Biomaterials
Editorial overview

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Biomolecular materials and engineering

Biomolecular materials are materials that result from research at the interface between materials science and engineering and biology [1]. This highly interdisciplinary research enterprise claims practitioners from diverse disciplines, including soft condensed matter physics, complex fluids, biophysics, chemical synthesis, biochemistry, molecular cell biology and genetic engineering. In most cases a common structural feature of such materials is self-assembly, in particular, into higher-ordered supramolecular materials.

Among the most interesting biomolecular materials are those made up of self-assembled and functionalized interfaces where the fuctionality is derived from biomolecules which may be manipulated at the molecular level. For example, two-dimensional self-assemblies of membrane-proteins, such as bacteriorhodopsin and similar native bacterial self-assemblies of surface protein layers, are of current interest for the development of biomolecular materials in technological areas as diverse as molecular electronics and optical switch applications [2], to uses as molecular sieves [3,4] and the lithographic fabrication of nanometer scale patterns [5]. Functionalized biomolecular interfaces which include receptor proteins, could form the basis for developing advanced materials that serve as chemical and biological sensors [6], or those with controlled interfacial properties such as adhesion and lubrication [7]. In many cases the materials have uses in biomedical applications, for example, in tissue engineering [8–10] or in developing self-assembled drug and gene delivery vehicles [11].

Lasic and Papahadjopoulos (pp 392–400) describe recent exciting developments in cleverly designed drug delivery systems currently used in biomedical applications consisting of self-assemblies of lipid and biocompatible polymer-lipids. Following this article, Bruinsma and Pincus (pp 401–406) describe our current theoretical understanding of protein–protein interactions leading to aggregation in membranes. Membrane-protein aggregation occurs in many vital biological processes including adhesion and cell–cell recognition events of the immune system. Their article is particularly timely as it builds on the considerable knowledge regarding the statistical mechanics of fluid membranes and the undulation interaction between membranes as originally described by Helfrich [12,13] and verified in numerous experiments during the last decade [14–23]. In contrast to the many studies of fluid membranes, there are very few experiments on solid membranes. Recent theoretical work on solid membranes, in particular, by Nelson and coworkers [24,25] and Aronovitz and Lubensky [26], has shown that in addition to the usual compression and shear elastic modes of a two-dimensional lattice, a third mode due to undulations and the effect of its coupling to the in-plane strains has profound implications. In the case of locally rough but globally flat tethered membranes, either thermally-induced or due to random quenched roughness, theory predicts the possibility of producing a novel new state of matter with anomalous elasticities [24–26]. These theories may turn out to describe the behavior of two-dimensionally ordered protein self-assemblies derived from bacterial coats [3,4] and two-dimensional red blood cell membrane skeletons [27].

Tirrell and Tirrell (pp 407–411) describe recent work on biopolymer syntheses which use extremely novel methods involving a combination of biological and chemical processes. As is evident from their article, a large gap exists between biologically produced materials and the polypeptides which can be produced synthetically, the best synthetic procedure being the ring-opening polymerization of α-amino acid N-carboxyanhydrides (NCAs). Chemical synthesis allows exceptional versatility in the types of amino acid monomers which can be utilized [28], yet the resulting polymers are almost exclusively homopolymers, random copolymers, or graft copolymers. To address these issues Deming [29] at the University of California at Santa Barbara is attempting to synthesize polypeptides via transition metal catalysis to control polymer end-group reactivity and prepare copolypeptides with controlled sequence and composition (e.g. diblock and triblock, and alternating copolymers) which will be able to self-assemble and display interesting properties.

Gittes and Schmidt (pp 412–424) bring the reader up to date on biological motors — a class of protein-based molecular machines — which are used in numerous cell-life processes involving the conversion of chemical energy into mechanical motion. The area of 'molecular-based bioenergetics' — the conversion of one form of energy

into another by utilizing molecular machines in general — has an unlimited potential in future biomolecular materials research [30]. Aside from the motors described by Gittes and Schmidt, researchers have been working with a large set of energy transducing machines. Bacteriorhodopsin, a membrane-protein from an Archeabacteria [2] converts light energy (photons) into electrochemical energy by pumping a proton across its membrane. The F_0F_1 ATPase complex (a membrane-associated protein localized in the thylakoid membranes of chloroplasts) converts the electrochemical energy of a proton into chemical bond energy in the synthesis of ATP [30]. These machines are extremely compact occupying volumes of order $(100\,\text{Å})^3$. Coupled molecular machines which convert one form of energy into another will most probably be utilized in future miniaturized robots. For example, a combination of such machines could be used to generate mechanical motion by an initial light (photon) trigger in a volume less than a cubic micron.

