Biomaterials
Editorial overview

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Abbreviation
CL cationic liposomes

Biomolecular materials and engineering

In this second year of the section Biomaterials we find once again that the articles derived from practitioners in the wide ranging fields of complex fluids, biophysics, biomolecular materials and biotechnology, are integrated with physical, chemical and biological and engineering concepts and methods in the elucidation of the properties of natural and engineered biomaterials [1]. This interdisciplinary field is currently undergoing a renaissance with physicists, chemists, biologists, and engineers actively working side by side with biomedical and biotechnological researchers. The four review articles covering 'soft' biomaterials deal specifically with the science and technology of firstly, polymer supported membranes; secondly, proteins interacting with polymer coated surfaces; and thirdly, lipid based drug delivery and actin gels. Additionally, the last two years has seen an enormous increase in experimental and theoretical studies of biomaterials based on DNA and we will give a brief overview on this activity.

This year the reviews begin with Rädler and Sackmann's (pp 330–336) which describes methods of functionalizing surfaces by the sequential layering of soft polymeric ultrathin films, followed by the deposition of self assembled monolayer or bilayer lipid molecules. The highly compressible hydrated composite films are reminiscent of the cell plasma membrane (and its polymeric underlayer) which of course has evolved to be the ideal environment for a variety of membrane-associated proteins. These proteins, in turn, have key functional roles in cell activities including cell-cell recognition, molecular transport across membranes, and ligand-receptor interactions among others. The authors give a comprehensive overview of the technological and scientific importance of these functionalized surfaces. For example, while they are technologically important in the development of chemical and biological sensors (with the sensing activity derived from proteins) by virtue of their ability to mimic the extracellular matrix (EM) when they are immobilized by EM biopolymers, they also provide ideal model substrates for animal cells for *in vitro* studies of tissue/organ development and engineering [2,3], which involve cell proliferation, locomotion, and adhesion.

The second review, by Szleifer (pp 337–344), briefly describes some recent experimental results on the interactions between water soluble proteins and polymer coated surfaces and follows with a thorough numerical investigation of the data and clear predictions of the adsorption properties of proteins to polymer surfaces. The intense recent interest in elucidating the properties of polymer coated membranes is mostly due to the finding of enhanced lifetimes in the blood when drug carrying vesicles (closed bilayer shells of lipid molecules) are covered by a monolayer of polymer-lipids with hydrophobic anchors [4]. The prevention of protein adsorption on these sterically stabilized vesicles is thought to lead to enhanced lifetimes and drug delivering capabilities. The review by Szleifer gives the reader a deep insight into this important problem. While both the Rädler and Sackmann and Szleifer articles review the properties of rather complex multicomponent heterogeneous protein/polymer membrane self assemblies, which have received a high level of attention due to their potential technological and drug delivery applications, it is important to keep in mind that from a scientific viewpoint the statistical mechanical and interaction properties in even simpler systems [5–7] involving polymer coated membranes are far from being understood.

The article by Zasadzinski (pp 345–349) gives a comprehensive review of the field of lipid based drug delivery, beginning with the earliest work with simple vesicles shortly after their discovery by Bangham. The review quickly turns to very recent work where engineering at the molecular level; is now the focus of most of the research in developing enhanced drug delivery vesicles with enhanced efficacy and biocompatibility (i.e. materials which do not evoke immune reaction). After describing the recent success with polyethylene glycol lipid (PEG-lipid) sterically stabilized vesicles in drug delivery applications, Zasadzinski focuses on new vehicles involving multiple vesicles bound together via reversible ligand-receptor interactions. These so called vesosomes which are beautiful examples of nanoengineering are further stabilized by a single outer bilayer and mimic eucaryotic cells comprised of many internal organelles. Because of the inherent compartmentalization vesosomes

should find important use in drug delivery applications involving multiple drugs.

The MacKintosh and Janmey (pp 350–357) review on filamental actin gels constitutes an important example of work at the interface of polymer science, biophysics, and biology. Filamental actin is one of the three major filamentous groups (i.e. F-actin, microtubules, intermediate filaments [IF]) of the cell cytoskeleton that coexist and almost certainly interact in eucaryotic cells. The cytoskeleton components are responsible for a variety of cellular functions including mechanical stability, cell locomotion, intracellular trafficking and signal transduction. While the rheological properties of F-actin in the cell cytoskeleton are crucial to cell survival and function, until recently, very little was understood about the viscoelastic properties of these gels measured *in vitro* under physiological conditions. For example, classical rubber elasticity is not able to account for the unusually large shear moduli observed in experiments. The authors review recent models of semi-flexible actin gels and in particular describe the successful ones which have been found to be consistent with the novel viscoelastic properties of F-actin gels measured *in vitro*.

