J Musculoskelet Neuronal Interact 2005; 5(4):313-315

Original Article

Hylonome

Sacrificial bonds in the interfibrillar matrix of bone

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Keywords: Bone, Facture Resistance, Noncollagenous proteins, Molecular Sacrificial Bonds, Atomic Force Microscopy

A remarkable self-healing, toughening and strengthening system, the sacrificial bonds and hidden length system, is widespread in nature: especially in "glues" in remarkably tough nanocomposite biomaterials such as bone, abalone shell and diatoms. Abalone shell is 97% crystalline calcium carbonate plates by weight, but is 3,000 times more fracture resistant than pure calcium carbonate[1,2]! The other 3% is an interstitial organic matrix, which contains an extremely efficient "glue" using the sacrificial bond and hidden length system[3].

It takes enormous energy to fracture an abalone shell because the protein-based "glue" between the mineral plates stretches and holds on even if the spacing between the plates increases from 1 nm to beyond 100 nm. The system dissipates large amounts of energy with entropic and enthalpic forces while stretching out the hidden length of polymers in the "glue" that is exposed when sacrificial bonds break[3]. Dissipating the energy from impacts in this way protects strong molecular bonds from irreversibly breaking. This mechanism works better in the presence of multivalent positive ions such as Ca^{2+} ions. Multivalent positive ions may be involved in forming bonds between negatively charged groups such as phosphorylated serine residues on the backbones of noncollagenous proteins. For example, both osteopontin and bone sialoprotein, which are prominent noncollagenous proteins in bone[4-7], have many phosphorylated residues that are negatively charged at physiological pH.

Evidence from Atomic Force Microscope indentation[8], pulling[8] and imaging[9,10] together with evidence from macroscopic testing[11] suggests that collagen fibrils and mineral plates are not the only components of bone with mechanical roles[12], as has long been assumed. Bone also contains a protein-based "glue" that uses the sacrificial bonds and hidden length system[13]. The "glue" binds mineralized collagen fibrils to other mineralized collagen fibrils and thus may also play a substantial mechanical role[13]. Order of magnitude calculations show that less than 1% by weight of this "glue" can have profound effects on the fracture resistance of bone, because it involves sacrificial bonds and hidden length[13]. As discussed above, the sacrificial bond-hidden length system can dissipate large amounts of work against entropic forces while stretching out the hidden length that is exposed when sacrificial bonds break. In bone, this appears to occur when mineralized collagen fibrils are torn apart or slid relative to each other during bone fracture. The sacrificial bond-hidden length system is also enhanced in bone with the presence of multivalent positive ions such as calcium ions. This dependence allows us to follow the involvement of the sacrificial bond-hidden length system right up to macroscopic fracture testing.

Many noncollagenous bone matrix proteins such as osteopontin and bone sialoprotein have negatively charged groups such as phosphate groups on phosphorylated amino acids at physiological pH that could be bound together into sacrificial bonds by multivalent positive ions, and are thus natural candidates for this "glue". We cannot, however, rule out a possible involvement of nonfibrillar collagen in the glue. We are embarking on further research to determine precisely which candidate or candidates are involved.

Acknowledgements

We thank Larry Fisher, Herb Waite, Galen Stucky, Dan Morse, Peter Fratzl, Ravi Nalla, John Kinney and Stephanie Lam for valuable discussions.

This work is supported by the National Science Foundation through the UCSB Materials Research Laboratory under Award DMR00-80034, by the National Institutes of Health under Award GM65354, by the NASA University Research, Engineering and Technology Institute on Bio-inspired Materials under Award No. NCC-1-02037, by a research agreement with Veeco #SB030071, by the U.S. Army Research Laboratory and the U.S. Army Research Office under contract number DAAD19-03-D-0004, SNF Postdoctoral fellowship PBEZ_105116 (P. Thurner), and FWF Project No. J2395-N02 (G. Schitter).

The authors have no conflict of interest.

