Biologically Inspired Materials Institute
NASA/URETI/BIMat NCC-1-02037
Bioinspired Design and Processing of Multifunctional Nanocomposites

Second Year Annual Report
September 1, 2003 – August 31, 2004

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Period of Performance: September 1, 2003 – August 31, 2004

Biologically Inspired Materials Institute:
Princeton University
Northwestern University
University of California, Santa Barbara
University of North Carolina – Chapel Hill
National Institute of Aerospace

Introduction

This report summarizes progress recorded during the second year of performance for NASA-supported research efforts being conducted by the University Research, Engineering, and Technology Institute (URETI) on Biologically Inspired Materials (BIMat). The research involves individual and collaborative efforts by and among researchers at Princeton University, Northwestern University, the University of California at Santa Barbara, the University of North Carolina – Chapel Hill, and the National Institute of Aerospace. It is being conducted to support recognized critical NASA needs in the areas of high-performance materials and advanced structures important to the successful planning and execution of a wide range of future agency space exploration missions.

The NASA vision for its future space exploration missions is one wherein new generations of space systems must be complex, multi-functional, intelligent and thinking systems that can adapt, form, evolve, and generally deal with changes and unanticipated problems in stressing environments over extended periods. High levels of capability will be achieved through the effective integration of advanced materials and structures with a variety of sensors, responsive control systems and actuators, and distributed power. Realizing unprecedented levels of reliability and functionality in these systems will not be achieved through costly over-design and redundancy. Rather, they will derive from the development of exciting new capabilities involving adaptability and self-repair, as widely manifested in biological systems. In addition, the materials, structures, and overall systems must also be designed to minimize weight, since weight is often the over-riding cost issue in conducting space missions. Biological systems offer inspiration here as well.

High-strength/high-toughness, multi-functional materials capable of providing orders-of-magnitude improvements in both performance and reliability compared to current systems will be a crucial element of these new space systems. Accordingly, the overarching objective of BIMat research is to achieve leap-ahead developments able to surmount current barriers in performance-to-weight ratios and to demonstrate high levels of reliability and functionality beyond any demonstrated to date. The research includes exploratory and focused activities in five principal areas: (1) Bioinspiration and Biomimetics, (2) High-Strength/High-Stiffness Nanocomposites, (3) Sensing and Actuation, (4) Self-Regulating and Self-Healing Systems, and (5) Computational Modeling. Significant progress and specific results recorded in each of these areas during the second year of performance are provided in the following sections.

The comprehensive and extensive results described in this report represent the work of numerous researchers, including faculty, post-doctoral fellows, graduate students, and undergraduate students at the five institutions comprising the BIMat Institute. The work is under the direction of numerous Principal Investigators at these institutions, who are identified below.

Princeton University:

Professor Ilhan Aksay, BIMat Director
Professor Roberto Car
Professor Jeffrey D. Carbeck
Professor Jean H. Prévost
Professor Robert K. Prud'homme
Professor Dudley A. Saville

Northwestern University

Professor Rodney Ruoff, BIMat Associate Director
Professor Ted Belytschko
Professor L. Catherine Brinson
Professor Isaac M. Daniel
Professor Phillip B. Messersmith
Professor George C. Schatz

University of California at Santa Barbara

Professor Daniel E. Morse, BIMat Associate Director
Professor Timothy Deming
Professor Paul Hansma
Professor Galen D. Stucky
Professor J. Herbert Waite

University of North Carolina – Chapel Hill

Professor Edward T. Samulski, BIMat Associate Director
Professor M. Gregory Forest
Professor Eugene A. Irene
Professor John M. Papanikolas
Professor Michael Rubinstein
Professor Richard Superfine
Professor Yue Wu
Professor Otto Zhou

National Institute of Aerospace

Dr. Cheol Park, BIMat Associate Director
Dr. Vasyl M. Harik
Dr. Kristopher Wise

Bioinspiration and Biomimetics

Lessons in Materials Design from Nature. The overall mission of BIMat researchers in the Waite lab at UCSB is to discover and characterize the macromolecular basis for self-healing in materials constructed by living organisms. The work has a particular focus on marine model organisms because they have successfully solved a major problem in materials fabrication—that of building robust composites (organic/inorganic) underwater. One model organism for this work was selected at the beginning of the project—the marine mussel (*Mytilus californianus*); the other, the sandcastle worm (*Phragmatopoma californica*), was selected about one year ago. The progress reported here deals entirely with tube construction by *Phragmatopoma*.

