ELSEVIER

Surface Science 500 (2002) 656–677

SURFACE SCIENCE

www.elsevier.com/locate/susc

Biological surface science

Bengt Kasemo *

Department of Applied Physics, Chalmers University of Technology and Göteborg University, S-412 96 Göteborg, Sweden

Received 13 December 2000; accepted for publication 5 July 2001

Abstract

Biological surface science (BioSS), as defined here is the broad interdisciplinary area where properties and processes at interfaces between synthetic materials and biological environments are investigated and biofunctional surfaces are fabricated. Six examples are used to introduce and discuss the subject: Medical implants in the human body, biosensors and biochips for diagnostics, tissue engineering, bioelectronics, artificial photosynthesis, and biomimetic materials. They are areas of varying maturity, together constituting a strong driving force for the current rapid development of BioSS. The second driving force is the purely scientific challenges and opportunities to explore the mutual interaction between biological components and surfaces.

Model systems range from the unique water structures at solid surfaces and water shells around proteins and biomembranes, via amino and nucleic acids, proteins, DNA, phospholipid membranes, to cells and living tissue at surfaces. At one end of the spectrum the scientific challenge is to map out the structures, bonding, dynamics and kinetics of biomolecules at surfaces in a similar way as has been done for simple molecules during the past three decades in surface science. At the other end of the complexity spectrum one addresses how biofunctional surfaces participate in and can be designed to constructively participate in the total communication system of cells and tissue.

Biofunctional surfaces call for advanced design and preparation in order to match the sophisticated (bio) recognition ability of biological systems. Specifically this requires combined topographic, chemical and visco-elastic patterns on surfaces to match proteins at the nm scale and cells at the micrometer scale. Essentially all methods of surface science are useful. High-resolution (e.g. scanning probe) microscopies, spatially resolved and high sensitivity, non-invasive optical spectroscopies, self-organizing monolayers, and nano- and microfabrication are important for BioSS. However, there is also a need to adopt or develop new methods for studies of biointerfaces in the native, liquid state.

For the future it is likely that BioSS will have an even broader definition than above and include native interfaces, and that combinations of molecular (cell) biology and BioSS will contribute to the understanding of the "living state".

Keywords: Adhesion; Biological compounds; Biological molecules – nucleic acids; Biological molecules – proteins; Solid–liquid interfaces

1. Introduction

Imagine the following six situations where the properties of solid surfaces are or may become important in practice:

* Tel.: +46-317723370; fax: +46-317723134.
E-mail address: kasemo@fy.chalmers.se (B. Kasemo).

0039-6028/01/$ - see front matter
PII: S0039-6028(01)01809-X

(i) A patient with severely degraded dental status is treated by a surgical procedure, where one or several dental implants, made from metal or ceramic, are implanted into the jawbone so that they after some healing in period can function as artificial teeth (Fig. 1). Other types of implants are shown in Fig. 2.

(ii) A blood or urine sample is distributed over a suitably prepared biosensor or biochip surface in order to diagnose a patient's health status or alternatively for a forensic identification (Fig. 3).

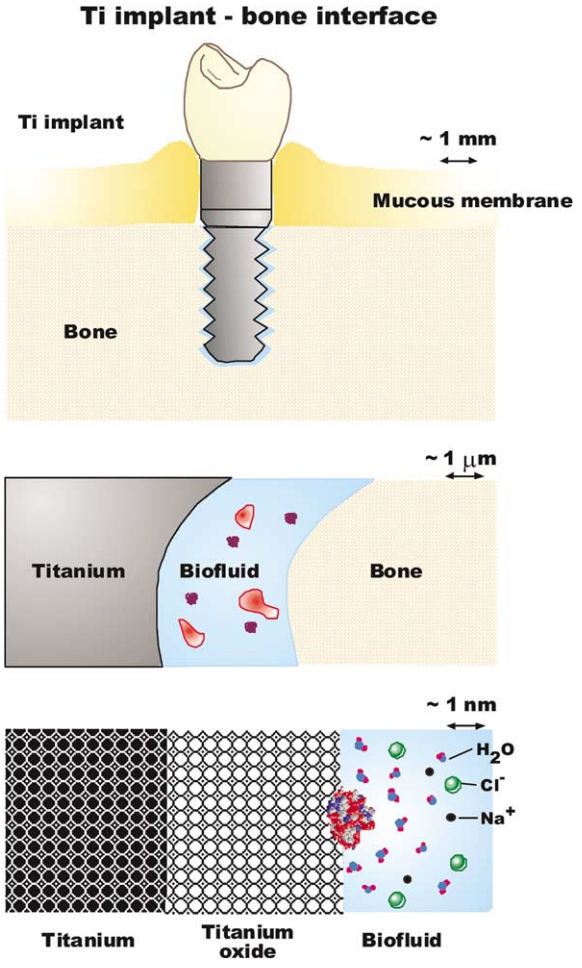

Fig. 1. Schematic illustration of the interface between a dental implant and the jawbone into which it is implanted, at different magnifications. After the surgical procedure the surface is first exposed to water, then to proteins, and eventually to cells (see Fig. 7). See Fig. 2 for other types of implants.

(iii) A small number of living cells of a particular kind, maybe a tissue culture or so-called stem cells for a certain type of human tissue, are placed in a scaffold made from some synthetic material, with the intention to make the cells grow in number and differentiate ex vivo into a new functional tissue, which later is placed in a patient, in order to repair a lost or degraded body function (Fig. 4).

(iv) Neural cells (neurons) are placed on a micropatterned surface, where they self-organize into a functioning neural network, which can be addressed chemically and/or electronically by in–out (I/O) connections so that a cell-based bioelectronic circuit is achieved.

(v) A particular kind of photosensitive, charge transfer proteins are attached to and organized on a specially designed material surface in order to harvest the energy of sun light with a high efficiency, thereby converting the light into chemical or electrical energy in a process mimicking the photosynthetic process of green plants or certain bacteria.

(vi) A surface is microfabricated with an array of specially architectured, soft protrusions in the micrometer size range, mimicking a shark or dolphin skin and thereby providing a dramatic reduction in hydro- or aerodynamic friction.

The *medical implant* example [1,2] is a clinical reality since many years and hundreds of thousands of patients have been treated with a major increase in life quality. There are many other examples of medical implants (Fig. 3), each one uniquely different from the others in the biological/clinical details e.g. artificial hip and knee joints, artificial blood vessels and heart valves, and synthetic intraocular lenses. Together they represent a large number of industries with total turnover approaching or even exceeding a hundred billion dollars per year.

The *biosensor* example [3–5] is representing an area, where commercial products already exist both for single sensors and array type sensors [6] (e.g. so-called DNA chips) which are used clinically or at the R&D level in biomedical industries and academia. Figs. 4 and 5 illustrate some of the current sensing principles and surface immobilization strategies. This area is in extremely rapid

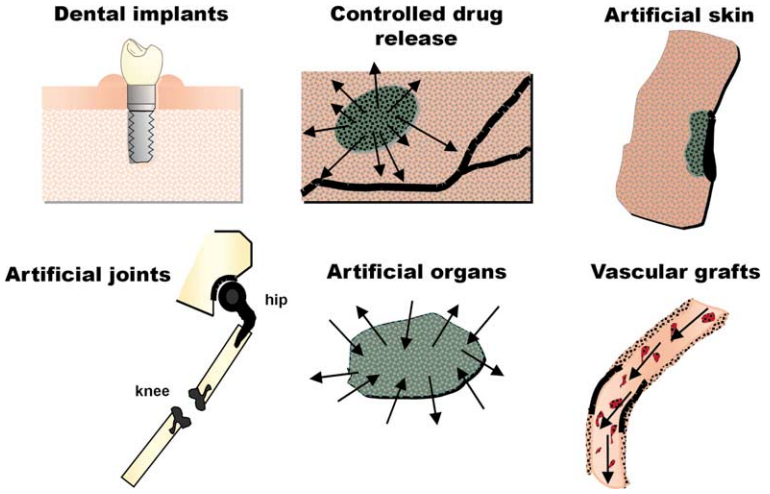

Fig. 2. A selected set of different medical implants.

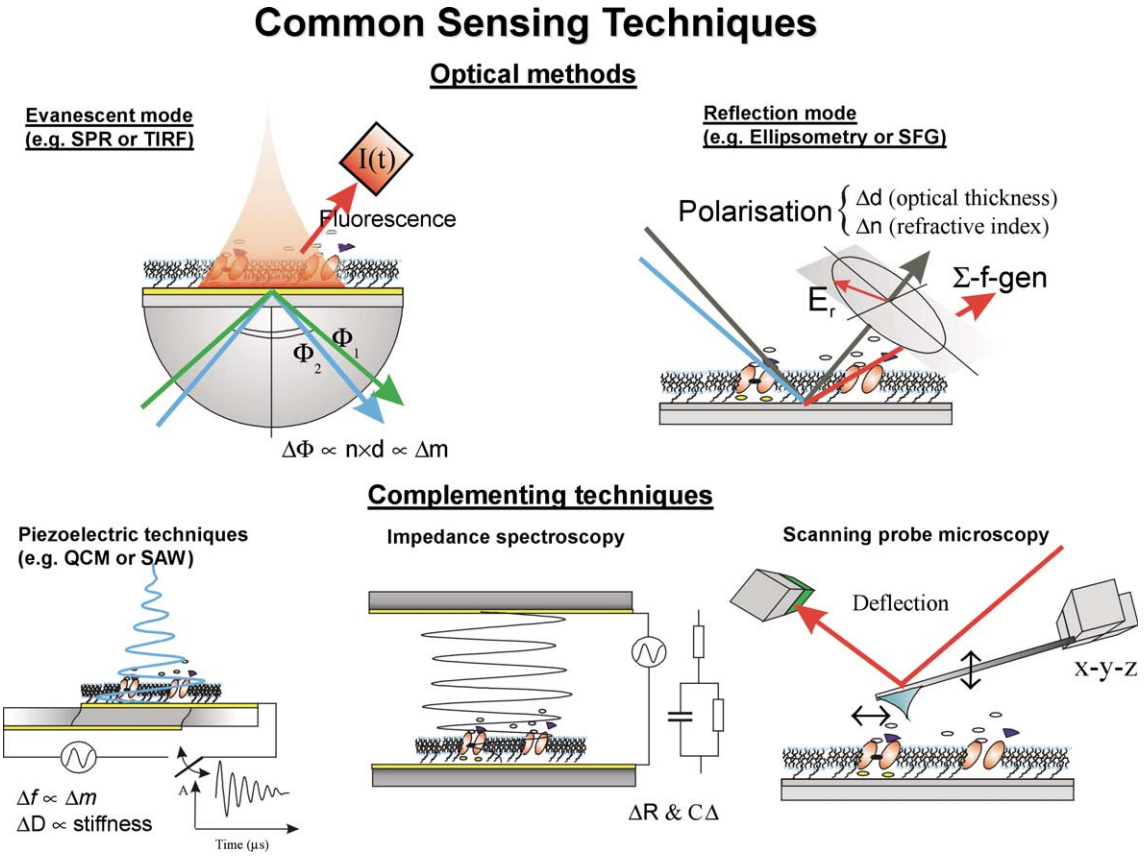

Fig. 3. Some common detection principles for biosensors and biochips.