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Accepted 31 July 2005

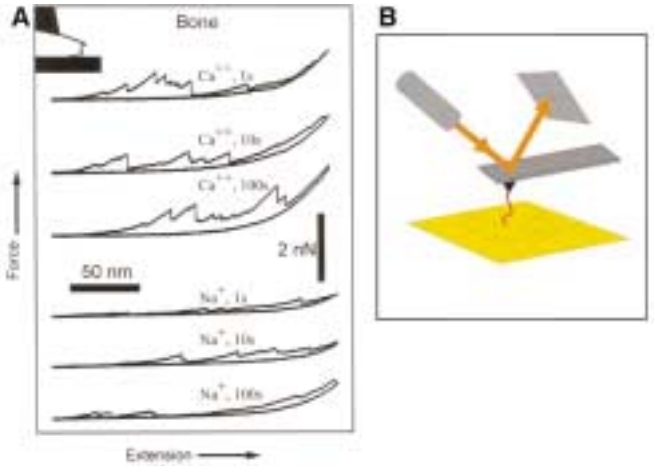

Figure 1. A. Atomic Force Microscope (AFM) force vs extension curves of pulled bone molecules demonstrate that the sacrificial bonds of polymers of the organic matrix of bone are much harder to rupture in the presence of Ca^{++} ion than an Na^{+} buffer. (Thompson et al. Nature 2001; 414:773-776). **B**. AFM molecular pulling: a cantilever stretching a molecule deflects according to the molecule's properties; a laser beam focused on the cantilever and reflected to a diode records the cantilever's deflections.

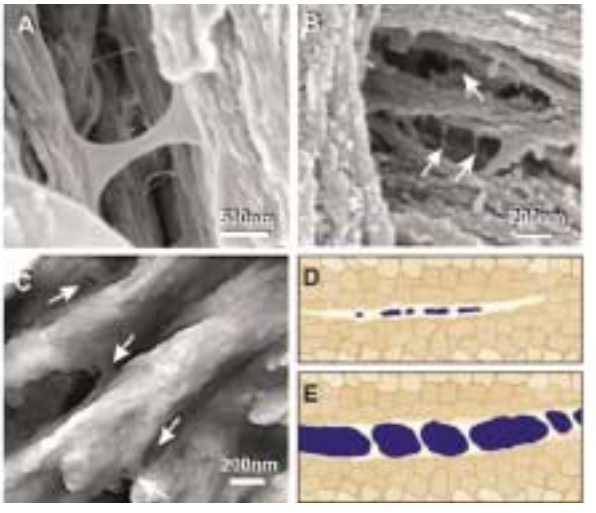

Figure 2. High-resolution Scanning Electron Micrographs (A and B) and AFM (C) show glue resisting fracture in bone. Cartoon (D) shows two adjoining mineralized collagen fibrils at rest; (E) shows the glue resisting the formation of microcracking. (Fatner et al. Nature Materials 2005; 4:612-616).

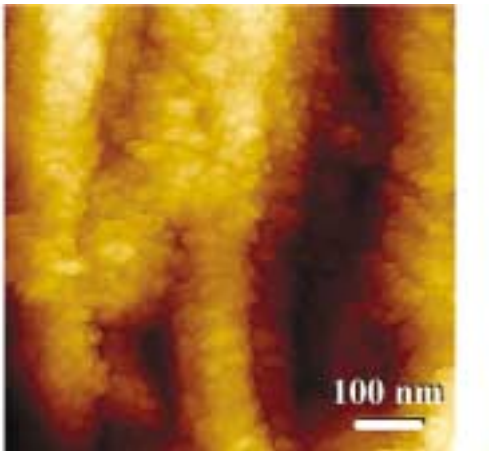

Figure 3. AFM imaging reveals shows bone structure at the nanoscale: collagen fibrils are coated with small mineral particles that appear to be coated with "glue", since the crystal lattice of the mineral, which should be easily observed with AFM, is not visible on these particles.

Figure 4. Hypothesized structures of assembled glue molecules, with sacrificial bonds (represented by *x*'s) along the backbone of glue molecules resisting rupture of the molecules as they are stretched during microcracking. There are three types of sacrificial bonds, shown in the following order from left to right in the insets: 1) Within one molecule. 2) Between two molecules. 3) Between a molecule and the surface of a mineral crystal. (Modified version of Figure 3 in Fatner et al. Nature Materials 2005; 4:612-616).

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