The overall approach to the UCSB work involves: (1) biochemical characterization of the components of a material, although given the costs involved in protein characterization this typically involves only partial sequencing; (2) deduction of a complete primary structure from a partial database provided from component biochemical characterization using recombinant cDNA technology; and (3) fine-tuning an analysis of interactions between proteins discovered in the first two steps and inorganic surfaces. Highlights of work during the first year of BIMat support using this approach were:

- Discovery of a porosity gradient in the mussel adhesive plaques, from no pores at the interface to pore diameters of 1-2 μm in the bulk; this is considered to be a strategy to moderate "modulus mismatch";
- Discovery of a DOPA (3, 4-dihydroxyphenyl-L-alanine) gradient, highest at the interface and decreasing to 1 mole% or less in the thread;
- Discovery of a molecular gradient in mussel byssal threads with mechanical consequences in Young's modulus: the gradient was effected by gradually switching from stiff domains to softer ones in the block co-polymer proteins along the long axis of the threads;
- Discovery of a reversible yield in the threads at strains of 30% or more; recovery is attributed to breakage and reformation of histidine ligand interactions with transition metals, Zn^{+2} and Cu^{+2};
- Discovery that byssal threads were piezoelectric; although this is suggestive of multifunctionality, it is not a useful finding until it has been determined how piezoelectric properties are sensed and actuated by the mussel.

During the second BIMat year, research involving lessons in materials design from nature in the Waite lab focused on adhesion. Sticking to wet glass surfaces is a challenging technological problem.[1,2] The sandcastle worm, which builds tubes out of wet sand, does this very well. Its success depends on a cement that is a mix of two very positively charged DOPA-containing proteins with a negatively charged one that resembles polyphosphoserine. Mixing polyelectrolyte-like proteins to achieve phase separation at conditions where there is no net charge is called complex coacervation. Coacervation allows one to work with concentrated desolvated protein solutions that can be triggered to gel or cross-link. *Phragmatopoma* hardens its cement by oxidizing DOPA residues that then cross-link with cysteinyl groups. The coacervation and cross-linking themes resonate strongly with much needed technology for better wet adhesion.

Phragmatopoma cement consists of proteins and significant levels of phosphate, calcium, and magnesium.[3-5] Two of the cement proteins, Pc-1 and Pc-2, known from an earlier partial characterization, resemble the byssal adhesives of mussels[6] in that they are basic and contain 3, 4-dihydroxyphenyl-L-alanine (DOPA).[7] Stewart et al.[5] recently observed that *Phragmatopoma* cement composition was consistent with protein coacervation and predicted the existence of a third, polyphosphoserine-rich protein to provide the polyanions needed to drive the phase separation of the basic Pc-1 and Pc-2 from the equilibrium solution. The aim of the present research was to find the missing third protein, to obtain full-length sequences of Pc-1 and Pc-2, and to gain some insights into the mechanism of cement solidification. Full-length sequences for cement proteins Pc-1, Pc-2 and Pc-3 were completed and found to be consistent with coacervation. Full sequences for variants of all three proteins now add considerable support to complex coacervation and reveal intriguing additional details about how the cement sets. Careful analysis of the cement indicates that at least half of the cysteine (C) residues in the Pc precursors are destined to react with DOPA-derived quinones, thus resembling, for example, curing in thermosets.