There have been, of course, numerous other very exciting developments in the past few years which will be outlined below. Tissue engineering is an extremely important and rapidly expanding field [8–10]. The goal of this research is to provide replacements for tissues and organs lost as a result of disease, aging, deformity, or accident. Among the current strategies are to seed a biodegradable polymer scaffold with desired cells and to optimize the conditions for cell proliferation. Once the tissue forms as a result of 'cell self-assembly', which usually involves molecular glues (i.e. cellular adhesion molecules), the plastic degrades. To date, artificial 'skin' and 'cartilage' have been successfully designed and used as replacement parts. This is an exciting research area that requires the participation of chemical engineers, complex fluid scientists, biologists and medical researchers.

In another area there have been new developments in the formation of new types of liposomes [31–36;P1] and emulsions [37–39]. Since their discovery, spherical liposomes (consisting of unilamellar or multilamellar vesicles) have received much attention because of their similarities to real cells and their encapsulation properties; they are likely to dramatically impact the medical field as drug and gene carriers as described in the article by Lasic and Papahadjopoulos. Recent groups have reported on the discovery of a new equilibrium phase of liposomes consisting of multilamellar tubular vesicles (L_{tv}) [31,32], not predicted by current theories describing fluid membranes. Among the very dilute phases which appear at equilibrium in surfactant solutions including the dilute lamellar L_α phases that are stabilized by the Helfrich undulation forces [14–21], the equilibrium L_4 phase of spherical vesicles [40,41], and the bicontinuous L_3 phases [42,43] all may be described by considering fluid membranes with physical properties described by Helfrich [12,13]. In contrast, the existence of the L_{tv} phase is not predicted with the Helfrich theory. Recent theoretical work which emphasizes a non-analytical bending energy term that appears to favor cylindrical and 'egg-carton' geometries may turn out to be the correct theory of the L_{tv} phase [44].

In a different vesicle system which is in a non-equilibrium regime, researchers have recently reported on a novel process for preparing multilamellar vesicles (MLVs) of controlled size [33–35;P1] These so called 'onion phases' are a result of a hydrodynamic instability when lyotropic lamellar phases are subjected to shear flow. The onion phases are currently used in cosmetics and chemical release applications [P1] and may have potentially important biotechnology applications. Under certain conditions, onion-like MLV phases may also occur spontaneously in mixtures of ionic and non-ionic surfactant membranes [36]. Dispersions made out of two immiscible liquid phases in the presence of surfactants are known as emulsions. By contrast with microemulsions, which form spontaneously, emulsions require external energy to form and are metastable. The intense interest in emulsion stabilization arises from their increased use in the cosmetics industries in formulations of lotions, gels, and creams. Recent novel new processing approaches to the development of emulsions made up of almost monodisperse droplets have paved the way for the unprecedented control of their stability and mechanical properties [37–39].

Recently, interesting results have been reported concerning biomolecular materials exhibiting higher-order self-assembly (i.e. self-assemblies of objects which themselves are self-assemblies of even smaller objects). Vesicles can be made to undergo a 'secondary' self-assembly into higher order structures in a controlled and reversible manner via 'site-specific' ligand–receptor coupling interactions [45,46]. The robustness, controlled versatility and reversibility offered by site-binding secondary self-assembly opens the way for the development of stable biomimetic structures consisting of microcompartments and membranes that could be designed to perform different functions (e.g. binding, biocompatibility, and controlled permeability and solute release) which cannot be easily carried out by single-membrane structures. Another example of the unexpected effects of higher-order self-assembly is the discovery of an exceedingly high temperature stable regime of the membrane-protein bacteriorhodopsin in stacked two-dimensional films [47–49]. The findings suggest general methods for the temperature stabilization of proteins and enzymes in artificially assembled multilayers of ordered arrays of biomolecules.