Aside from the current review articles the last few years has seen an explosion of activity in biomaterials based on DNA. This recent activity owes much of its development to earlier work on DNA carried out over the last twenty years. Structural studies have been designed to elucidate the various mechanisms by which giant DNA molecules are able to undergo dense conformations, with possible similarity to their biologically active native state, either induced by multivalent cations [8], or in high density liquid crystalline phases, both *in vitro* and *in vivo* packing of DNA in eucaryotic cells [9,10].

The chiral properties of densely packed aligned polymers (e.g. DNA) are theoretically predicted to lead to a variety of novel new phases, which still need to be verified experimentally. Two of these phases include a tilt-grain-boundary phase where the polymers rotate about an axis perpendicular to their direction of alignment, and a moiré phase, in which the columnar hexagonal lattice rotate about an axis parallel to the columns [11,12]. In both cases, rotation is produced by twist grain boundaries. More recent work appears to have found a novel phase of rope-like structures consisting of twisted columns (ropes) of DNA which further self assemble into an hexagonal lattice work (G Yan, R Kamien, TC Lubensky, personal communication). Chirality favors the creation of the rope-like structures.

There have also been some interesting new theoretical developments in understanding the interactions between charged macromolecules. The effective interaction between DNA (charged polyelectrolyte rods) embedded in a solution of their counterions was studied numerically by Brownian particle dynamics by Gronbech–Jensen, Mashl, Bruinsma and Gelbert [13]. A significant range of attraction between the rods was identified for physically relevant parameters, as arising from the microscopic ordering of counterions in the vicinity of the macromolecules. Thus, the macroscopic interactions between the DNA rods in solution are dictated by the short range microscopic order rather than by the average of the microscale. Systems of three rods with divalent counterions have been simulated identifying angular dependence of the effective potentials between rods (N Gronbech–Jensen, personal communication). This suggests that simple pair potentials may not be sufficient to describe an ensemble of rods.

The interaction between DNA rods electrostatically adsorbed on a *deformable* two-dimensional lipid membrane has been theoretically studied by Dan [14]. The model which balances the electrostatic repulsion between DNA molecules and an attractive interaction induced by the local membrane deformations, predicts the existence of a membrane-bound DNA condensed phase with fixed interaxial spacing. Recent AFM studies of DNA adsorbed onto membrane supports show a fixed interaxial spacing [15,16]. In membrane self assemblies researchers are investigating the renormalization of the internal structure of charged bilayer membranes as an oppositely charged liposome is adhering (A Lau, P Pincus, personal communication). More specifically they have found that the head group areas may change significantly; for example, by as much as 100%, as a cationic and anionic membrane stick together under the influence of Coulomb forces. In neutral fluid membranes composed of equimolar mixtures of cationic and anionic phospholipids in the Debye–Huckel approximation researchers have determined that two such parallel membranes have an unusually strong long-range attraction originating from correlated charge density fluctuations (S Safran, P Pincus, personal communication).

In another area involving DNA, cationic liposomes (CL) complexed with DNA (CL-DNA) have been shown to be promising synthetically based nonviral carriers of DNA inside animal cells for gene therapy [17]. The entire field of 'gene therapy based on synthetic carriers of DNA' has undergone a renaissance since the initial seminal paper by Felgner *et al.* which was soon to be followed by numerous other groups demonstrating gene expression *in vivo* targeted organs and in human clinical trials [18,19]. However, the mechanism of action by CL-DNA complexes remains largely unknown with transfection efficiencies varying up to a factor of one hundred in different cell lines. This unpredictability, which is ubquituous in gene therapy, may be attributed to a lack of knowledge regarding the interactions between DNA and CLs and the resulting structures of CL-DNA complexes. In their paper Felgner *et al.* had assumed that the complexes were comprised of liposomes randomly

attached to the oppositely charged DNA; that is, bead on a string structure [20].