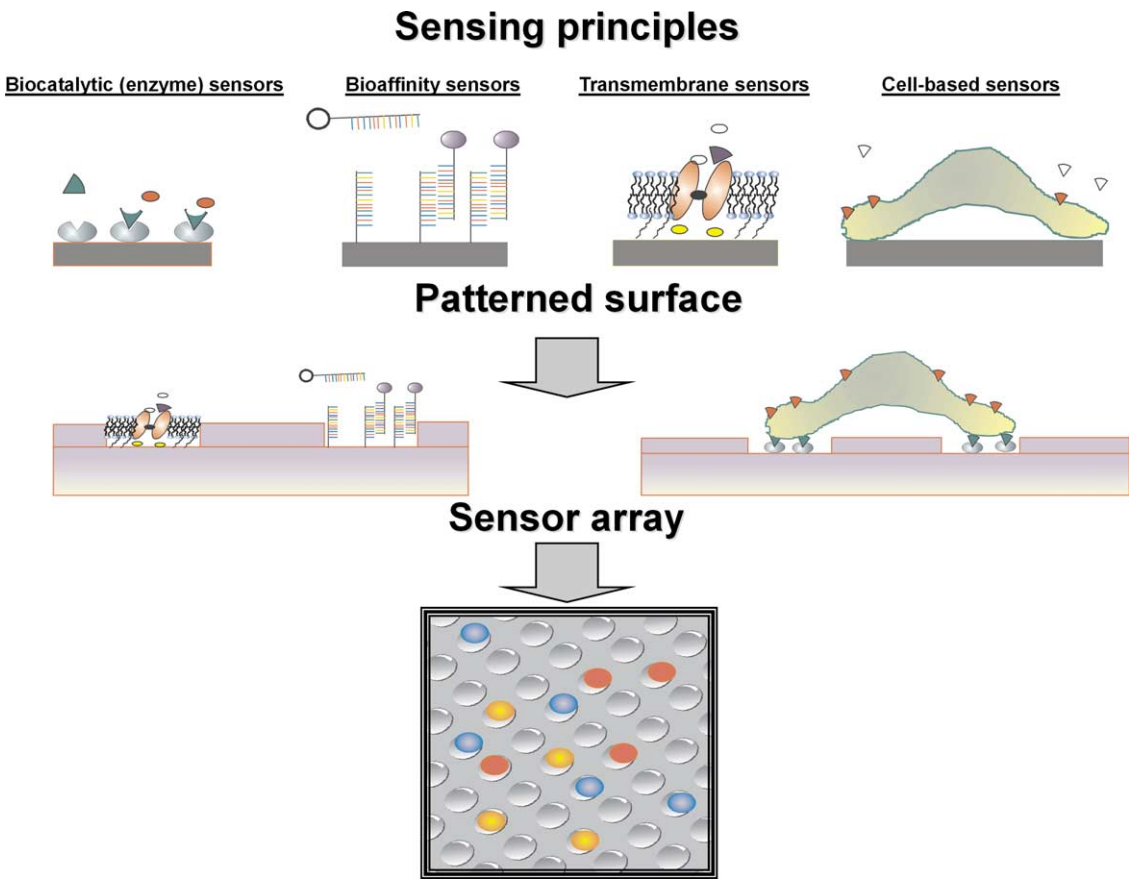

Fig. 3 (*continued*)

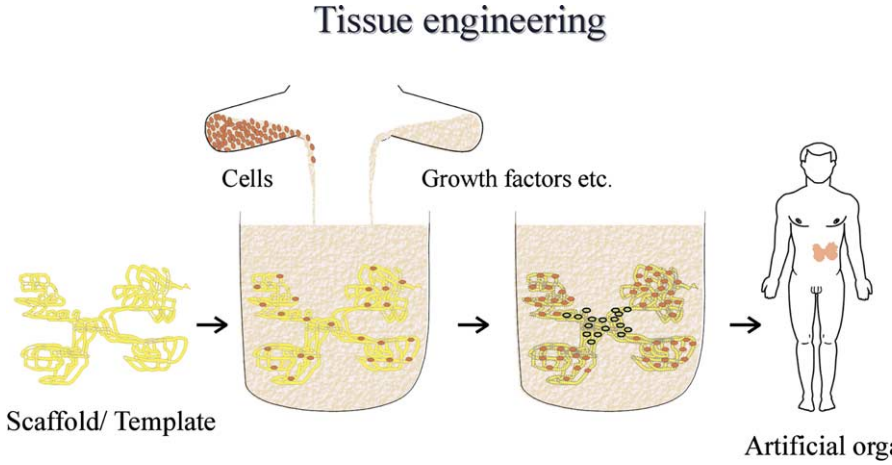

Fig. 4. Tissue engineering scaffold. A template of a synthetic material forms the "host" for a cell or tissue culture. The scaffold should promote the development of the culture into a functioning tissue.

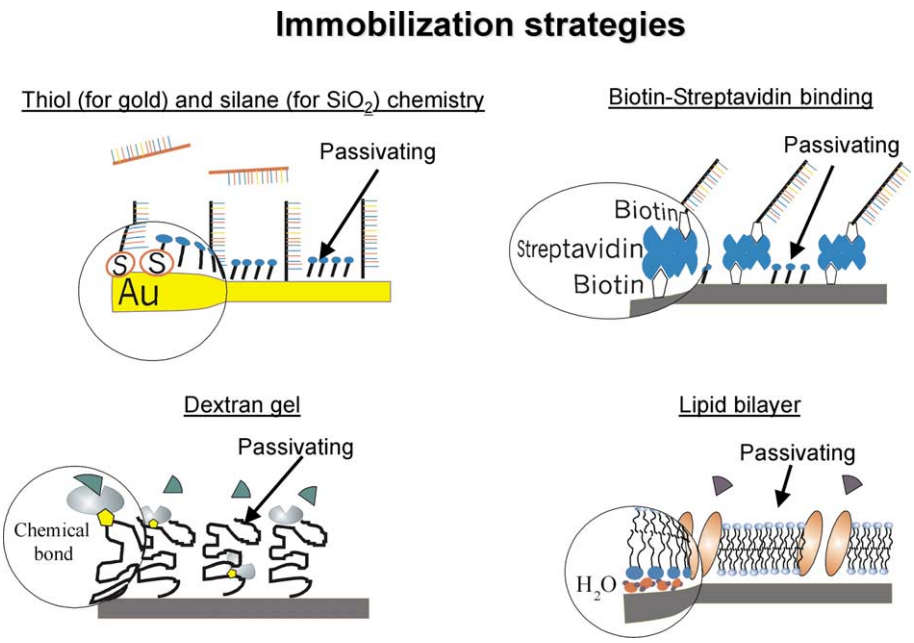

Fig. 5. Immobilization strategies for biomolecules on surfaces to achieve biosensor and biomedical diagnostic functions.

development with an estimated market growth for labchips/bioarrays of 40% per year. Most of its impact, technically and commercially, is yet to come in the wake of the so-called molecular biology or biotechnology revolution. The product will have important input from modern microelectronics, materials and surface science, advanced micro- and nanofabrication, and bioinformatics. The perspective for the future is unprecedented precision and speed in multiple (broad band), and individualized clinical and eventually "near patient" (home) diagnostics. Other important applications are expected in e.g. environmental control and food production.

Although the focus up till now has been primarily on DNA-based diagnostics by large sensor arrays, the future sensing arrays will also address biologically more "down stream" components in the cell expression and cell–cell communication processes, typically peptides and proteins (the latter area is sometimes referred to as "proteomics"). Even further down the line lies (potentially) cell-based diagnostics (cellomics).

Another type of sensors is the kind that can be implanted and used in feed back systems in vivo e.g. for real-time control of drug administration. One such example is the search for a reliable glucose sensor for real-time control of insulin administration for diabetes patients, via an implanted, sensor controlled insulin pump.

Tissue engineering [7,8] is an emerging area where most of the impact is also still to come, very much fueled by the molecular and cell biology revolution. The basic strategy for repair of lost body functions by way of tissue engineering is different compared to medical implants: In the latter case a synthetic component is fabricated that replaces the function of the lost or degraded part of the body, such as the metal ball head on the hip implant, and a matching counterpart made from high molecular weight polyethylene (HMWPE), constituting an artificial hip joint. In the tissue engineering strategy the idea (Fig. 4) is instead to produce a real functioning tissue from a cell culture "seed", by making it grow ex vivo in a suitable environment. The latter requires a synthetic scaffold or template for the growing cells/tissue, whose macroscopic geometry, microscopic topography and surface chemical properties cooperate with other stimuli such as extracellular signal

substances and the composition of the physiological solution etc., to make the cell culture develop into a functioning tissue, ready for implantation. Practical tissue engineering is today realized only for a few "simple" tissues like skin and, to some extent, cartilage, while large research efforts are focussed on e.g. liver, pancreas, blood vessel, bladder, and heart valve tissues.