Some important new developments have occurred in the area of natural [50,51] and synthetic biogels [52]. Many biological cells experience shear stresses of order 100–1000 Pa. Their elastic resistance to such stress is largely due to a gel-like network of semiflexible actin filaments formed at the cell periphery. Despite some similarity of actin networks to conventional polymer gels

and rubbers, classical rubber elasticity cannot account for the large shear moduli required of the actin cortex *in vivo* and observed [50] for physiologic concentrations of actin solutions *in vitro* (1000 Pa for 10 mg ml^{-1}, whereas rubber elasticity would predict moduli of order 1 Pa). A novel new model for the elasticity of actin networks can account for both the magnitude of the shear modulus and its observed dependence on concentration and strain [51].

A recent discovery describes a new class of synthetic-based lamellar biogels made up of fluid membranes of lipids and surfactants with small amounts of polymer-lipids [52]. These gels are quite distinct from the 'onion-like' multilamellar vesicle phases which may also exhibit strong viscoelastic behavior with a finite yield stress [33–36;P1]. The gel phase is characterized by a highly defected microstructure made up of a network of connected membranes with the polymer-lipid stabilizing the high curvature regions, which on a semi-macroscopic length scale leads to random layer orientation domains. These lamellar biogels exhibit striking differences with isotropic hydrogels of polymer networks where the gel phase can be formed by adding water to the liquid-like lamellar phase, and, furthermore, gels with larger water concentrations require less polymer-lipid concentrations to remain stable. The underlying fluid membrane allows for the incorporation of membrane-proteins that are biologically active, thus providing a way in which to deliver such molecules in a stable gel.

In the next few years many researchers of biomolecular materials will almost certainly be working in the area of 'non-viral-based' gene therapy. Somatic gene therapy depends on the successful transfer and expression of extracellular DNA to the nucleus of eucaryotic cells, with the aim of replacing a defective gene or adding a missing gene in corrective molecular-level biomedical applications [53]. Although viral-based carriers of DNA are presently the most common method of gene delivery, there has been a tremendous recent surge in activity in the development of synthetically-based non-viral vectors. In particular, an important recent breakthrough involves the use of cationic liposomes (CL) as non-viral transfer vectors (i.e. carriers) of recombinant DNA molecules [54]. While significantly improved in transfection efficiency, compared to other non-viral carriers, at present, CL vectors exhibit much lower efficiencies than do viral vectors. This low efficiency is related to the lack of fundamental knowledge regarding the mechanisms which underlie transfection via cationic liposomes [53–56]. In particular, what is the precise nature of the interactions and resulting structures of the CL–DNA complexes? Also, what is the nature of the interactions of the complex with intracellular organelles and the mechanism of transfer across the nuclear membrane? Some recent experiments are now beginning to properly address the structure of the complexes through quantitative X-ray diffraction studies; they reveal a well defined higher-ordered liquid crystalline structure of the CL-DNA complexes (JO Radler, I Koltover, T Salditt, CR Safinya, unpublished data; DD Lasic, R Podgornik, H Strey, PM Frederik, personal communication). Meanwhile, on the theoretical side a recent elegant work shows that even in the absence of lipid self-assemblies, DNA molecules may have a tendency to condense into a new (as yet unobserved) state with a 'braided chiral' structure with crystalline order [57].

The obvious complexity in biomolecular materials research makes the field especially pleasing because of the seemingly unlimited number of very important unresolved problems. Furthermore, while almost no problem in this area will be completely solved due to its complexity, because of the importance of the field, even small progress towards their solution tends to lead to large gains from a scientific, technological, medical and environmental prospective. For future workers of this field it is important to realize that the highly interdisciplinary nature of the field means that physical and chemical scientists and engineers will only make real significant progress if they not only have an up to date appreciation of the relevant biological issues associated with the problem, but are also ready to invest a substantial amount of their research time working with biologists and medical researchers on some purely biological aspects of the problem.

