J Mater Sci (2007) 42:8765–8770
DOI 10.1007/s10853-007-1952-8

Nano- and micromechanical properties of hierarchical biological materials and tissues

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Published online: 7 August 2007

Abstract The mechanical properties of biological materials have been the focal point of extensive studies over the past decades, leading to formation of a new research field that intimately connects biology, chemistry and materials science. Significant advances have been made in many disciplines and research areas, ranging throughout a variety of material scales, from atomistic, molecular up to continuum scales. Experimental studies are now carried out with molecular precision, including investigations of how molecular defects such as protein mutations or protein knockout influence larger length- and time-scales. Simulation studies of biological materials now range from electronic structure calculations of DNA, molecular simulations of proteins and biomolecules like actin and tubulin to continuum theories of bone and collagenous tissues. The integration of predictive numerical studies with experimental methods represents a new frontier in materials research. The field is at a turning point when major breakthroughs in the understanding, synthesis, control and analysis of complex biological systems emerge. Here we provide a brief perspective of the state of this field and outline new research directions.

Introduction

The field of mechanical properties of biological materials has seen an exciting development over the past several years, partly due to the emergence of physical science based approaches in the biological sciences, leading to cross-disciplinary investigations of materials, structures, diseases as well as the development of new treatment and diagnostics methods.

This special issue of the *Journal of Materials Science* entitled "Nano- and micromechanical properties of hierarchical biological materials: Linking mechanics, chemistry and biology" constitutes a snapshot of the current status, new directions and research opportunities in this field. The focus of the collection of papers is on experimental, computational and theoretical efforts that contribute to a more quantitative understanding of biological materials and the interaction of biological materials with their environment, across various scales. This includes the deformation and fracture behavior of biological materials, with a particular focus on nanoscale features and materials, and its relationship with diseases.

The issue also contains contributions that focus on the synthesis and growth of biological tissues and materials, in particular such methods that advance the ability to control nano- and micro-structural features more quantitatively. Individual contributions emphasize on linking the chemical or molecular, and mesoscopic structures of these materials to macroscopic engineering properties, across various scales, including the impact of genetic mutations, solvent conditions and other chemical stimuli on the macroscopic behavior of the material. Specific topics include elasticity, deformation and fracture of biological and biomimetic materials including bone, tissues and scaffolding materials, interactions of cells with materials under mechanical loading, mechanics of hierarchical biological materials as well as the mechanics of single molecules. Development of experimental protocols to study the human stratum corneum, addressing the role of misfolded proteins in Alzheimer's

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disease, development of theories of the properties of viral capsids and elucidation of the statistical mechanics principles of stretching biopolymers in geometric confinement represent a broad range of topics.

Materials science of biological materials: challenges and opportunities

Historically, the use of classes of materials has been used to classify stages of civilizations, ranging from stone age more than 300,000 years ago, to the bronze age, and possibly the silicon age in the late 20th and early 21st century. However, a systematic analysis of materials in the context of linking chemical and physical concepts with engineering applications has not been achieved until very recently. For instance, 50 year ago, E. Orowan, M. Polanyi and G.I. Taylor have discovered dislocations, a concept proposed theoretically in 1905 by V. Volterra. It was discovered that dislocations represent the fundamental mechanism of plastic deformation of metals [1, 2]. Remarkably, it was not until dislocations and other nano- and microscopic mechanisms have been understood theoretically that major breakthroughs have been possible that utilize this knowledge, to enable building airplanes, cars, space shuttles and more recently, nanodevices, through synthesis of ultra-strong and heat resistant materials, for instance.

Perhaps, today we stand at another cross-road: Biological materials and systems are vital elements of life, and therefore, a rigorous understanding of the matter that makes life "work" is in reach. This may enable us eventually to integrate concepts from living systems into materials design, seamlessly. Optical, mechanical and electrical properties at ultra-small material scales, their control, synthesis and analysis as well as their theoretical description represent major scientific and engineering challenges and opportunities. However, just like in the case of more conventional materials, these breakthroughs will probably only be accessible provided that the fundamentals are understood very well. Characterization of the materials found in biology within a rigorous materials science approach is aimed towards the elucidation of these fundamental principles of assembly, deformation and fracture of these materials.