Bioelectronics [9–11] is still only at the very early, explorative research stage and it is too early to judge if it will or will not become a practical reality in the future. The basic idea is to take advantage of some unique aspects of nature's own way of information storage and processing. This can alternatively be done at the cellular level (sometimes referred to as "reverse engineering"; see below), where the primary building stones are networks of real cells, or by "forward engineering" at the biomolecular level, where the building stones are peptides, proteins, DNA strings etc., and combinations of them. Both in the cellular and molecular approach the biological components are likely to be steered into a suitable pattern in or on a microengineered template or substrate surface, providing chemical and/or electrical I/O communication. Bioelectronic components and circuits made by the molecular level, forward engineering approach are likely to resemble today's microelectronics circuits in some respects, but will also have new unique characteristics originating from the biomolecules' highly specialized functions, including their replication and recognition ability, and self-organization. The cell level, reverse engineering approach will result in entirely new circuit, component, and functional concepts. The idea is to "automatically" achieve signal- and information-storage pathways from the internal self-organization, architecture and multiple functions of living cells, and from the biosystem's inter- and intracellular communication pathways. The latter have evolved over billions of years, and thus one does not need to invent these functions and architectures again (the latter is the prime reason for calling this approach reverse engineering).

As in the bioelectronics case, biomimetic or *artificial photosynthesis* [12] is still an area at its infancy. The basic mechanism is known by principle (Fig. 6); it is based on electron excitation by incident, photons in suitable proteins or protein arrays, followed by charge separation (preventing charge recombination and concomitant energy loss), and finally redox reactions creating useful, energy rich (fuel) molecules. However, biomimetic structures that have the quantum efficiency, sustainability, and a self-repair ability approaching those of natural systems, and that can produce useful fuels (like hydrogen) are still just a vision and goal for future research. A strong driving

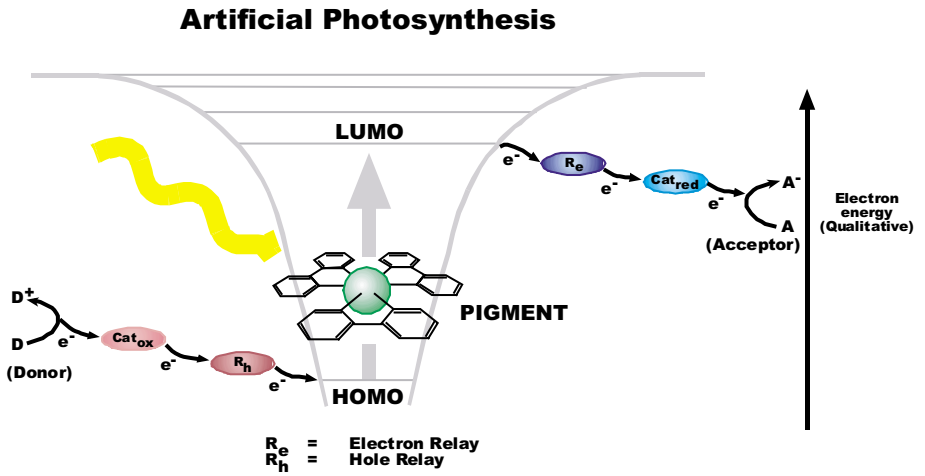

Fig. 6. The principle of photon to electron energy conversion in photosynthesis.

force is the rapidly growing need for sustainable energy systems. One strategy to realize such structures is to immobilize the photon-harvesting and redox protein arrays of real plants, or their synthetic counterparts, on surfaces with suitable chemical and electronic properties. These surfaces need to be functionilized both with respect to nano- and microtopography and with respect to surface chemistry. Another approach is to build entirely synthetic nanostructures mimicking the photosynthetic systems.

The low friction mimic of a sharkskin surface constitutes just one example of *biomimetic materials science* [13]. In biomimetic materials science the idea is to mimic the functional properties of biological materials/components or the processes by which they are manufactured in nature, in order to achieve new, superior materials properties or manufacturing advantages. A major driving force to attempt mimicking the sharkskin is the potentially much lower energy consumption of airplanes and sea vehicles that would be obtained. A more exotic example, already realized in real products, is swimsuits for faster swimming and low friction hull coatings on sailboats. Other biomimetic materials examples are antifreeze materials, utilizing the special features of some water holding proteins that can lower the freezing point of water in certain arctic fishes below 0 °C, and self-cleaning surfaces like some green leaves (the lotus leaf), and self-repairing surfaces.

1.1. Biological surface science

In all six examples above, each representing large and growing fields of applications and associated basic R&D, surfaces play a very important role. As a generic name for this branch of surface science I use *biological surface science* (BioSS) [14] (it includes the sub-area of medical applications; *biomedical surface science*).

The importance and function of synthetic solid surfaces in biological and medical contexts, is the subject of the next paragraph (Section 2), with special focus on the six examples or sub-areas mentioned above. In Section 3 the large variety of model systems available for BioSS studies, of different complexity (from water and amino acids to living cells) are briefly discussed. The same paragraph also discusses different types of surfaces and surface modifications, and analytical and preparation methods for biofunctional surfaces. However, these paragraphs are intentionally very short, since the focus is on general aspects and concepts rather than specific surfaces or methods. Finally some comments are attempted regarding the likely future development of BioSS.

The intention with the article is not to present a comprehensive review of the field, but rather to identify some important trends, directions, and concepts in BioSS and particularly to outline the opportunities for graduate students and surface scientists to contribute to this exciting field. References are therefore chosen primarily to provide starting points for further reading, and there is no ambition to cover all previous work in BioSS (i.e. the reference lists in the quoted references are just as important as the textual content).

2. The role of surfaces

2.1. Biorecognition

In most of the examples above, especially for (i)–(iv), *biorecognition* is a central component. Any attempt to make a sophisticated, functional surface for biointeractions must take into account the highly developed ability of biological systems to recognize specially designed features on the molecular scale. Famous examples are antibody–antigen, enzyme–substrate, and receptor–transmitter recognition (e.g. in cell membranes). The recognition is programmed into the molecules through the combination of their 3D topographic architecture, the superimposed chemical architecture, and the dynamic properties. Consequently an optimally designed surface for specific biological function must take these aspects into account.

Although the fundamental interactions occur on the molecular scale there is an interesting and unique synergistic connection between the nanometer and the micrometer length scales [1,15,16] when cells are present, as e.g. in the cases of medical implants, tissue engineering and cell-based bioelectronics. This is schematically illustrated in

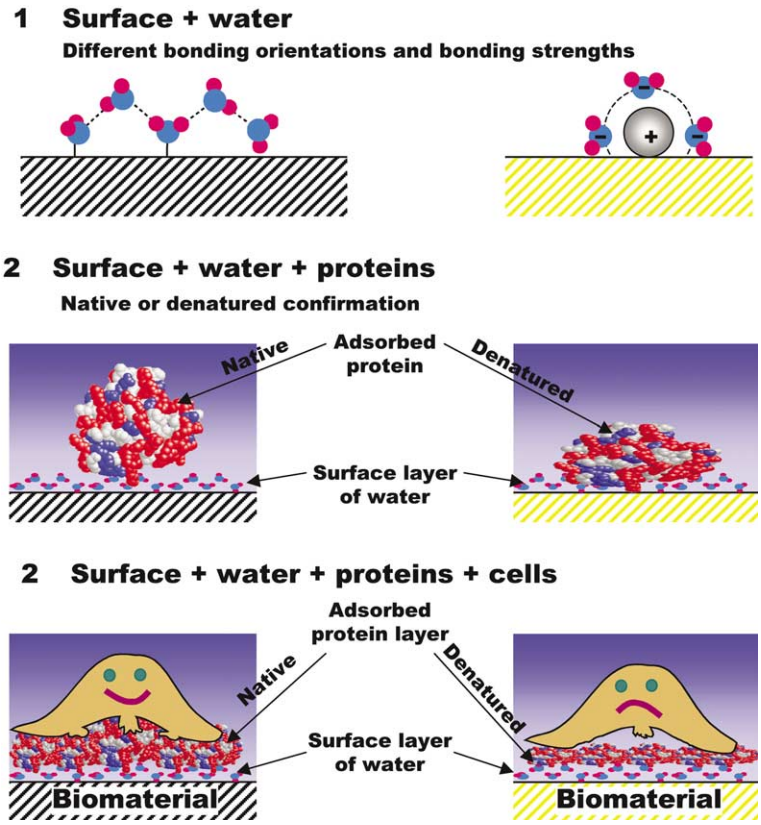

Fig. 7. Schematic illustration of the successive events following after implantation of a medical implant. The first molecules to reach the surface are water molecules (ns time scale). The water shell that is formed affect the protein interaction starting on the micro- to millisecond time scale, and continuing for much longer times. The water shell on the surface affects the protein interaction. Eventually cells reach the surface. Their surface interaction takes place via the protein coating whose properties is determined by the surface and water adlayer properties.

Figs. 7 and 8. The first picture (Fig. 7) shows the sequence of events after a surface has suddenly been placed in a biological milieu containing cells. The first molecules to reach the surface (time scale of order ns) are water molecules. Water is known to interact and bind very differently at surfaces depending on the surface properties (see Section 3). The properties of the surface water "shell" are an important factor influencing proteins and other molecules that arrive a little later. These water-soluble biomolecules also have hydration (water) shells and the interaction between the surface water shell with the biomolecular water shell influences the fundamental kinetic processes and the thermodynamics at the interface. For example, it may determine if proteins denature or not, their orientation, and coverage etc.

When cells arrive at the surface they "see" a protein-covered surface whose protein layer has properties that were initially determined by the preformed water shells. Thus, when we talk about cell–surface interactions, it is ultimately an interaction between cells and surface bound proteins (or other biomolecules). The latter is illustrated in Fig. 8, where the clean surface in the illustration is deliberately covered with a native or artificial biomembrane (supported bilayer), containing embedded receptors that can specifically interact (bind to and/or provide I/O signals etc.) with cells approaching the surface.