Using quantitative synchrotron X-ray diffraction Rädler, Koltover, Salditt and Safinya were able to solve the self assembled structure of CL-DNA complexes in solution demonstrating that they consisted of a higher ordered multilamellar structure with DNA sandwiched between cationic bilayers [21]. This structure was observed not only in higher concentration precipitates, but also by using the high brightness of the synchrotron source, *in extremely dilute suspensions of globules used in gene therapy applications.* The structural results should ultimately correlate to transfection efficiencies in gene therapy (i.e. the expression of the introduced foreign gene which results in protein synthesis); in particular, they should be directly relevant to our understanding of the interactions of the complex with cellular lipids and the mechanism of DNA transfer across the nuclear membrane [21,22]. These purposefully designed series of X-ray diffraction experiments [21] led to the observation of a variation in the DNA interaxial distance as a function of the lipid to DNA (L/D) weight ratio in multilayers, which umabiguously showed that the X-ray technique was directly probing the DNA structure in multilayer assemblies. Furthermore, the work showed that linear DNA confined between bilayers forms an expanding one-dimensional lattice of chains; that is *a novel 2D smectic phase* resulting from long range electrostatic repulsions. Analytical studies (RF Bruinsma, J Mashl, W Gelbart, personal communication) and numerical Brownian particle dynamical studies (N Gronbech–Jensen, personal communication) show the existence of long-range repulsive electrostatic interactions.

Aside from the interest in developing synthetically based nonviral carriers of DNA for gene therapy, because CL-DNA complexes are self assemblies where the DNA adsorbs in an aligned orientation onto the membrane [21], there are potentially many important technological implications of the work in developing nanoscale masks in lithography and molecular sieves with nanometer scale cylindrical pores.

Recent theoretical work appears to lead to a variety of novel new phases in DNA-lipid complexes (TC Lubensky, C O'Hern, personal communication). The ground state of these systems is considered to be a mixed columnar-lamellar phase in which the lipid forms a regular smectic phase and the DNA forms a periodic aligned columnar lattice with a single periodic row between each pair of lipid lamellae. This phase would have the symmetry of an anisotropic columnar phase. At higher temperatures positional coherence between DNA columns in adjacent layers could be lost but without destroying orientational coherence between layers. Or both positional and orientational coherence between layers could be lost. Preliminary calculations indicated that the orientationally ordered but positionally disordered phase can exist and that there can be a phase transition between it and the columnar phase. This would be a remarkable new phase of matter if it exists. Orientational coherence between layers can also be lost at sufficiently long length scales where nonlinearities and dislocations and disclinations in the 2D smectic lattice of DNA become important. This system shares many fascinating similarities with flux lattices in superconductors.

Finally, two other areas involving DNA in biomaterials development deserve mention. First, an interesting advance has been in the description of DNA based techniques for self assembling nanoparticles with tailored novel optical or electrical properties into macroscopic aggregates. This has been made possible by using specifically synthesized DNA with sticky oligonucleotide overhangs as the linking molecular glue between the nanoparticles which are themselves coated with oligonucleotides complementary to the DNA ends [23,24]. The second is in the development of high density DNA arrays or so called 'gene chips' [25,26]. These biochips, which result from interdisciplinary work combining molecular biology with microfabrication and nanotechnology, are produced by combining photolithography techniques and combinatorial light-directed synthesis of oligonucleotide (single-stranded) DNA probes and are well on the way to revolutionizing DNA sequence analysis through the sequence hybridization methods.

Hard biological materials

Many biological hard tissues, such as vertebrate bone and teeth, shells and many others, can be defined as ceramic composite materials. They consist of a pliant matrix of biological macromolecules, mainly fibrous proteins and/or polysaccharides, inside which mineral particles are induced to grow.

Echinoderm spines and test, sponge spicules and others, are fiber-reinforced composite materials, where biological fibers (proteins) are emdedded inside a continuous hard and brittle host matrix (often a single crystal of calcite).

Probably one of the most interesting examples of biogenic composite materials is the sea urchin tooth [27,28]. Each single tooth combines in a few millimeters a gradient fiber-reinforced ceramic matrix composite and a laminated ceramic composite. Continuous self-sharpening is achieved through an accurately balanced combination of the geometrical shape of the main structural elements and their spatial arrangement, the interfacial strength between structural elements, and the hardness gradient extending from the 'stone' part to the surrounding zones.