Deformation and fracture properties are intimately linked to the atomic microstructure of the material. Whereas crystalline materials show mechanisms such as dislocation spreading or crack extension, biological materials feature molecular unfolding or sliding, with a particular significance of rupture of chemical bonds such as hydrogen bonds, covalent cross-links or intermolecular entanglement. Much different mechanisms operate at larger length scales, where the interaction of molecules with cells and of cells with one another, different tissue types and the influence of tissue remodelling become more evident. The dominance of specific mechanisms is controlled by geometrical parameters as well as the structural arrangement of the protein elementary building blocks, across many hierarchical scales, from nano to macro (Fig. 1).

It is known from other fields in materials science that nano- or microscopic structures control the macroscopic material behavior: For example, grain size reduction or confinement leads to an increase of the strength of crystalline metals [3–6]. Deformation maps have been proposed to characterize material properties for engineering applications [7]. Discovering similar insight for biological structures and materials represents and important frontier of research. A particularly challenging question is the elucidation of the significance and role of nanostructures for macroscopic properties, that is, carrying out sensitivity analyses that show how small-scale features influence larger scale properties.

A major trait of biological materials is the occurrence of hierarchies and, at the molecular scale, the abundance of weak interactions (e.g. H-bonds). The presence of hierarchies in biological materials may be vital to take advantage of molecular and sub-molecular features, often characterized by weak interactions, and multiply their properties so that they become visible at larger scales, in order to provide a link between structural organization and function [8]. Utilization of weak interactions makes it possible to produce strong materials at moderate temperatures and thus with limited energy use. An important distinction between traditional and biological materials is the geometrical occurrence of defects. While defects are often distributed randomly over the volume in crystalline materials, biological materials consist of an ordered structure that reaches down to the nano-scale. In many biological materials, defects are placed with atomistic or molecular precision, and may play a major role in the material behavior observed at larger scales. These features have been observed in bone, nacre, collagenous tissue or cellular protein networks.

The mechanical properties of biological materials have wide ranging implications for biology. In cells for instance, mechanical sensation is used to transmit signals from the environment to the cell nucleus in order to control tissue formation and regeneration [9, 10]. The structural integrity and shape of cells is controlled by the cell's cytoskeleton, which resembles an interplay of complex protein structures and signaling cascades arranged in a hierarchical fashion [9]. Bone and collagen, providing structure to our body, or spider silk, used for prey procurement, are examples of materials that have incredible elasticity, strength and robustness unmatched by many man-made materials, mainly attributed to its structural formation with molecular precision [11–19]. The transfer of concepts observed in biology into technological applications and new materials design remains a big challenge, with potential huge payoff.