P. californica's successful sand masonry is likely to be largely determined by the adhesive properties of its cement, but there is still much uncertainty regarding what makes a good underwater adhesive. With respect to the interface between a conventional glue and its substratum, the absence of weak boundary layers, good spreading of the adhesive, formation of extensive interfacial interactions, and uniform setting or curing are widely considered to be prerequisites for effective practical adhesion.[1] If fulfillment of these prerequisites also applied to underwater adhesion by *P. californica* the challenges would appear insurmountable. Water, the most subversive of weak boundary layers,[2] is everywhere. Spreading of adhesive would be impeded by the affinity between water and the sand particles. Strong noncovalent interfacial interactions would be compromised by the hydration of the silica surface as well as by the high dielectric constant of water.[8] In addition, cross-linkers needed for curing would continually be lost to the surrounding medium by diffusion.

To overcome these challenges, *P. californica* was proposed to bind together grains of sand by exploitation of a protein-based complex coacervation.[5] By mixing Pc-1, -2 and –3 in the cement gland under appropriate conditions of pH and ionic strength such that the mixture has no net charge, the proteins phase separate or coacervate from the aqueous equilibrium phase. It is noteworthy that negative charge in Pc-3 depends entirely on post-translational phosphorylation. Future studies should address what adaptive effect, if any, differential phosphorylation has on coacervation. Using a variety of biomacromolecules, Bungenberg de Jong[9] established that complex coacervation is endowed with several attributes advantageous to underwater adhesion: a somewhat dehydrated protein content, fluidity that can be triggered to gel or set, density greater than water, high internal diffusivity for good mixing, as well as a very low interfacial tension to enable coacervates to spread over most suspended particles. The mineral-binding capabilities of DOPA are an added bonus.[10]

Although simple coacervation has been implicated in the formation of elastin,[11] this is the first report to identify the molecular players in a biological load bearing material formed by complex coacervation and the reaction by which solidification is triggered. Complex coacervation is already extensively used in colloid chemistry for the microencapsulation of pharmaceuticals, flavors, pesticides, and explosives,[12] but its occurrence in the construction of

sandcastles by *P. californica* holds promise for inspiring a new generation of synthetic composite materials as well.

Continuing research in the third year will explore whether polyphosphoserine proteins are also involved in mussel adhesion, compare the abundance of cysteinyl-DOPA based cross-links with other covalent and noncovalent strategies, include mechanical testing of tube adhesion with and without chelation treatment, and begin construction of a phase diagram for adhesive proteins.

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Hassenkam, T., Gutsmann, T., Hansma, P., Sagert, J., and Waite, J. H. (2004), Giant bent core mesogens in the thread forming process of marine mussels. *Biomacromolecules* 5, 1351-1355.

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Stewart, R.J., Weaver, J.C., Morse, D.E., and Waite, J.H., The tube cement of *Phragmatopoma californica:* a solid foam. *J. Exp. Biol***. 207**, 4727-4734 (2004*).*

Discovering Nature's Mechanisms for Material Toughness. The hypothesis behind this research, being conducted in the Hansma lab at UCSB, is that the fracture behavior of mineralized tissues, particularly bone, critically depends on a molecular "glue" that binds together mineralized collagen fibrils and mineralized particles in reversal/cement lines. This

hypothesis is based on high-resolution imaging studies of bone at UCSB using the Atomic Force Microscope (AFM),[1] on force spectroscopy of bone that revealed "glue-like" molecules,[2] and on high-resolution imaging and mechanical testing of bone with a degraded organic matrix.[3] Further, single-molecule force spectroscopy in a related system revealed that the molecular mechanistic origin of the toughness of abalone shell is due to its microstructure together with a molecular "glue."[4] Just a few percent by weight of that "glue" makes abalone shells, which are 97% crystalline carbonate, 3,000 times more fracture resistant than crystalline carbonate!