Hard biological materials

The last few years have witnessed an increasing interest in the study of the strategies adopted by organisms in building their skeletal hard tissues, in order to provide scientists with ideas for the production of sophisticated artificial materials. Indeed, the world of biomineralization presents a varied range of solutions to the problems of scaffolding, support, abrasion and protection of soft tissues encountered by organisms of all species. These solutions, perfected over millions of years of evolution, are centered around the production of composite materials of minerals (amorphous or crystalline) and biological macromolecules. The uniqueness of these materials lies in the sophisticated control and construction strategies, rather than in the choice of the materials used.

The four remaining topics in this section cover the section on 'hard' biological materials, summarizing research performed on biomineralization and its synthetic counterparts, each from a different perspective.

Intuitively, the best way to fill a space with compression-resistant structural materials would appear to be to provide a solid space filling by *in situ* polymerization of readily available and transferable monomeric units. The amorphous precipitate may then be modelled into the desired shapes by accurate control of growth and aggregation of the polymer into nanometer- and micrometer-size particles. The strategy is adopted in biogenic silica materials and biomimetic silicate synthesis, reviewed here by Zaremba and Stucky (pp 425–429). The authors draw attention

to the extreme conditions required in the traditional synthesis of solgels and ordered silica-based materials, in contrast to the physiological conditions used by organisms. Moreover, although solgel-produced silica is industrially important, chemists have achieved little control over its physical properties. Therefore, new growth techniques, under organic preserving conditions, are sought after. Zaremba and Stucky review the biomineralisation techniques applied to silica synthesis and compare them to their biological counterparts. These include the introduction of organic frameworks such as surfactants, the use of *in situ* solid state phase transitions and *in situ* coassembly techniques.

Recent discoveries and characterization of biopolymers (proteins and carbohydrates) associated with biosilicates may result in a new model for biolsilicate formation. It is thus expected that biomineralization methods will be incorporated increasingly in silicate materials of the future.

The materials most widely used by organisms to build their hard parts, however, involve crystalline rather than amorphous minerals. Among these calcium carbonate and phosphate are the most studied. The use of such crystalline minerals has required the evolution of complex techniques to achieve complete control over their deposition. The interfacial aspects of these control techniques are reviewed by Hunter (pp 430–435). The processes of control over crystal orientation, nucleation, growth habit and polymorphism are examined separately in calcium carbonate and phosphate biomineralization and biomimetic crystallization. Interestingly, Hunter claims, in calcium carbonate biomineralization the control processes are reasonably well understood, although the organic matrix components are not well characterised. In contrast, the main proteins of bone, enamel and dentin (phosphate biominerals) have been sequenced, but a better understanding of the processes awaits consensus on the functions of the proteins involved. Hunter concludes that the rapid progress in elucidating events occurring at the organic matrix–crystal interface hold promise that a combination of techniques and approaches will soon produce a detailed understanding of mineralization in organisms "from mollusk to man".

From the egoistic human standpoint, understanding bone mineralization and the development of new bone biomaterials are a priority. Cuisinier (pp 436–439) examines the present degree of understanding of the initial events in bone crystal formation and the role of organic bone compartments in the control processes, with a view of the possible pathways for the development of bone biomaterials. A four stage development model is presented, mainly derived from HRTEM observations, which includes adsorption of ions, followed by formation of crystalline nanometer-size particles, their growth and lateral fusion. Work on matrix vesicles and some studies performed in cell cultures are also reviewed.

The question of how the final product of the biomineralization process fulfils its function is finally addressed by Currey (pp 440–445) in his review on micromechanics of biological hard tissues. Currey claims, and with good reason, that understanding the micromechanics of hard tissues has been hampered until recent years by the lack of a detailed understanding of their microstructures: a deficiency that is being rapidly remedied. From this starting point, Currey thus examines the current state of the art in the understanding of the microstructure in various vertebrate tissues. The mechanical parameters describing the material and its behaviour upon failure with microcrack formation are then considered. A review of the remarkable properties of other hard tissues, including nacre, enamel, and beetle wings follow. Currey concludes that biological materials have such complex microstructures that often they do not lend themselves to be studied with the tools developed for manmade composite materials. The transfer of information from biology to artificial materials should, however, yield valuable information about fabrication and micromechanics.

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