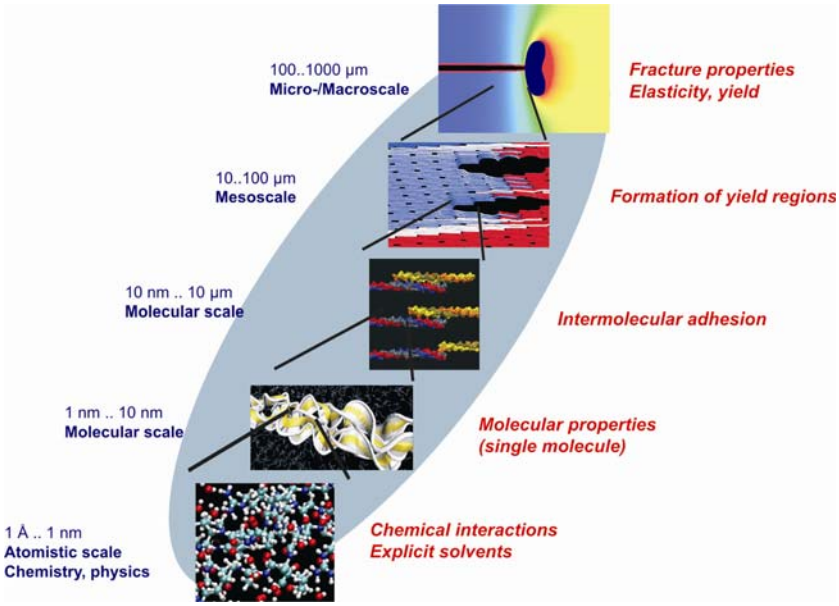

Fig. 1 Overview over different material scales, from nano to macro, here exemplified for collagenous tissue [11–15]. Biological materials such as collagen, skin, bone, spider silk or cytoskeletal networks in cells feature complex, hierarchical structures. The macroscopic mechanical material behavior is controlled by the interplay of properties throughout various scales. In order to understand deformation and fracture mechanisms, it is crucial to elucidate atomistic and molecular mechanisms at each scale, and to appreciate the cross-scale interaction of these mechanisms. Our ability to synthesize, characterize and control such materials in their native environment or in technological applications depends critically on the theoretical foundation of its mechanical behavior

In particular, the combination of nanostructural and hierarchical features into materials developments could lead to significant breakthroughs.

What are the most promising strategies in order to analyze these materials? Perhaps, an integrated approach that uses experiment and simulation concurrently could evolve into a new paradigm of materials research. Experimental techniques have gained unparalleled accuracy in both length- and time scales (see Fig. 2), as reflected in development and utilization of Atomic Force Microscope (AFM) [20, 21], optical tweezers [22, 23] or nanoindentation [24] to analyze biological materials [25]. At the same time, modeling and simulation have evolved into predictive tools that complement experimental analyses (see Fig. 2). It is now achievable to start from smallest scales—considering electrons and atoms, to reach all the way up to macroscopic scales of entire tissues [26], by explicitly considering the characteristic structural features at each scale. Even though there are still major challenges ahead of us, this progress is amazing and provides one with infinite possibilities and potentials, transforming materials science as a discipline through increased integration of computational approaches in scientific research.

Linking the scales: cross-scale interactions

A central theme of the efforts in developing the materials science of biological materials is to appreciate the structure-property or structure-processing-property paradigm, constituting the heart of the materials science community. This paradigm has guided materials science for many decades. For biological materials, there are many challenges that make developing these rigorous links increasingly difficult.

For example, bond energies in biological materials are often comparable to the thermal energy, as for instance in the case of hydrogen bonding, the most abundant chemical bond in biology. Biological materials show highly viscoelastic behavior, since their response to mechanical deformation is intrinsically time-dependent. In many cases, biological structures contain extremely compliant filaments, in which entropic contributions to free energy are important and can even control the deformation behavior. Many material properties are also length scale dependent and can vary significantly across various length scales. Quite often, this can be quite perplexing, since measuring different volumes of material lead to different values of Young's modulus. Size effects very strong and possibly utilized systematically to ensure physiological functioning of the material in its biological context. However, why and how these size effects are exploited within this context remains less understood. The presence of hierarchical structures calls for new paradigms in thinking about the structure-property paradigm, since corresponding concepts must include an explicit notion of the cross-scale and inter-scale interactions [27].

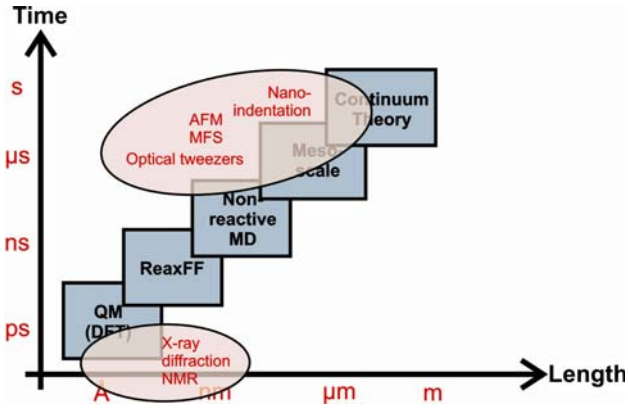

Fig. 2 Overview over various computational and experimental tools. Hierarchical coupling of different computational tools can be used to traverse throughout a wide range of length- and time scales. Such methods enable to provide a fundamental insight into deformation and fracture phenomena, across various time- and length-scales. Hand-shaking between different methods enables one to transport information from one scale to another. Eventually, results of atomistic, molecular or mesoscale simulation may feed into constitutive equations or continuum models. While continuum mechanical theories have been very successful for crystalline materials, biological materials require statistical theories. Experimental techniques such as Atomic Force Microscope (AFM), Molecular Force Spectroscopy (MFS), nanoindentation or optical tweezers now overlap into atomistic and molecular approaches, enabling direct comparison of experiment and simulation