Part of the UCSB work on bone is based on a recent observation[4] that "sacrificial bonds that tie up "hidden length" within one polymer molecule or between molecules can increase the energy to break the polymer by orders of magnitude. Though this observation was made for abalone shells, it may be the molecular mechanistic origin of the toughness of other natural adhesives, fibers and composites—such as bone. This observed mechanistic phenomenon has been dubbed the "sacrificial bond – hidden length" system. An important complementary aspect of the work continues to be the development of AFMs that are faster, more accurate, and able to provide ever-higher resolution. Significant progress in near-video rate AFM is expected over the next year based on preliminary results with a new scanner and head.

Much progress has been recorded in both areas since the creation of the BIMat URETI in 2002. During the first year, research focused on the remarkable strength and elasticity of spider-web silk threads, in particular capture-silk threads: the very sticky, strong and elastic spiraled material in the webs of orb-weaving spiders. Force spectroscopy experiments with the AFM, pulling bulk threads, and amino acid sequencing led to the formulation of models for molecular and multimolecular structure of the silk.[5] Structure-function relationships and mechanical properties of collagen, primarily the collagen of rat-tail tendon fibrils[6,7], were also investigated. AFM work led to innovative implementation of small cantilevers to achieve higher resolution with less disruption from overall noise due to the attainment of higher resonance frequencies.[8] This development has enabled the performance of novel experiments for characterizing proteins and other fine molecular structures, particularly for force spectroscopy experiments to characterize mechanical properties. This innovation led to a patent for an optical high sensitivity device to sense the deflection of an AFM cantilever through a laser incident beam (US Patent 6,455,838). The device enables AFM users to adjust the size, position and power of the incident beam with better ease and accuracy.

In the second BIMat year, work in the Hansma lab focused mainly on "glue" in bone and other mineralized tissues, as well as other structural determinants of material strength. This work included ***the first AFM study of living diatoms in ambient conditions – diatom "glue"***. As noted for the work cited above, engineering stable underwater adhesives currently poses a major technical challenge. Most man-made adhesives fail in wet conditions due to chemical modification of the adhesive or its substrate. Diatoms, however, produce adhesives that are extremely strong and robust in both fresh- and seawater environments. Phase-imaging and force-pulling experiments at UCSB have revealed key characteristics of these natural adhesives that might be of use in designing man-made analogs that function in wet environments.

The AFM allows for investigations of micromechanical properties of the cell surface (e.g., viscoelastic properties, adhesion forces and hardness measurements) in physiological conditions. Effortless sample preparation and unprecedented resolution on insulating materials are other benefits. UCSB researchers conducted the first *in vivo* study of diatom adhesion with AFM during this period. The experiments involved three species that produce outstanding

natural adhesives: *Eunotia sudetica*, *Navicula seminulum,* and a yet to be identified species. The siliceous skeleton of the diatom is enveloped by an organic coating consisting essentially of polysaccharides and proteins and some lipids. All three diatom species investigated form chains by adhesion of their valve faces and were strongly attached to the glass sample slides, either with their valve face (*Navicula seminulum*) or with part of their girdle bands and valve sides (*Eunotia Sudetica* and the yet unidentified diatom species). AFM phase images depict the phase delay between the drive and response of the cantilever. These images contain information about the energy dissipated during the interaction of the AFM tip with the sample, and enhance understanding of the viscoelastic and adhesion properties of the surfaces being investigated, specifically of the organic material responsible for diatom adhesion.