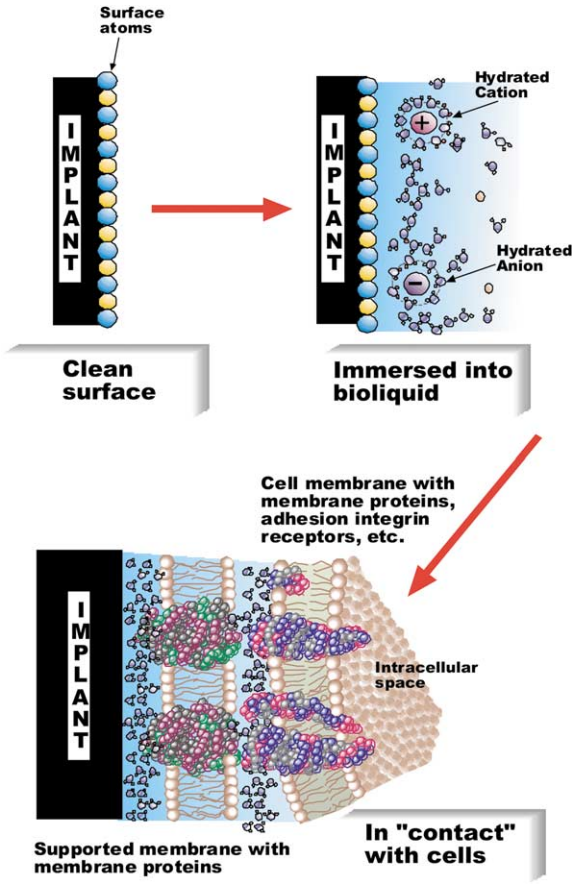

Fig. 8. A conceptual approach to convert a synthetic surface to a biomimetic surface by coating it with a supported biomembrane with built-in functional membrane-bound proteins.

Figs. 7 and 8 emphasize the above mentioned large size range that the functional units in biology cover, from water molecules and small proteins at sub-nanometer sizes, via cell membranes and supra-molecular complexes in the 10 nm range, to cells in the micrometer range and finally fully organized tissue and organs at the macroscopic size level.

2.2. Medical implants

In the case of medical implants (Figs. 1 and 3) the importance of surface science is quite obvious [1,2,14–16]. In 15–20 years time biomaterials research and development has transformed from only marginally applying surface analytical techniques to study medical implant surfaces, to a current situation where such use is the rule and mandatory for high quality research, and also for legal control, standardization and quality control of commercial products. Let us briefly inspect how surfaces come into play.

For a dental implant (Fig. 1), which is just one of many examples of so-called bone anchored implants, the clinical goal is to obtain a long term (i.e. life long) secure anchoring of the implant. The latter includes e.g. the implant's ability to carry and sustain the dynamic and static loads that it is subject to. It is obviously (for the patient and for cost reasons) important to achieve this function with the shortest possible healing time, with a very small failure rate and with minimal uncomfort for the patient. The implant is put in place after a surgical procedure typically involving drilling a hole in the cheekbone, threading of the hole, and then placing the screw-shaped end of the implant into the threaded hole.

The surface design is one of many components contributing to the clinical goal fulfillment. An inappropriate material choice or surface coating might lead to a too strong inflammatory response that adds on top of the already initiated inflammatory process by the surgical procedure. Then connective tissue rather than bone is formed around the implant, with concomitant loss of mechanical stability and eventual implant failure. A less perturbing surface may lead to a bone healing process, which is similar to the one in the absence of an implant i.e. as if the implant were a fairly passive spectator (sometimes this is referred to as inert materials or bone accepting materials, although no material is really inert in an absolute sense). Some surface coatings appear to have the ability to induce bone formation, for example the calcium phosphate materials usually referred to as hydroxyapatites.

More recently there has also been attempts to speed up the bone healing beyond the normal healing rate by adding so-called bone growth factors. The latter can e.g. be implemented by using a porous surface with a slow release of the desired biomolecules. Engineering of such surfaces requires a whole set of surface science tools. Exam-

ples of used surface science methods are coating technologies to deposit the desired surface material on a bulk carrier; surface spectroscopies like XPS, AES, and SIMS to control e.g. the Ca/P ratio; SEM or scanning probe techniques (SPMs) to control surface structure and chemistry, both of which influence the biological response; micro- and nanofabrication or other techniques to make porous surfaces; surface impregnation by molecules that are released at a pre-programmed rate to promote healing/bone formation, FTIR to measure adsorbed biological molecules etc.

Another challenging task for surface engineering of biomaterials is blood-compatible materials, such as the inner walls of artificial blood vessels, and the blood contacting surfaces of heart valves, dialyses equipment, and heart–lung machines. Normal blood is, naively speaking, a metastable state stabilized between two opposing driving forces—coagulation and anticoagulation. Almost any external perturbation in the form of an artificial surface tends to trigger the coagulation system, i.e. blood clotting may occur. The extent to which this happens, and is detrimental for the patient, depends on a number of factors such as where the surface is placed in the blood stream, the local hydrodynamics, the flow speed, contact time between the surface and blood and, of course, on the surface chemistry.

There is today clear evidence that the latter has a major influence on blood compatibility and that surfaces can be engineered to suppress blood coagulation, at least for considerable time. However, there is still a long way before surfaces that mimic the blood compatibility of the endothelial cells of normal blood vessel walls are achieved. For the surface scientist this task involves designing durable surface coatings that have the right chemical and micromechanical (visco-elastic) properties, which in turn may require a better understanding than today of how the native system works, so that a true biomimetic approach can be implemented.

2.3. Biosensors and biochips

A variety of surface based detection principles are employed for biosensors/biochips (Figs. 2 and 3). A common principle of a biosensor/biochip is that "detector molecules" are attached/immobilized on a solid surface in such a way that a specific signal is obtained from the sensor when the detector molecules *selectively* react with (bind to) the biomolecules they are designed to detect. In other words, the detection relies on the biorecognition between pre-designed native or synthetic reagent molecules and unknown sample molecules. The biorecognition event typically occurs when the precoated sensor is exposed to blood or urine samples or extracts thereof.

The detector read-out signals may be electrical, optical, or some other signal that is convertible into a measurable electrical signal. The detection may occur in real time, i.e. just when the recognition event occurs, or the sensor can be read-off at a later time. Two common examples are (i) antigens are pre-adsorbed on the sensor surface to detect the corresponding antibody(ies) in a clinical sample for diagnostics; (ii) a single strand DNA segment (i.e. a nucleic acid chain without cross-linking), representing a specific, known part of the genetic code, is attached to the surface so that all the dangling bonds of the non-hybridized, single strand are exposed and available for binding to a matching DNA strand. When the sensor is exposed to a DNA sample to be analyzed, one wants to obtain a specific response when there is perfect matching between the detector molecule and some sample molecules, while a different response should be obtained when the matching is imperfect. On a DNA chip many different such DNA segments are placed at different locations and thus many different DNA segments can be analyzed in one "shot" (bioarray, biochip). When the sensor array is combined with a sophisticated microfluidics system often utilizing functional hydrophobic–hydrophilic surfaces—we call the device a lab-on-a-chip or just labchip.

Other sensing principles are to e.g. employ enzymes that are immobilized on a surface with preserved function, and which can recognize specific biochemical substances, or the use of MALDI-MS to identify proteins and antibody–antigen complexes.

Surface science tools and expertise come into play in many ways in the preparation and use of biosensors and biochips. Immobilization of the

detector molecules in such a way that they preserve their original recognition specificity, requires surface pre-coatings that simultaneously allow sufficiently strong binding of the detector molecules, and sufficiently weak and gentle perturbation that the molecules retain their functionality (Fig. 5). For example, the strong polarization forces at metal surfaces and the strong ionic or covalent interactions on many inorganic metal oxides and semiconductor materials may cause denaturation and loss of specificity of biomolecules. At the same time such materials may be desirable "platforms" for sensor arrays. A solution is then deposition of special spacer and/or linker molecules (typically some type of polymer or a native or synthetic biomembrane) on the original sensor substrate.

The preparation and characterization of such layers for useful deposition of detector molecules, obviously require sophisticated surface science input. The same holds for the preparation of the part of the sensor that provides a measurable signal. The most common detection approaches so far are based on optical detection in the visible or infrared. Optimization of the optical detection requires deposition of optically suitable layers e.g. regarding reflectivity, absorption properties, plasmon properties, field enhancements etc., which are all typical components in thin film design of optical coatings. Consequently advanced surface spectroscopy and microscopy come into play both for understanding of how the matrix and detector molecules bind to the sensor surfaces and for practical diagnostics. Regarding biochips, arrays of detector "spots" are required and here surface science plays a similar role as it does for the multiple microfabrication steps in semiconductor/microelectronics technology.

2.4. Tissue engineering

To reach the goal of tissue engineering—to grow a functional tissue ex vivo that can later be implanted into a human body—requires control over a large number of external parameters. Examples are the nutrients, extracellular signal substances, antibiotics, and the dynamic mechanical force fields etc. in the bioreactor where the tissue is grown. The surface of the template or scaffold material (Fig. 4), on which the original cell culture(s) are deposited and eventually develop into a tissue, must be specifically designed to promote, and not compromise, the tissue evolution. This calls first of all for a non-toxic surface for the cell culture, and for suitable protein adsorption properties, since the protein adsorption layer that always forms on the surface influences the cell–surface interactions. Since differentiated tissue is an ultimate goal, different parts of the scaffold may require different surface functionalities, i.e. different surface chemistries and topographies.

Cells in a tissue culture communicate with each other by sending out and receiving signaling molecules to/from the extracellular matrix. The total spectrum of such signals is influenced by the surfaces onto which cells attach. Thus, the surfaces of tissue engineering scaffolds are an integral part of the communicating and self-organizing tissue.

A common—but not the only one—concept in tissue engineering is a scaffold that eventually is dissolved as the tissue "matures". This calls for a sophisticated "programming" of the material so that it dissolves into harmless products at the right pace, and maybe also exposes different functional surfaces at different times. (The latter may also be a desired function of medical implants.) Many different surface technologies are potentially suitable for such surface functionalization, especially coating technologies that can be applied to 3D curved surfaces and porous surfaces, but also the standard surface characterization methods come into play. The 3D surface topography, which is very important for cell–surface interactions, may require micro- and nanofabrication methods.

