1 is a component of the mineralized matrix of bone and dentine and, as shown recently,<sup>9</sup> forms part of the pericellular matrix surrounding the osteocytic canalicular network. DMP-1 has multiple calcium-binding sites and is thought to promote mineralization by nucleation of hydroxyapatite crystals.<sup>11</sup> ARHR is characterized by rickets, osteomalacia, elevated FGF-23 levels, and hypophosphatemia.<sup>9,10</sup> Generation of DMP-1-null mice that display a similar phenotype to that of ARHR<sup>9</sup> has provided further definition of the pathways involved. The rickets caused by DMP-1 loss in the null mice was alleviated by dietary phosphate supplementation, suggesting that DMP-1 loss caused increased FGF-23 release, thereby resulting in decreased phosphate uptake in the kidney, hypophosphatemia, and rickets. However, failure of phosphate supplementation to correct osteomalacia in the DMP-1-null mice suggests that DMP-1 plays an additional independent role in the control of

bone structure, probably by regulation of osteoblast-to-osteocyte differentiation.<sup>9</sup>

The complex canalicular network morphology of osteocytes and their extensive surface contact with bone matrix have led to consideration that osteocytes are able to sense bone mineral needs and bone quality. These recent reports provide substance for that belief. The information obtained will form the basis to define the details of a long sort pathway connecting osteocytes with the regulation of bone matrix structure by the kidney.

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### **The Road for Wear Debris Disease Leads to RANK and RANKL**

The cell signaling required to develop osteoclastogenesis has been linked to RANK, that is, the receptor activator of nuclear factor kappa  $\beta$  (NF- $\kappa$ B). RANK is on the surface of preosteoclasts and mature osteoclasts; it binds to RANKL, the ligand found on the surface of stromal and bone-lining cells. Many different factors can lead to bone loss; these include parathyroid hormone, tumor necrosis factor, and interleukin-1, all of which stimulate the production of RANKL. Osteoprotegerin is a free-circulating ligand that blocks the RANK/RANKL cell association by attaching to RANK.

The increasing number of joint arthroplasties done each year, both in younger individuals and older recipients who are living longer, has resulted in an increase in the number of prostheses that fail because of "wear debris disease." During the early history of total prosthetic replacement of the hip joint, the phenomenon of the cavitory bone loss associated with loosening was initially mistaken for an occult infectious process. However, it became clear that bone loss could occur in the absence of infection. The term

aseptic loosening was used for some time because the biologic cause was not completely known. By the 1980s, however, it became clear that the aseptic loosening of the prosthesis was somehow related to cellular response to wear particles.<sup>12</sup> Although osteoclasts are required to remove bone, the biochemical mechanism leading to the osteoclastosis was poorly understood. At the end of the 20th century, the principal deterrent for wear debris disease was to decrease wear by improving polyethylene plastics and using alternative bearings.<sup>13</sup>

A hip or knee revision holds a very reasonable chance of success in the healthy adult. Unfortunately, an increasing number of individuals are reluctant to undergo additional surgery or are too ill to have a revision. Thus, continued efforts have been made to determine a means of preventing osteolysis in the presence of wear debris. One strategy has been to use various bisphosphonates to block osteoclastic absorption. Inhibitors to the cell-surface adhesion molecules called integrins have been tested to prevent osteoclast resorption. Integrins are required for osteoclasts to recognize and attach to the bone matrix. Inhibitors to cathepsin, which is secreted by osteoclasts for the breakdown of collagen, have been studied in animals and show effective antiresorptive properties.<sup>13</sup>

The importance of the RANK/RANKL cascade in wear debris disease is now clear. NF- $\kappa$ B is involved in several signals to the nuclear DNA that upregulate several mRNA transcriptions, some of which are important in osteoclastogenesis. In the absence of RANK (the receptor), the debris-induced pathway to osteoclastogenesis is eliminated.<sup>14</sup> The search for safe ways to interfere with this pathway may become important in the management of focal wear debris disease and, perhaps, of systemic disease, such as osteoporosis.

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