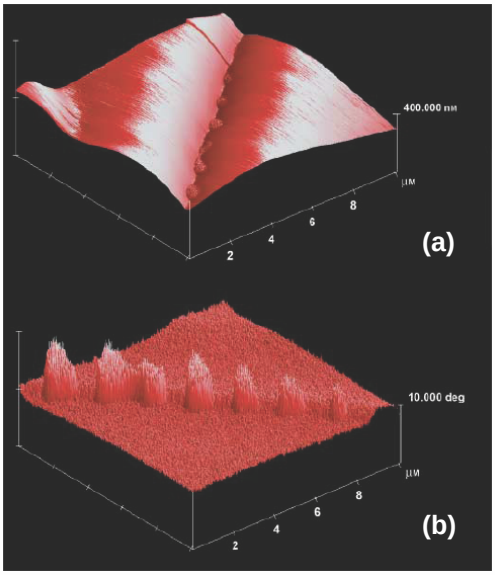

Figure 1. Topographical (a) and phase (b) images of diatom adhesive between two cells. In (a), the adhesives in the contact region of two cells of *Eunotia sudetica* are apparent as small topographic features on the slightly undulated cell interface. The corrugation of the bead-like structures is between 10 and 20 nm, and their lateral dimension and spacing is ~1 µm. In the phase image (b), these features are far more striking. The diatom adhesive causes a phase lag of about 10° compared with the rest of the frustule surfaces, where on a single frustule it is within 1°. Note the 2° interfrustule phase step, which reveals slightly different viscoelastic properties of the two neighbouring valves. Note that for a better view, (b) is rotated clockwise by 90°compared with (a).

Understanding the mechanics of living bone continues to be a major scientific challenge. An important aspect of this challenge is understanding the nanoscopic interplay between the basic building blocks of bone. It is known that bone is primarily composed of mineralized collagen fibrils. Imaging these mineralized fibrils is a daunting task since both the fibrils and the mineral plates are very small. There has been recent progress, however, with light, electron, and scanning probe microscopy, both on bone. Nevertheless, tapping the structural layout of mineralized collagen fibers, within their natural environment of healthy bone, remains a challenge. Recent work at UCSB has involved ***high-resolution AFM imaging of intact and fractured trabecular bone and study of the "glue" in bone.***

Bone is a three-phase composite material composed of mineral plates, tube-shaped collagen fibrils, and other organic matter made up almost entirely of proteoglycans and glycoproteins, which has been termed "the organic matrix." Previous work on the organic matrix involved characterization of its components and their roles in mineralizing bone; its possible mechanical role as "glue" or otherwise has not been investigated outside of the UCSB group. The work at UCSB has been inspired by earlier work there on the "glue" in abalone shell and its importance to shell mechanical properties.

Unlike many previous studies of structure-function relationships in bone, the possible structural facets of the organic matrix were given careful consideration. The UCSB study has produced—and continues to obtain—the highest resolution images of bone to date. There has been much speculation and theorizing on the size and shape of bone components, such as the mineral plates coating the collagen fibrils. Images of bone components have been obtained previously using Transmission Electron Microscopy (TEM) and Scanning Electron Microscopy (SEM); however, these techniques require fragile biological sample to be dried, coated in metal, and then imaged in vacuum. AFM images obtained at UCSB have revealed a number of interesting features:

- On pristine fractured surfaces, a densely woven structure of collagen fibrils, banded with a 67-nm periodicity, and densely packed mineral plates;
- The mineral plates on collagen fibrils overlap and exhibit a large range of plate diameters from 30 to 200 nm. Ranges were also determined for other dimensions;
- In a few isolated locations, short sections of bare collagen fibrils are visible;
- In other regions, the existence of the underlying collagen fibrils can be inferred from the linear patterns of the mineral plates;
- On collagen fibrils, small nodular features, spaced 20–30 nm, run perpendicular to the fibrils. In some cases, these nodules are also seen on filaments extending between collagen fibrils; mostly, these aggregates form bridges between individual fibrils. The protrusions are thought to be noncollagenous proteins such as proteoglycans and may have collapsed into compact structures when the sample was dried; these nodules are hypothesized to contain "glue";
- Fractured samples, rinsed to remove mineral plates, reveal separated collagen fibrils on the fractured surfaces; these fibrils are often covered with protrusions similar to those observed on the exterior surfaces but are less organized;
- In addition, as on the exterior surfaces, there are sometimes small filaments extending between neighboring collagen fibrils, about 20nm in diameter – some of these filaments are collagen fibrils, some are other organic polymeric matter.