2.5. Bioelectronics

Bioelectronics based on living cells (neurons etc.) call for patterned surfaces where the cells can be confined to certain geographic locations (in order to make a reproducible circuit), and where they can be kept alive and functional, which in turn requires suitable surface chemistries and topographies. Regarding the basic control of cells there is actually large similarities between cell-based electronics and tissue engineering. The surface patterns for bioelectronics require in addition

adequate I/O connections for electrical or chemical communication. Similar requirements are at hand for bioelectronic devices based on biomolecules rather than whole cells, albeit at a generally smaller length scale (see also the text under artificial photosynthesis below). Consequently bioelectronic circuits require a combination of molecular cell biology with surface preparation/characterization and micro- or nanofabrication techniques.

2.6. Artificial photosynthesis

From a surface design point of view there are many similarities between biomolecule-based bioelectronics and artificial photosynthesis based on biomolecules. One common denominator is charge transfer within or between biomolecules, and between biomolecules and surfaces, either induced by external electrical fields (voltage bias) or by the electromagnetic field of light in the visible regime (e.g. photoexcitations of electron hole pairs). The relationship between the two areas becomes especially obvious when the bioelectronics concepts are extended towards opto(bio)electronic devices.

For the surface scientist a major contribution can be to produce chemical–topographic patterns on surfaces that adsorb and retain the biomolecular building blocks of the circuit elements at prescribed locations and in the right orientations (self-organization), without loss of functionality. In artificial photosynthesis with e.g. proteins (Fig. 6) there is also a major challenge to find and prepare surfaces that match the electronic states of the charge transfer proteins, e.g., as electron/hole acceptors or donors. Further requirements of the surface may be to make field enhancement structures that increase the quantum efficiency of "photon harvesting"; perhaps similar to the ones that produce the recently discovered giant surface enhanced Raman effect. Other ideas that come into play are to use optically active quantum dot arrays to catch and convert photons to energetic and "useful" electrons.

2.7. Biomimetic materials

This heading covers a large number of widely different scientific and technical applications. Examples involving surfaces as key elements are mimics of the lubrication and wear resistance of artificial joints, the low drag friction of shark and dolphin skin, the self-cleaning action of some green leaves, and threads made to mimic the strength and flexibility of spider web.

3. Model systems, research topics, and ongoing research

3.1. Biological model systems

In this section model systems are discussed and brief references are made to recent or ongoing research, illustrating the diversity of research areas and the challenging research problems in BioSS. The biologically relevant model systems available for surface science approaches cover such a broad range that any taste should find its topic of choice. Some of these areas—such as water and simple amino acids—are immediately approachable by existing UHV- and surface science preparative and analytical techniques. Others require entirely new or modified experimental approaches, such as those requiring wet preparation and in-liquid analyses or e.g. freeze-drying techniques, to maintain the biological properties and function of the studied systems. The model systems contained in the following list should be read with the accompanying label "... at surfaces":

- water
- amino acids, nucleic acids, lipids
- peptides, DNA segments
- proteins
- DNA
- lipids, biomembranes
- cells
- tissue, in vitro
- tissue, in vivo
- microorganisms

The list covers most but not all biointerfaces where there is an *interface between a synthetic (i.e. non-native) material surface and some component(s) of native biological systems*. I call them *hybrid biointerfaces*, in contrast to *native biointerfaces*,

because they consist of one native biological component and one synthetic, non-native component. I will not treat native biointerfaces, such as cell–cell or microorganism–cell interfaces, although they are closely related to the hybrid interfaces; it is actually very likely that the knowledge and methods for these two types of interfaces will mutually interact and cross-fertilize in the future. For example, studies of properties and processes in real cell membranes and in so-called supported biomembranes (see below) are clearly benefiting from each other.

3.1.1. Water

Water at interfaces is an area where surface science studies will contribute significantly in the future, e.g. to the understanding of what is sometimes called ''biological water''. The latter refers to the special water structures that are formed near surfaces or at narrow interfaces between biological components, such as the hydration shells of proteins, DNA and biomembranes. Biological water at surfaces, or in small confined volumes, is in principle closely related to the special water structures that form at solid synthetic surfaces. They obtain their unique interfacial properties through a combination of the local physical–chemical interactions (the thermodynamics) at the interface, including the spatial constraints caused by the surface, and the kinetic constraints. They are also related to the hydration shells of solvated ions and to the Helmholtz layers at electrode surfaces in electrochemical cells, an area where surface science has contributed with significant new insight in the past two decades.

Since the presence of water has such a profound influence on both the thermodynamics and kinetics at biointerfaces it is a safe prediction that it will be a major topic in BioSS for a decade or more ahead. Recent conceptual and theoretical considerations can be found in the series of papers published by Vogler and collaborators (see Ref. [17] and references therein). Experimental work includes both direct studies of the force interactions at hybrid interfaces, as described by e.g. Israelachvili and collaborators [18], and kinetic and spectroscopic studies of interfacial water, using both biological and non-biological substances. There is also current progress in the modeling of water hydration shells on protein resistant self-assembled monolayers by Gruntze and co-workers [19], which is an important component both for understanding of what the requirements protein resistant surface is, and for understanding protein adsorption. It is also a central component for blood-compatible surfaces.

3.1.2. Amino acids and peptides

Amino acids are the building stones of one of the most elementary functional units in biology, namely proteins. Mapping their interactions with surfaces is therefore, apart from the inherent basic research interest, a precursor to the understanding of how peptides and proteins interact with surfaces. In the recent years a number of papers have been published [20–24] addressing how submonolayer to multilayer deposits of simple amino acids (glycine, alanine, ...) interact with single crystal surfaces or polycrystalline surfaces in vacuo. These studies include standard spectroscopic techniques, such as SIMS, HREELS, FTIR, and synchrotron light based spectroscopies applied to amino acid adsorbate layers. Some of these systems have also been characterized by LEED and TDS. There are also recent attempts to describe the amino acid–substrate interactions (including adsorbate–adsorbate interaction) by first principle calculations.

The amino acids—at least the simpler ones—are compatible with UHV evaporation techniques. For example glycine and several other amino acids can be evaporated from a simple Knudsen source at low temperature. More complex (fragile) amino acids may require deposition from the liquid phase, and then similar techniques are needed as have been developed for UHV studies of electrode double layers in electrochemistry. Important questions concern (i) how the amino acids bind to the surface, if the bonding is non-dissociative or dissociative, where and how the bonds are established (at the amine group, and/or at the carboxylic group, and/or at the R-side group etc.), (ii) strength and character of adsorbate–adsorbate interactions, (iii) molecular orientations, (iv) 2D crystal structure (when ordered layers are formed), and (v) the influence of water molecules on these

properties. Another class of questions regards kinetic processes, e.g. to what extent can surfaces catalyze polymerization (e.g. forming peptide bonds) and other reactions between amino acids and co-adsorbates.

The same questions as above can be formulated for peptides, which are polymer chains of amino acids. They are important functional units in biology per se, and also constitute the amino-acid-chain sub-units of proteins. Studies of peptides are therefore one step up in complexity from amino acids, but also a step closer towards "real biology". (By analogy the step from single nucleic acids at surfaces to DNA segments at surfaces is a step both in complexity and towards biological relevance.) A natural extension of studies of amino acid monolayers is thus di-peptide, tri-peptide etc. adsorption studies. Up to date no systematic peptide studies, like the ones for amino acids, have to my knowledge been done in UHV, with state of the art surface science techniques. In contrast there is large ongoing activity focussing on liquid phase deposition of various peptide depositions and formulations on biomaterial surfaces in order to make them more functional for e.g. medical implant and tissue engineering applications. These depositions still lack the rigorous control that can be achieved in UHV, but surface spectroscopies like XPS and FTIR can and are applied, after deposition, to control the deposited layer composition.

3.1.3. Proteins

Proteins, which are (polymerized) peptide chains wrapped folded into special 3D structures (conformations), have since long been, and is currently the subject of extensive surface studies [25–42] due both to their central role in all biological processes and due to their importance in the context of bioengineering, e.g. for biosensors, biofouling and tissue engineering. The vast majority of past studies have focussed on the (macroscopic) kinetics, and to some extent on the energetics of adsorption on various surfaces, and under different conditions of liquid properties (protein concentration, pH, salinity, ...). A typical experiment records the kinetics by which a surface is gradually covered by a single protein type or by proteins that compete for the surface sites. Common techniques are surface plasmon resonance, ellipsometry, fluorescence detection, and other optical methods, and gravimetric (QCM, QCM-D, SAW-devices) and radiolabelling methods. Protein adsorption is for most surfaces irreversible through additive contributions from e.g. van der Waal's interaction, polar and charged groups on the proteins, hydrogen bonding, and hydrophobic interactions.

The relative strengths of these contributions depend on protein type, on surface properties and on pH and salt concentration. Binding energies are difficult to measure for irreversible adsorption. A few surfaces bind proteins sufficiently weakly that reversible adsorption occurs, whereby heats of adsorption can be obtained from the kinetic measurements.

Very few surfaces are so-called protein resistant surfaces (see also under biomembranes below). Protein resistance means that the adsorbed amount, even at relatively high protein concentration in the bulk liquid, is below or near the detection limit. Theoretically it is still a major challenge to understand the mechanisms that contribute to protein resistance of surfaces, and even more so to understand the detailed contributions to strong(er) protein–surface bonds. Recent experimental work by Whitesides and co-workers [40–42] and by Gruntze and co-workers [19,39] constitute important steps towards understanding of protein adsorption, and protein resistance and ultimately of protein binding more generally.

Important challenges, not at all clarified at the molecular level, are to quantify the relative and absolute contributions of the various protein–surface interaction terms, and to ultimately understand how the relatively fragile protein structures (usually they denature well below 100 °C) are affected by the perturbation exerted by a solid surface. There is no doubt that the water shell of the surface and that of the (water soluble) proteins, and their mutual interaction, play an important role, which can be expressed in terms of hydrophobicity–hydrophilicity at the macroscopic scale, and in terms of water–water versus water–surface bonding strengths at the molecular level.