Biological and hard materials have been thus regarded as an inspiration for novel materials with superior mechanical properties. Organisms control, however, the deposition of more than sixty different minerals, not all of which are used for skeletal support. Just one well known example;

the single domain crystals of magnetite that impart to magnetotactic bacteria their navigational ability are accurately programmed and assembled magnetic nanopraticles. Within the last few years the scope of biologically inspired materials science research has expanded far beyond mechanical properties to include structural, electronic, and optical properties of mesoscale composites reviewed by Manne and Aksay (pp 358–364) and Fendler (pp 365–369).

In the range of mineralized biological materials, as in many different fields, nature thus serves as an almost infinitely variable source of ideas and inspiration. Our degree of knowledge and our abilities to program and execute sophisticated construction projects are, however, ridiculously inadequate, when compared to those manifested in nature. This basic truth should be kept in mind when using the word 'biomaterials'. Still much has to be understood about the design strategies used by organisms, from the molecular to the structural and ultrastructural level. The effort must be convergent; trying to better understand natural materials, while at the same time building new ones that incorporate even only one of the lessons learnt. This is the 'halfpenny philosophy' behind the choice of the subjects for this issue.

Most of the subjects of last year's issue concentrated on the mineral component of mineralized tissues and on the properties of the finished product. The fibrous matrix that constitutes the predeposited scaffold of many tissues and within which mineral deposition occurs, was hardly touched upon. The components of these matrices vary from polysaccharides, such as chitin and cellulose, to proteins such as silk and collagen, each organized at different hierarchial levels.

The review by Veis (pp 370–378) addresses studies on the collagen fibraller structure in mineralized and nonmineralized tissues. Collagen is the main component in skin, and one of the main components in vertebrate bone, tendon and dentin. The collagen molecules assemble into fibrils that aggregate into fibers. The fibers are organized in complex ultrastructural designs that eventually govern specific tissue properties.

The basic organization of the collagen fibril, was elucidated more than thirty years ago [29]. The manner in which the fibrils are assembled into fibers has also been studied to a certain extent. It is in the intermediate range, namely the order within a fibril and the influence of this order on the deposition of the crystals in the mineralized matrices, that most progress has been made in recent years. Veis identifies a number of parallel topics: the variability of local stability of the triple helix influencing its ability to accommodate distortions within the basic structure, the order-disorder characteristics in the fibrillar radial packing, and the structural analysis of inter-molecular cross-linkages. Interestingly, the cross-link pattern is found to change not only between nonmineralized and mineralized tissues, but within the same tissue as mineralization proceeds. The incorporation of oriented crystals may finally improve the triple helix structure and thus the crystallinity of the fiber matrix, reducing the equatorial liquid-like disorder, change the molecular organization within a fibril and cause rearrangement of the intermolecular cross-linking pattern. All of these are far reaching consequences, the full implications of which are not yet completely clear.

The approach of biomineralization-inspired preparation of new advanced materials is presented in the two remaining reviews by Fendler and Manne and Aksay.

Fendler addresses the organized assembly of nanoparticles under monolayers, in reversed micelles and in layers of nanoparticulate films. The main interest here, is directed at the synthesis of semiconductor or metal nanoparticles. One procedure involves *in situ* nucleation, growth and coalescence of nanoparticles induced by the direct interaction of the monolayer headgroups with ions in solution. Surface charge and a structural lattice match between the monolayer and crystal are the leading principles. Alternatively, particles can be formed inside separate compartments such as surfactant or reversed micelles, and assembled in a second stage onto a substrate.

Manne and Aksay review recent work on thin films and nanolaminates incorporating organic/inorganic interfaces. In their broad overview, the particles range from carbonates, insulating oxides, clays and silicates to metals and semiconductors; and the techniques from Langmuir–Blodgett deposition to self-assembly, from sequential layer-by-layer adsorption of inorganic particles on polyelectrolytes to infiltration of organic molecules into the interlayer spaces of layered inorganics. The target properties may be electronic, mechanical or optical, including substrates for surface enhanced Raman scattering and solid electrolytes, humidity sensors, nanocapacitors and others.

Clearly, the potential is enormous and although much progress is being made the area is still mostly *terra incognita*.

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