This work may have clarified a number of issues that have been debated in the literature. The size of the mineral crystals observed ranged from 20-200 nm in diameter, 550-200nm in width (with an average of ~100 nm), and approximately 20 nm thick. The length of the collagen fibers were difficult to estimate from the AFM images due to the woven structure of the composite, but the fibrils were at least several micrometers long. In addition, the vast majority of the mineral crystals were plate-shaped, not needle-shaped. One other important insight gained relevant to the development of new, biologically inspired materials, is that in the central region of trabeculae, collagen fibrils are woven in an interlocking meshed pattern. This is a region of maximum stress experienced during falls and other impacts. These studies provide important new information regarding the nanostructured architecture of this complex biocomposite; even on the nanoscale, the material can vary dramatically in its structure.

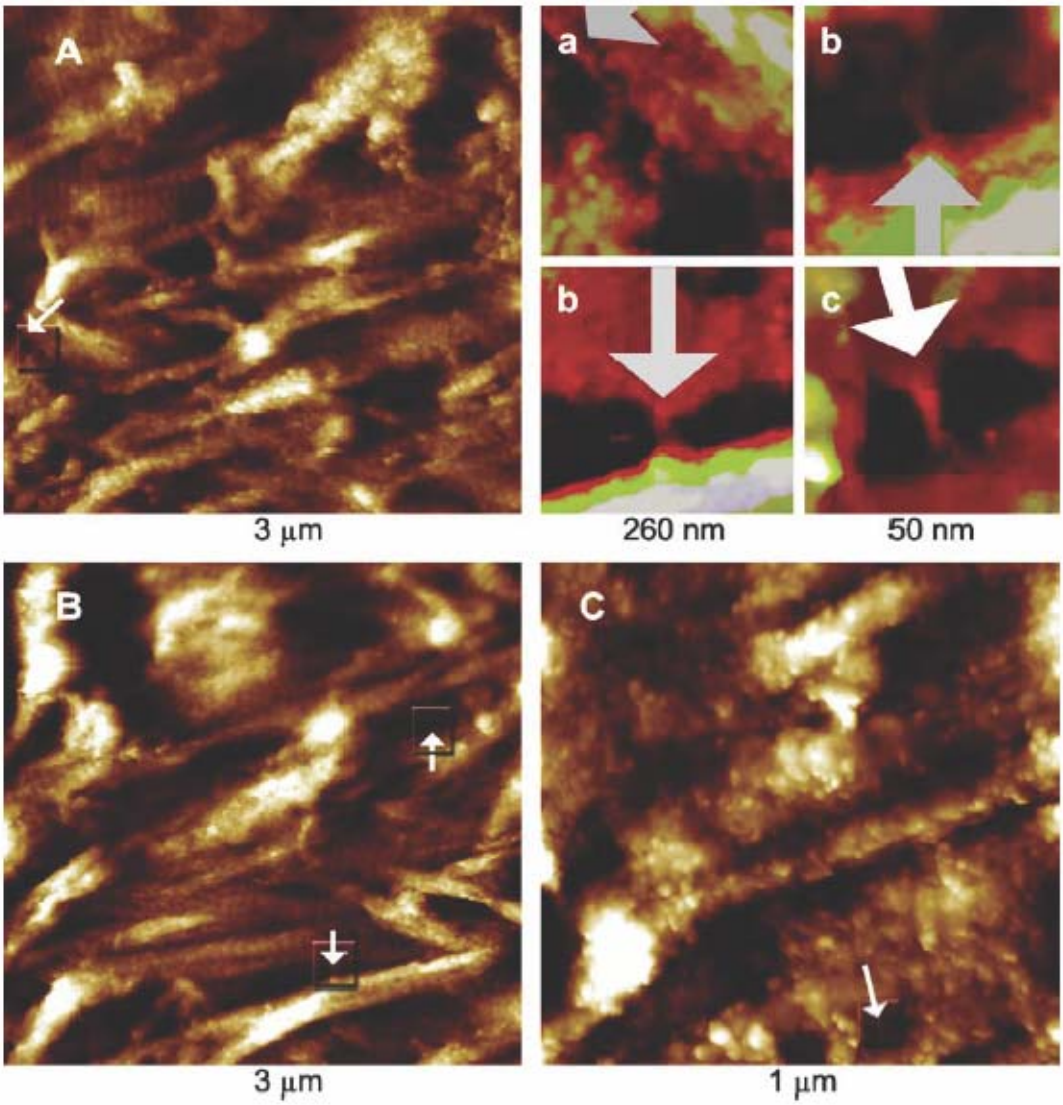

Figure 2. AFM topography images from different regions of the fracture surface of a trabecula that has been partly demineralized by rinsing in water. The 67-nm banding pattern in the collagen is evident in all the fibrils. The small protrusions covering the collagen fibrils are probably noncollagenous proteins attached to the fibrils though there may be some remaining mineral plates as well. In some cases, bridging aggregates between individual collagen fibrils is clearly visible (see arrows in the full frame images and in the magnified and computer-enhanced small images; a is from A, b is from B, and c is from C).

A third area of investigation in the Hansma lab at UCSB during the past year has involved study of ***toughness mechanisms in marine organisms.*** Tremendous mechanical stresses can be experienced in the area of the interface of the hard and soft tissues that comprise most organisms. Little is known about how nature works to mitigate these stresses. The work at UCSB on two noncellular tissues, mussel byssus and polychaete (worm) jaws, suggest that one natural strategy to relieve interfacial stresses between adjoining stiff and soft tissue appears to be the creation of a "fuzzy" boundary, which avoids abrupt changes in mechanical properties, and graduates the transcendence from stiff to soft properties. This observation has relevance to the development of new synthetic materials. In manufacturing such materials, two methods can be