For example, a material surface of low polarizability in the bulk with a strongly bound water mono- or bilayer on the surface, is likely to only weakly perturb a protein with predominantly hydrophilic surface domains, while a hydrophobic surface is likely to strongly perturb a protein with mixed hydrophilic and hydrophobic domains, via hydrophobic interactions with the latter. These ideas have recently been quantified theoretically in some simple cases. Furthermore there are important entropy effects in the energetics of protein bonding at surfaces due to the highly organized and multiple state structures of proteins, and also due to the many different water structures that are possible.

Finally kinetic effects are of primary importance; the final structure of an adsorbed single protein, or protein adlayer, is almost always a long-lived metastable state, rather than the lowest energy state. In other words there is usually a sufficiently large activation barrier towards formation of the lowest energy states, so that further conformational changes are prevented. A common experimental manifestation of this is that the final structure of a protein adlayer often depends on the rate by which it is formed; at low bulk concentration of proteins the adsorption is slow and there is then longer time available for conformational changes to occur, in comparison with high bulk concentration and high adsorption rates, when neighboring molecules may sterically prevent slow conformational changes. The issue of protein conformation and denaturation at surfaces connects protein adsorption to the broad field of protein folding–unfolding in the bulk phase (see e.g. Refs. [38,43] and references therein).

A quantitative first principle description of protein bonding at surfaces lies far into the future. It will be preceded by corresponding descriptions of adsorbed amino acids and di- and tri-peptides (and the influence of water on such bonding). It will also require a quantitative first principles total energy description of proteins in the bulk phase, including the description of dynamics and multiple states. Spectroscopic techniques such as non-linear optical techniques and other laser based techniques (SFG, SHG,...), synchrotron based spectroscopies, neutron scattering, NMR, and scanning probe techniques are already addressing some of these questions. There are major challenges to adopt these techniques to the special "wet" requirements for proteins. The theoretical work ahead is equally demanding and important as the experimental work and will be absolutely necessary for progress in this area.

3.1.4. DNA

Studies of the DNA molecule at surfaces [44,45] is driven both by the inherent interest in understanding different aspects of this molecule, and by its importance for medical diagnostics using DNA biochip arrays. More recently this interest has been stirred by potential applications of DNA in (biomimetic) materials science and in molecular electronics. Generally, much less has been done regarding basic adsorption studies of nucleic acids and DNA compared to amino acid, peptide and protein adsorption, except in the sub-area related to DNA biochips. As a rule direct adsorption of DNA on such surfaces is of less interest than adsorption on pre-adsorbed spacer layers. The reason is that the surface perturbation from, say a metal, oxide or semiconductor surface, turns out or is suspected to be too strong to preserve the full function of the DNA molecule. For example, it is very likely that the dangling bonds of a non-hybridized DNA segment will bind to the surface and then be useless for detection of matching segments. The maintenance of the full recognition function of e.g. a single strand DNA segment is crucial for diagnostic applications as on biochips. Therefore various spacer layer and linker molecule strategies are currently developed to achieve both localization of the diagnostic DNA segments at different spots on a surface array, and preserved functionality.

The molecular electronics potential of DNA is currently a hot topic. Specifically there is intense research addressing the question whether the DNA molecule is a 1D conductor or not, and in that case, what the conductivity mechanisms are. Recent literature reports both high conductivity and insulating properties. Although some reports may be plagued by experimental difficulties there is another aspect; the question of conductivity or not must be more specific, and discriminate between

different types of molecules, and take into account if the measurement is made with or without presence of the water shell of DNA etc. Microscopic (e.g. by SPMs) and spectroscopic characterization of the actual DNA configurations, preferably in the liquid phase, is thus vital for progress in this field.

3.1.5. Biomembranes

Biomembranes are and will be a future "hot" topic for BioSS and many other sub-disciplines, due to their enormous importance as functional units in living cells, and because of their potential role in many applications [46–53]. Biomembranes (Fig. 8 lower picture, Figs. 9 and 10) are self-organized structures of amphiphilic lipid molecules, the latter consisting of a hydrophobic (weakly interacting with water) lipid tail (linear hydrocarbon chain) and a hydrophilic (strongly interacting with water) terminal group such as a phosphate group (phospholipid molecules). In water solutions the thermodynamics drive these amphiphilic molecules to (self)assemble into structures that minimize the water interaction of the hydrophobic tails with surrounding water, and maximize the exposure of the hydrophilic end groups towards the water. This can result in many different self-assembled structures of which so-called unilamellar or bilayer membranes are the most interesting in the present context. They typically consist of a double layer of phospholipid molecules, with opposite orientations of the two layers i.e. with two consecutive layers of parallel hydrophobic tails in the interior, terminated on both sides by the hydrophilic end groups.

A specific structure occurs when the bilayer forms a closed spherical shell, with water both on the inside and the outside. This structure is named a vesicle or liposome, an entity that is a first crude approximation of a closed cell membrane, however, lacking the functional units of real cell membranes such as membrane-bound proteins, ion channels etc. One could refer to them as a primitive, "empty cell" that can be filled by both membrane-bound functional units (membrane proteins) and intracellular components, in order to

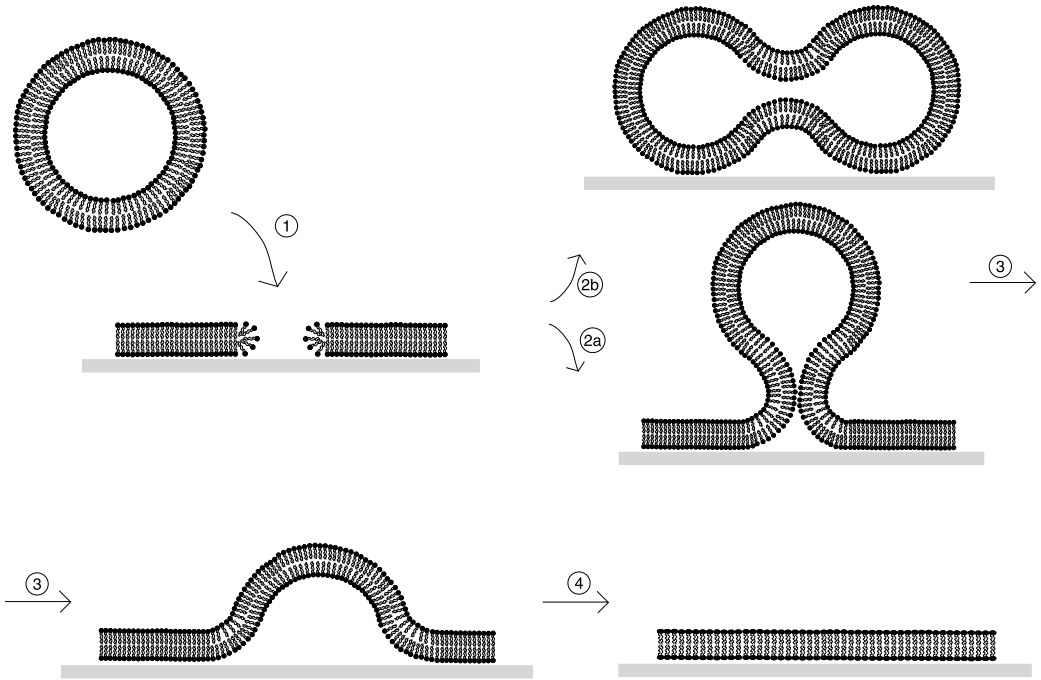

Fig. 9. Formation of SPB from vesicles (schematic; not proven by experiments).

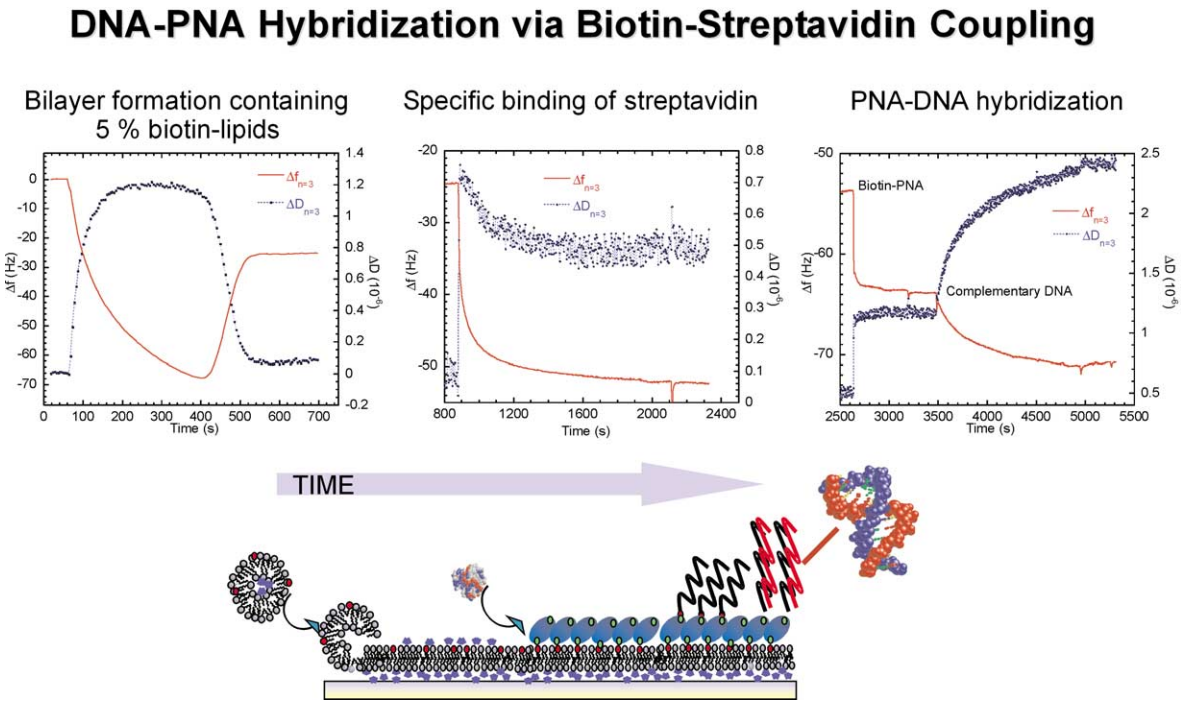

Fig. 10. A sequence of preparation steps that have been experimentally implemented [50] to build up a biofunctional surface for e.g. cell culturing or biosensing. The first step consists of formation of a biotin doped, phospholipid bilayer from vesicles, on top of which (2nd step) a 2D crystalline protein layer (streptavidin) is adsorbed. The third step is adsorption of DNA or PNA molecules, which are biotin coupled to the remaining free attachment sites on the streptavidin molecules. This platform can then be used e.g. for discriminative recognition of perfectly and non-perfectly matching DNA segments.

successively build up more cell-like structures. Another common structure is the planar bilayer membrane, which is called a *supported (bilayer) membrane* when deposited on a suitable surface. Such supported membranes are of extensive interest both as key elements of biosensor devices, and as mimics of "empty" or real cell membranes, the latter after they have been "doped" by incorporating membrane proteins etc. When the bilayer is made up of phospholipids, it is called a supported phospholipid bilayer or biomembrane (SPB). Such bilayer membranes are also of interest as functional coatings on medical implants, cell culture surfaces, and tissue engineering scaffolds.