It has become evident that the atomistic scale, and in particular the notion of a chemical bond, provides a very fundamental, universal platform at which a variety of scientific disciplines can interact. Chemists, through the molecular structure of proteins, physicists, through the statistical mechanics of a large number of atoms, and materials scientists through analysis of phenomena such as elasticity, optical properties, electrical properties or thermodynamics, linking structure and function (see Fig. 3).

A particularly exciting aspect of the materials science of biological materials is that it is interdisciplinary, by nature. Nature doesn't know of scientific disciplines, since they were invented by humans many centuries ago. Performing research in this field thus often means to overcome barriers between scientific disciplines and to develop strategies that enable us speak to each other more openly. Structures in universities and research institutions may have to be modified to facilitate such investigations.

It is vital overcome the barrier that currently separates the scales, through development of new methods, better model systems and an advanced appreciation for a multi-scale view, in order to fully understand multi-scale or cross-scale interactions. To facilitate these developments, we must also develop a proper nomenclature to capture the various scales involved in a material. Current terminologies referring to atomistic, meso, micro and macro are insufficient to capture the subtleties of the various scales. Research should address the question, what are the opportunities in integrating nanoscience and nanotechnology into biological research? What will and can our impact be, in a long perspective, in understanding fundamental biology? For instance, is the nanomechanics of protein materials significant for biology, and have biologists missed out on important effects due to lack of consideration of the nanomechanics? How does Nature design materials that are environmentally friendly, lightweight and yet tough and robust and can serve multiple objectives? How is robustness achieved? How do universality and diversity integrate into biological structures?

From a theoretical viewpoint, major challenges are the development of new materials theories that include atomistic and statistical effects into an effective description,

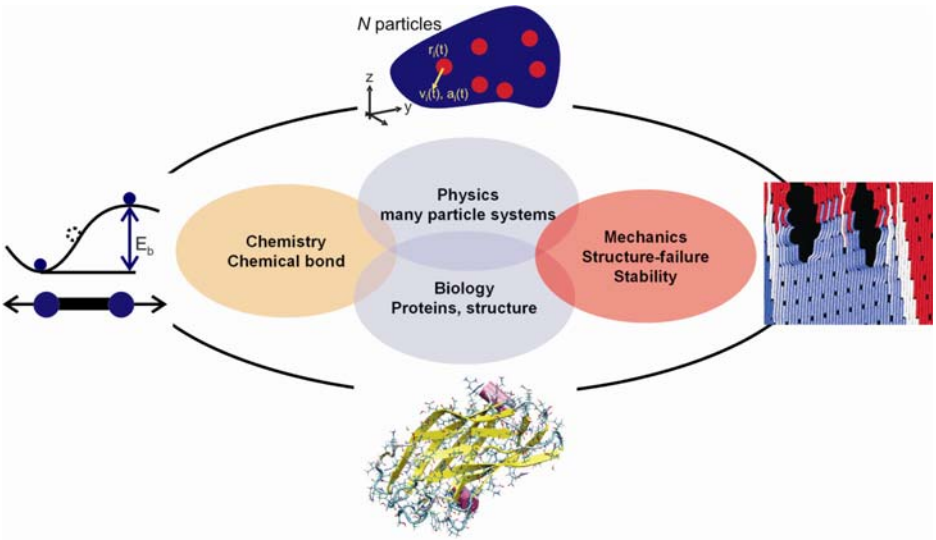

Fig. 3 Chemistry is the most fundamental 'language' of materials science. Many other disciplines can link up with the notion of a 'chemical bond' that defines the structure and eventually the properties of materials, thereby representing a joint root for these disciplines

while retaining a system theoretical perspective [28–30], maybe eventually leading to a merger between system biology and materials science.