employed to mitigate interfacial stresses: (1) increasing the energy of interaction across the contact zone between areas of different mechanical properties, or (2) avoiding sharp boundaries between materials. Although the first option may increase the stress at failure, it does not decrease interfacial stresses. It has been practiced for many years and is the basis for "priming" or surface-coupling treatments of glass or metals that precede bonding with polymers. The second option, which has surfaced only recently, is the manufacture of functional gradients. In other words, if material A can be processed so that it gradually or incrementally becomes material B (and vice versa), then interfacial stresses can be dispersed over a much larger surface area and volume.

The byssus is a connective tissue peculiar to mussels. It is remarkable because, although it is remarkably stretchy, it is also stiff and strong.[9] It is deposited outside the confines of living tissue and contains no cells for maintenance or repair. Byssus securely attaches mussels to rocks and pilings against the strong, repetitive forces generated by waves. The byssus thus mediates between a very stiff inert material (e.g., rock) and very soft living tissue. Because mussel byssus is a bundle of several hundred threads, each measuring between 2 and 4 cm in length and 50 μm in diameter (in *Mytilus edulis* and *M. galloprovincialis*), every individual thread represents a unit of attachment with the distal end bonded to rock and proximal end inserted into living mussel tissue. Previous scanning electron microscopy and biomechanical studies have established that it is much more than a tether. It represents a mechanically graded fiber that is significantly stiffer at the distal end where it joins to rocks than at the proximal end where it joins to living tissue.

Much recent work has focused on the fibrous proteins that make up the different portions of the byssal thread. The simplest unit of structure is a trimer of preCOL chains (~80 kD each). The proteins composing byssal threads were characterized, and their locations along the thread, their mechanical and chemical properties, and their contributions to the graduating mechanical properties of the byssal thread have been reported.[10] Recent work by Waite et al.[11] on the collagen-containing threads from both *M. edulis* and *M. galloprovincialis* showed that the ultimate or breaking strain is between 100 and 200% in the proximal portion and is an important feature of thread toughness. This strain greatly exceeds the ultimate strain of ~10% for more typical collagenous structures such as tendon.[9] Toughness in a variety of biological materials seems to be linked to the reversible sacrificial unfolding of modular domains to provide extra length during deformation, for example in titin.[12] In work conducted in the Hansma lab at UCSB, individual byssal threads from intact byssus shed by marine mussels (*Mytilus galloprovincialis*) were dissected and imaged using an AFM. Examination of preCOL-NG assemblies in the proximal thread revealed many levels of hidden extra length that may contribute to the extraordinary observed strain.