Supported bialyer membranes can be formed on a surface starting from vesicles/liposomes (see Refs. [46–50], and references therein). Fig. 10 shows in schematic form how such supported bilayers may form. Supported bilayers with incorporated membrane proteins or protein or DNA layers on top of the membrane, can also be formed [48–53]. Proteins are e.g. incorporated into the membrane by placing them inside the vesicles/liposome membrane prior to surface deposition. The proteins incorporated in the membrane can then in turn act as a binding site for other proteins on top of the membrane, e.g. through antibody–antigen binding. A beautiful example reported by Reviakine and Brisson is the 2D protein crystals on top of a biotin doped bilayer imaged by AFM [49]. By suitable linker chemistry one can continue to add additional layers, to achieve a specific desired function. One such sequence of preparation steps executed by Höök et al. [50] is shown in Fig. 11, beginning with a supported membrane containing biotin, followed by binding a 2D (crystal) of streptavidin on top, and completed by binding another biotin layer linked to a single strand DNA or PNA segment in

the outermost layer. This structure can then be used to detect recognition events between the single strand DNA segment and its matching counterpart, forming a double helix. For sensor applications it is important to find methods to pattern cells at surfaces. Such patterning has recently been demonstrated by several groups, see e.g. Ref. [53] and references therein.

3.1.6. Cells

Cells at surfaces is a fundamental ingredient both with regard to the cultivation of cell cultures in vitro, tissue engineering, and medical implants. In the future it may also be employed in cell-based biosensors and bioelectronics. The role of the surface chemistry and topography for the evolution and function of single cells and cell assemblies has been demonstrated in many experiments [54–63] but is still poorly understood. The recent rapid development in the area of so-called stem cells is particularly interesting in this context. Stem cells are cells that have not reached a high degree of specialization, but can "choose" to differentiate in different specialized directions depending on the local environment. Depending on the latter stimuli the cell can thus differentiate into different functions. It has also been shown that some stimuli can make a cell "go backwards" from a specialized to less specialized state.

The chemistry and topography of surfaces on which cells grow/divide are part of the total environment for the cells and can potentially influence their differentiation, i.e. they are part of the total cell–cell signaling/communication system. One key research area for the future is therefore how tailored surfaces should be designed to act as stimuli to guide cell differentiation, e.g. in tissue engineering, bioelectronics and cell-based sensors. For the surface scientist the chemical and topographic patterning of surfaces, and the associated characterization is a major opportunity and challenge for future research. Another is to use SPMs to probe or stimulate specific cell functions. Yet another one is non-invasive, spatially resolved spectroscopies and microscopies to study special fractions of living cells or cell membranes at high spatial resolution (bioimaging) and sensitivity (single molecule detection).

3.1.7. Tissue

Tissues at surfaces, in vitro and in vivo, have been demonstrated to be sensitive to the adjacent surfaces, but the mechanisms are understandably even less known than in the case of single cells and cell monocultures. Current research on tissue-material interfaces in vivo follows several parallel lines. One major direction is chemical modification of surfaces to enhance their biocompatibility and/or to provide controlled release of substances that promote the healing and sustained function of implants. Another one focuses on topographic modification of surfaces to promote desired cell–surface responses, and to some extent also protein–surface interaction. In some studies these surface modifications, i.e. chemical and topographic, are combined to achieve optimal performance of the surface. Additional functions of the surface may be to e.g. act as a reservoir that can release certain desired substances, such as growth hormones, in a time-programmed manner.

In the rapidly expanding tissue engineering field the questions and challenges are to some extent similar as for the in vivo situation with medical implants. There are however also entirely new challenges connected with the fact that tissue engineering demands that a given cell or tissue culture shall evolve into a desired tissue without the support from the living organism that is the host for in vivo implants. Thus tissue engineering demands scaffolds that cooperatively with other factors (nutrients, extracellular chemicals, force fields, temperature, ...) steer the tissue culture in the required direction. In some sense one can say that the bioreactor must do for the tissue engineering scaffold + cell culture, what the living organism does for the medical implant during the healing-in period. However, the total demand is much higher, namely to make the orininal cell/tissue culture develop into a functioning organ. This may require special 3D architectures ranging from macroscopic dimensions to micrometer and nanometer dimensions, with superimposed chemical patterns and (bio)chemical signals, and probably with a time-programmed degradation rate of the templates as the tissue matures. The research opportunities for surface scientists in this area are similar to those for cell–surface interactions (see above).

3.1.8. Microorganisms

Microorganisms at surfaces are important e.g. in marine biofouling and bacterial infections on implant surfaces or in tissue cultures. They are also potentially of interest for growing simple organism cultures as model systems for a variety of biological experiments e.g. related to food production, development and evaluation of pharmaceuticals, and biomimetic energy production. Colonization of surfaces by e.g. bacteria (biofouling) is surface specific and can be influenced by surface chemistry, surface topography, and surface visco-elasticity. The settling down of microorganisms on a surface is—as in the case of cell adhesion—almost always preceded by protein adsorption. There is thus a causal relationship between protein adsorption and biofouling by microorganisms, in a similar way as sketched earlier for cell adhesion on a surface. Also the basic research opportunities and tools are similar as for cell culture growth and tissue engineering, while the more applied research may require much more specific and specially adapted tools, e.g. to study "real" marine biofouling or bacterial colonization on medical implants.

4. Surfaces

The material surfaces include almost all material types; metals, ceramics, carbon materials, polymers and composite materials. Although many native materials are currently used in practice, such as titanium for dental implants, stainless steel for orthopedic implants, PTFE for blood vessel replacements, silicones for internal drainage, PMMA for intraocular lenses, and so on, future more advanced materials and applications will often require the build up of sandwich type overlayers, with specific topographies and patterns, on the native surfaces, to obtain the desired function. This is actually already the case e.g. for intraocular lenses made from PMMA and almost always the case for advanced biosensors. In addition we know that surface patterns, topographically and chemically, on different length scales generate different responses of the biological system. This requirement of sophisticated surface structures and patterns derives from the very advanced recognition power of biological systems discussed earlier.

Native material surfaces are very unsophisticated compared to the biomolecular chemical + topographic architectures they are intended to interact with, and will in most cases at best cause a mild negative response in the biological system. In order to obtain a positive and selective response the molecular architecture should in general match some recognition sites of biomolecules on the biological side of the hybrid interface, so that it generates desired signal patterns to adjacent biomolecules or cells or sensing devices. This type of topographic + chemical microarchitectures are necessary both for applications and understanding-oriented basic research of biointerfacial processes.

Consequently the future development of biologically functional surfaces demands a very sophisticated machinery for surface preparation and characterization, including dry and wet chemical adlayer depositions, and nano- and microfabrication.

5. Methods

Since this article is more concept and idea oriented than method oriented this paragraph is deliberately very brief. A general statement about surface science methods and biointerfaces would be too trivial, since it would conclude that essentially all methods of surface science come into play in some contexts.

Well-established examples on the *analytical* side are the use of established surface spectroscopies (XPS, AES, SIMS,...) to characterize medical implants after manufacturing and sterilization, but before clinical insertion, and also after varying periods in vivo (retrieved implants). Synchrotron based spectroscopies will no doubt be important tools both for spectroscopic characterization, structure determination, and for imaging of biological components on surfaces. With regard to in situ characterization of surface bound biomolecules and larger entities, and especially for real-time recording of kinetic processes, the optical techniques are of prime importance. This includes

both linear and non-linear (laser) techniques, and all spectral regions from UV and down in wavelength to the far IR. There is no doubt that scanning probe techniques will continue to increase in importance for studies of biomolecules at surfaces. This includes both STM and AFM [64,65] and spatially resolved optical techniques like SNOM. Complementary techniques that provide unique information in special cases, when sufficient surface sensitivity is achieved are e.g. NMR, neutron scattering and ESR.

Preparation by thin film deposition methods (PVD, CVD) and by glow discharge plasma methods are common in R&D on hybrid biointerfaces. Wet chemical methods such as self-assembled monolayers, colloidal chemistry, microemulsions, and micelles and vesicles come into play because they have the ability to produce interesting chemical and topographic patterns on surfaces. In addition they are usually fast and often inexpensive. Synthetic polymer, protein and oligonucleotide chemistry are increasingly important because they can produce ligands that can selectively anchor desired polymer chains, peptides, oligonucleitides or whole proteins to surfaces.

The whole range of nano- and microfabrication methods constitute important tools for the functional patterning of surfaces. They include electron beam and photolithography and the soft lithographies, like stamping and imprinting.

Interesting to note are the special demands on methods for BioSS, set by the fact that one deals with very fragile matter, often requiring a liquid environment for meaningful studies. This calls for new developments and/or to adaptations of experimental techniques to work non-invasively with e.g. cells under liquid environments, or perform sophisticated freeze drying to retain the features to be studied by methods that require a vacuum environment. For smaller entities like adsorbed proteins and biomembranes, both the microscopic inspection by SPMs and the spectroscopic characterization under water are key issues. In order to address the important and challenging task of dynamics of e.g. proteins and supported membranes, femtosecond techniques are required, in the aqueous environment. Consequently scanning probe techniques and time resolved laser spectroscopies will be central tools in the development of BioSS.