Similar to dislocation mechanics for metal plasticity, what is the theoretical framework for biological materials and structures? It is possible that statistical theories may evolve into the theoretical language of nanomechanics. Atomistic simulations of complex protein structures with explicit solvents are often prohibitive, and coarse-graining techniques are often used. However, how effective are coarse-graining techniques? Can we indeed average out over atomistic or mesoscale structures? How important are atomistic features at macroscale? What are the best numerical strategies to simulate the role of water in very small confinement? How does confined water influence the mechanics of natural and biological materials?

Progress in these various challenging fields will probably occur specific to problems and applications, perhaps in those have most impact in medical or economic fields. Eventually, we must generalize our insight into the formulation of a *holistic theory* that extends the current nomenclature, theory and experimental thinking. These efforts will provide the scientific and engineering fundamentals to develop and maintain the infrastructures to enable and evolve modern civilization. Materials—and materials science—will surely play a seminal role in these developments.

Future directions, challenges and impact

Over the last centuries, engineers have developed understanding of how to create complex man-made structures out of a diverse range of constituents, at various scales (machines, buildings, airplanes, nuclear reactors and many others). Increased development and research funding into these areas of research will lead to breakthroughs not only on the fundamental sciences, but also in technological applications. Research in the area of mechanics of biological materials will extend our ability to carry out structural engineering, as used for buildings or bridges today, to the ultimate scale—nanoscale, and may be a vital component of the realization of nanotechnology.

A better understanding of the mechanics of biological and natural materials, integrated within complex technological systems will make it possible to combine living and non-living environments to develop sustainable technologies. New materials technologies such as protein-based materials produced by recombinant DNA techniques represent new frontiers in materials design and synthesis [31, 32]. These questions have high impact in the understanding and design of environmentally friendly technologies and may enhance the quality of life of millions of people, through advances in the medical sciences as well as through improvements of the living environment. A currently pressing question is the development of new technologies to address the energy problem. Advances may be possible by utilization of bacteria to produce and process fuel from crops, or by enabling the synthesis of materials at reduced processing temperature.

Nanoscience and nanotechnology enable us to make structures at the ultimate scale (self assembly, recombinant DNA, utilization of motor proteins for nano-machines and many others). This will perhaps lead to novel complex structural materials, designed from nano to macro. The theoretical progress in understanding hierarchical biological materials will facilitate to use an extended physical space, through the use of multiple hierarchies, in an efficient and controlled manner, that is, lead to a bottom-up structural design on the sub-macroscopic scale, instead of trial-and-error approaches. For example, the extended hierarchical design space might serve as means to realize new physical realities that are not accessible to a single scale, such as material synthesis at moderate temperatures, or fault tolerant hierarchical assembly pathways [33], which enable biological systems to overcome the limitations to particular chemical bonds (soft) and chemical elements (organic) present under natural conditions [27]. The increased understanding of the hierarchical design laws might further enable the development and application of new organic and organic-inorganic multi-featured composites (such as assemblies of carbon nanotubes and proteins or polymer-protein composites [34–36]), which will mainly consist of chemical elements that appear in our environment in an almost unlimited amount (C, H, N, O, S). These materials might consequently help to solve human's energy and resource problems (e.g. fossil resources, iron etc.), and allow us to manufacture nanomaterials, which will be produced in the future by techniques like recombinant DNA [31, 37, 38] or peptide self-assembly [32, 39, 40], techniques where the borders between materials, structures and machines vanish.

Applications of these new materials and structures are new biomaterials, new polymers, new composites, engineered spider silk, new scaffolding tissues, improved understanding of cell-ECM interactions, cell mechanics, hierarchical structures and self-assembly. In addition to the long-term impact in biology, bioengineering and medicine, this research may eventually contribute to our theoretical understanding of how structural features at different scales interact with one another. In light of the 'extended physical design space' discussed above, this may transform engineering approaches not only for materials applications, but also in manufacturing, transportation or designs of networks.

Acknowledgements I'd like to sincerely thank all authors and contributors to this special issue for submitting excellent illustrations of exciting research work in this field to this special issue. I am delighted to have such a strong collection of papers from outstanding contributors, from many different countries, throughout a range of scientific disciplines.

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