Although much work has been done on the structure and formation of byssal threads, the specific shape of mesogenic units, the nature of their ordered assembly in the byssus, and how they deform under tension are still unknown. The gigantic banana-shaped protein mesogens used by marine mussels to make their byssal threads were characterized, and it was determined that the sacrificial bonds and hidden length system in these protein molecules contributes to the amazing strains the threads can withstand before yielding.[13] It is quite likely that nature has many other examples of fibers and films based on giant bent-core mesogens.[14,15] Fundamental studies of these could provide exciting bio-inspired design paradigms for a host of new materials properties and applications.

The well-developed jaws of the *Nereis* marine worm were also investigated. This worm uses its jaw to grasp, inject venom into, and dismantle prey. The jaws contain proteins with histidine-rich domains and transition metals, Cu and Zn, and nanoindentation experiments confirmed the distinct mechanical gradients the jaws display, with the jaw tips much stiffer than the jaw base. The chemical make-up of the jaws was studied, particularly the basis for the preference mussels and worms have for using histidine-metal groups to stabilize the structural proteins that make up most of the jaw, instead of covalently cross-linking them. Histidine-metal interactions can be robustly and instantly formed (e.g., by being triggered to be formed at pH changes); they are also reversible and so able to self-heal. The addition of only a few percent Zn ions with the jaw proteins makes the material 2-3 times harder and stiffer than the best organic synthetic polymers, and ranks it near dentin in hardness, even though dentin is over 70% mineral by weight, as reflected in Figure 3 below.

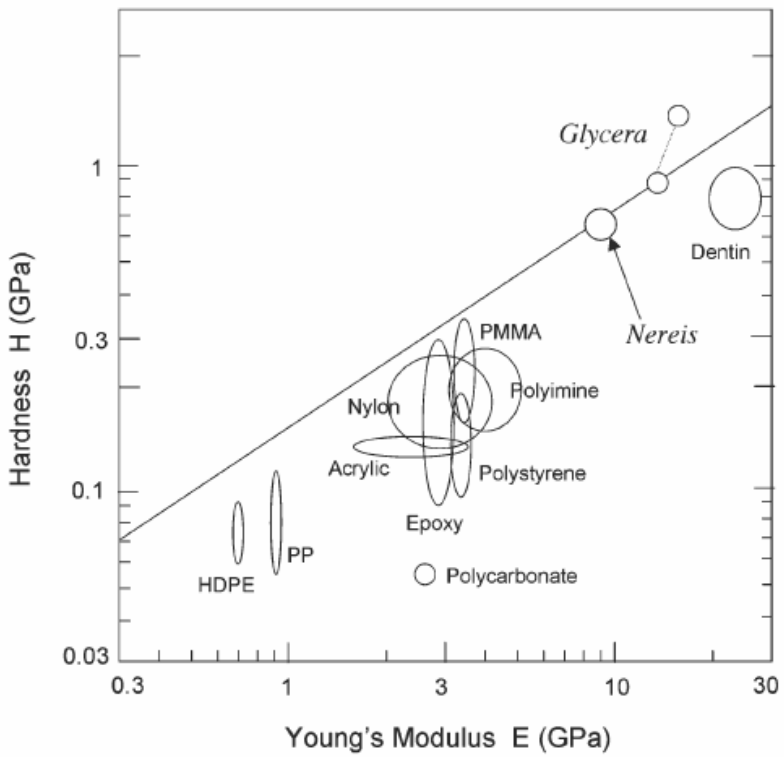

Figure 3. Relation between hardness and elastic modulus for various materials.

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