6. A look into the future

When surface science started to take off as a research field on its own, over thirty years ago, little was known even about the simplest model systems and the number and sophistication of the experimental and theoretical tools were very limited compared to today. The electronic structure of surfaces was largely unknown, we did not know e.g. how low energy electrons were scattered by surface atoms, hampering the use of LEED as a quantitative tool, the positions and orientation of CO on any surface were totally unknown, and we were very far from any quantitative potential energy surfaces for dissociation or reaction dynamics (even the 1D cuts were unknown). Total energies of chemical accuracy were impossible to calculate.

For simple systems the situation is today reversed; the total energies can be calculated, the electronic and atomic structures, and the lattice dynamics of surfaces are known in great detail. Potential energy surfaces can be calculated for dissociation and reaction of simple molecules, and the understanding is conceptually transparent even for much more complex systems. Important factors have been the development of new experimental probes, advanced preparation of well defined model systems, development of theoretical methods and simulation schemes to describe both the studied model systems and how the experimental probes (electrons, photons, ions, SPM tips etc.) interact with surfaces, and a vast increase in computational power. This development has allowed a broad and systematic, theoretical and experimental, exploration of model systems of varying complexity, which successively paved the way for a genuine, quantitative understanding today of the simpler model systems, and a semi-quantitative and/or conceptual understanding of quite complex systems.

This is the platform from which BioSS launches just now. We can foresee an exciting development

of new knowledge about biomolecules at surfaces, initially mainly conceptual and qualitative rather than quantitative, except for the simplest model systems like water, and amino and nucleic acids. Successively we will map out and understand the structure, dynamics and biological functions of supported biomebranes with and without membrane-bound proteins, charge transfer in electron transfer proteins, DNA and ion channels attached to surfaces etc. Eventually (probably a decade or two) we will see the beginning of a detailed understanding of cell–surface interactions.

These basic research endeavors will be driven both by curiosity and the large number of applications of this knowledge; tissue engineering, biosensors, DNA and proteomic chips for drug development and unprecedented precision and individualization in medical diagnostics, and bioelctronics based on cell–cell self-organization and communication.

On the way there will be a dramatic improvement of existing experimental methods, and development of new ones, to prepare and characterize surfaces on the atomic and mesoscopic scale, including measurements of the dynamics and kinetics. These methods must, to a large extent, be adopted for real-time measurements at solid–liquid interfaces. The influence of the theoretical methods and simulations is likely to be even stronger than in the development of surface science referred to above.

The development will take place through strong interaction with adjacent fields to surface physics and chemistry, for example molecular biology, nanoscience and polymer science. Ultimately, it is likely that these studies will not only produce a deep understanding of material-biosystem interfaces, but also about the biosystems themselves.

Acknowledgements

I am very grateful to all members of the Chemical Physics Group at the Department of Applied Physics, Chalmers, who have so kindly helped me with invaluable reference searches and constructive comments on the manuscript.

References

[1] B. Kasemo, J. Gold, Advances in Dental Research 13 (1999) 8, and references therein.
[2] B.D. Ratner, Molecular Recognition 9 (1996) 617.
[3] W. Göpel, Biosensors and Bioelectronics 13 (1998) 723.
[4] A.F. Collings, F. Caruso, Reports on Progress in Physics 60 (1997) 165.
[5] H.A. Fishman, D.R. Greenwald, R.N. Zare, Annual Review of Biophysics and Biomolecular Structure 27 (1998) 165.
[6] D.J. Graves, Tibtech 17 (1999) 127.
[7] R. Langer, Accounts of Chemical Research 33 (2000) 94.
[8] J.A. Hubbell, Current Opinion in Solid State and Material Science 3 (1998) 246.
[9] R. lyengar, Science 283 (1999) 381.
[10] P. Fromhertz, A. Offenhäusser, T. Vetter, J. Weis, Science 252 (1991) 1290.
[11] R.F. Service, Science 284 (1999) 578.
[12] A.J. Bard, M.A. Fox, Accounts of Chemical Research 28 (1995) 141.
[13] M. Sarikaya, Proceedings of the National Academy of Sciences 96 (1999) 14183.
[14] B. Kasemo, Current Opinion in Solid State and Materials Science 3 (1998) 451.
[15] B. Kasemo, J. Lausmaa, CRC Critical Review on Biocompatibility 2 (1986) 335.
[16] B. Kasemo, J. Lausmaa, Osseointegration in Oral Rehabilitation, 1993.
[17] E.A. Vogler, Advances in Colloid and Interface Science 74 (1998) 69.
[18] J. Israelachvili, H. Wennerström, Nature 379 (1996) 219.
[19] A.J. Pertsin, M. Grunze, Langmuir 16 (2000) 8829.
[20] S.M. Barlow, K.J. Kitching, S. Haq, N.V. Richardson, Surface Science 401 (1998) 322.
[21] M. Nyberg, J. Hasselström, O. Karis, N. Wassdahl, M. Weinelt, A. Nilsson, L.G.M. Pettersson, Journal of Chemical Physics 112 (2000) 5420.
[22] N.A. Booth, D.P. Woodruff, O. Schaff, T. Gießel, R. Lindsay, P. Baumgartel, A.M. Bradshaw, Surface Science 397 (1998) 258.
[23] X. Zhao, Z. Gai, R.G. Zhao, W.S. Yang, T. Sakurai, Surface Science 424 (1999) L348.
[24] P. Löfgren, A. Krozer, J. Lausmaa, B. Kasemo, Surface Science 370 (1997) 277.
[25] I. Lundström, Progress in Colloid and Polymer Science 70 (1985) 76.
[26] J.L. Brash, P.W. Wojciechowski, Interfacial Phenomena and Bioproducts (Edited by McGregor WC), Marcel Dekker, New York, 1996.
[27] T.A. Horbett, T.L. Brash, ACS Symposium Series 602, ACS, Washington, DC, 1995.
[28] V. Hlady, J. Buiks, Current Opinion in Biotechnology 7 (1996) 72.
[29] W. Norde, Macromolecular Symposium 103 (1996) 5.
[30] T. Zoungrana, G.H. Findenegg, W. Norde, Journal of Colloid and Interface Science 190 (1997) 437.

[31] H. Arwin, Thin Solid Films 313 (1998) 764.
[32] F. Höök, M. Rodahl, B. Kasemo, P. Brzezinski, Proceedings of the National Academy of Sciences 95 (1998) 12271.
[33] F. Höök, M. Rodahl, P. Brzezinski, B. Kasemo, Langmuir 14 (1998) 729.
[34] F. Caruso, D.N. Furlong, P. Kingshott, Journal of Colloid and Interface Science 186 (1997) 129.
[35] P. Tengvall, I. Lundström, B. Liedberg, Biomaterials 19 (1998) 407.
[36] H. Elwing, Biomaterials 19 (1998) 397.
[37] V. Hlady, J.N. Lin, J.D. Andrade, Biosensors Bioelectronics 5 (1990) 291.
[38] V.P. Zhdanov, B. Kasemo, Surface Review Letters 5 (1998) 615.
[39] P. Harder, M. Gruntze, J.H. Waite, Journal of Adhesion 73 (2000) 161.
[40] R.G. Chapman, E. Ostuni, S. Takayama, R.E. Holmlin, L. Yan, G.M. Whitesides, Journal of the American Chemical Society 122 (2000) 8303.
[41] R.G. Chapman, E. Ostuni, L. Yan, G.M. Whitesides, Langmuir 16 (2000) 6927.
[42] M. Mrksich, G.M. Whitesides, Annual Review of Biophysics and Biomolecular Structure 25 (1996) 55.
[43] V.P. Zhdanov, B. Kasemo, Proteins 29 (1997) 508.
[44] H.W. Fink, C. Schönenberger, Nature 398 (1999) 407.
[45] E. Braun, Y. Eichen, U. Sivan, G. Ben-Yoseph, Nature 391 (1998) 775.
[46] E. Sackman, Science 271 (1996) 43.
[47] J. Salafsky et al., Biochemistry 35 (1996) 14773.
[48] C.A. Keller, B. Kasemo, Biophysical Journal 75 (1998) 1397.
[49] I. Reviakine, A. Brisson, Langmuir 16 (2000) 1806.
[50] F. Höök et al., Langmuir, submitted for publication.
[51] W. Knoll, M. Zizlsperger, T. Liebermann, S. Arnold, A. Badia, M. Liley, D. Piscevic, F.J. Schmitt, Physiochemical and Engineering Aspects 161 (2000) 115.
[52] T. Stora, Z. Dienes, H. Vogel, C. Duschl, Langmuir 16 (2000) 547.
[53] J.S. Hovis, S.G. Boxer, Langmuir 16 (2000) 894.
[54] G.M. Whitesides, J.P. Mathias, C.T. Seto, Science 254 (1991) 1312.
[55] R.S. Kane, S. Takayama, E. Ostuni, D.E. Ingber, G.M. Whitesides, Biomaterials 20 (1999) 2363.
[56] C.S. Chen et al., Science 276 (1997) 1425.
[57] R.G. Flemming, C.J. Murphy, G.A. Abrams, S.L. Goodman, P.F. Nealey, Biomaterials 20 (1999) 573.
[58] Y. Xia, J.A. Rogers, K.E. Paul, G.M. Whitesides, Chemical Reviews 99 (1999) 1823.
[59] A.S. Curtis, C.D. Wilkinson, Journal of Biomaterials Science Polymer Edition 9 (1998) 1313.
[60] D. Brunette, B. Cheroudi, Journal of Biomechanical Engineering 121 (1999) 49.
[61] M. Mrksich, Cellular and Molecular Life Sciences 54 (1998) 653.
[62] R.S. Kane, S. Takayama, E. Ostuni, D.E. Ingber, G.M. Whitesides, Biomaterials 20 (1999) 2363.
[63] D. Qin, Y.N. Xia, B. Xu, H. Yang, C. Zhu, G.M. Whitesides, Advanced Materials 11 (1999) 1433.
[64] A. Engel, H.E. Gaub, D.J. Muller, Current Biology 9 (1999) R133.
[65] H.G. Hansma, K.J. Kirn, D.E. Laney, et al., Journal of Structural Biology 199 (1997) 99.