

Modern biomaterials: a review—bulk properties and implications of surface modifications

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Abstract This review concerns the importance of length and time on physicochemical interactions between living tissue and biomaterials that occur on implantation. The review provides information on material host interactions, materials for medical applications and cell surface interactions, and then details the extent of knowledge concerning the role(s) that surface chemistry and topography play during the first stage of implant integration, namely protein adsorption. The key points are illustrated by data from model *in vitro* studies. Host implant interactions begin nanoseconds after first contact and from then on are in a state of flux due to protein adsorption, cell adhesion and physical and chemical alteration of the implanted material. The many questions concerning the conformational form and control of bound proteins and how this may impact on cell adhesion in the first instance and later on cell signalling and implant integration can be answered by systematic investigations using model materials. Only then we will be in a more informed position to design new materials for use in the body.

Keywords Biomaterials · Cell–surface interactions · Protein–surface interactions · Topography · Implantation · Physicochemical

Introduction

This review highlights the importance/development of physicochemical interactions between living tissue and a biomaterial upon implantation. Of utmost importance are the length scales of the species involved and the time for specific interactions to occur. The bioactive nature of a material describes its positive effect or interaction with living tissue. A more precise definition for a specific material may include terms such as osteoconduction or osteopromotion, with the latter describing the directed growth of bone due to the presence of a specific material. Recent advances in the study of bioactive and biodegradable poly(α -hydroxyacids) composite materials are presented to illustrate the occurrence of macroscopic interactions between materials and living tissue. Then, the latest developments in the understanding of surface chemistry and topography at both the micron and nanoscopic scale on cell and extracellular protein matrix interactions are reported.

The bulk of a biomaterial presents physical and chemical properties of the material that remain during the lifetime of the implant. They can be altered to allow the biomaterial to mimic the physicochemical properties of tissues which they are meant to augment or replace. Advances in materials design have resulted in the development of products such as degradable sutures that naturally decompose after fulfilling their function. Importantly, the specific nature of a biomaterial surface both chemically and physically determines how the living host tissue and/or organism interacts with the implant. On a linear time scale, in the early stages of implantation (nanoseconds to minutes) protein adsorption takes place, which is affected by the chemical nature of the adsorption environment and nanoscale topographical features. Surface-bound proteins

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may then mediate cell attachment during the next stage of implant assimilation (hours to days) if they have the correct orientation and conformation to interact with cell receptors. In this way, the effects of surface chemistry are conveyed through the adsorbed protein layer and macro-scale topographical features can also effect cell–surface interactions.

We highlight in this review some of the fundamental principles which impact on the design of modern biomaterials. These have been categorised as those relating to bulk material properties, to cell–surface interactions and to protein–surface interactions. The field of biomaterials is a major developing area that will generate new and improved materials for use in the body.

Material–host interactions

The breadth of material types elaborated for biomedical applications reflects the increasing need, but also the difficulty of the field, namely the control of the interface between material and living host [1]. In the early days, materials assessed by trial and error experimentation were chosen to minimise perturbation. However, materials are never inert. Biocompatibility or the clinical success of a biomaterial is directly dependent upon the response of the host tissue to the perturbation brought about by the foreign material [2]. Biocompatibility is much dependent on the site of implantation, the function and size of the implant and the duration of implantation with a key issue being the time-scale required for material–host tissue interactions to become established [3].

The initial response to a material surface being placed in a biological milieu is for water molecules to reach the surface and create a water shell around the material on a time scale that is of the order of nanoseconds. This stage would be redundant if the material was primed for use in an appropriate medium prior to implantation. The extent and specific pattern of interaction of the water molecules with the surface is dependent on the surface properties of the material. This property also determines which proteins and other molecules will adhere following the formation of the hydration shell. In the second stage, from seconds to hours after implantation, the material becomes covered in an adsorbed layer of proteins initially present in the extracellular matrix. In the third stage, cells eventually reach the ‘surface’ interacting through the protein covering, thus cell–surface interactions can be described as the interaction between cells and surface-bound proteins. This stage occurs from as early as minutes after and up to days after implantation. As the time of material implantation increases from a few hours to several days, adhesion, migration and differentiation of cells occurs. This third stage is influenced by biological molecules (extracellular matrix proteins, cell membrane proteins and cytoskeleton

proteins), the biophysical environment and the evolving materials physicochemical characteristics at the surface (chemistry, nano and micro-topographies), the released soluble products from the material and its micro-structure (porosity) [4, 5]. The final stage in the useful life of the implant, which can last from a few days (biodegradable suture) or up to several decades (total hip replacement) is the continuing development of the early implant stages. Adverse responses (clots, fibrous capsule formation, etc.) and device failure can occur—processes that can be promoted by material degradation or mineralisation. The future development of improved and new biomaterials looks towards minimising such effects, promoting rapid and controlled healing and implant integration. The long-term success of a material in the body depends on the controlled macro-functional properties (mechanical as well as a match of tissues at the site of implantation) and the physicochemical properties of the material on the micron and nano-scale.

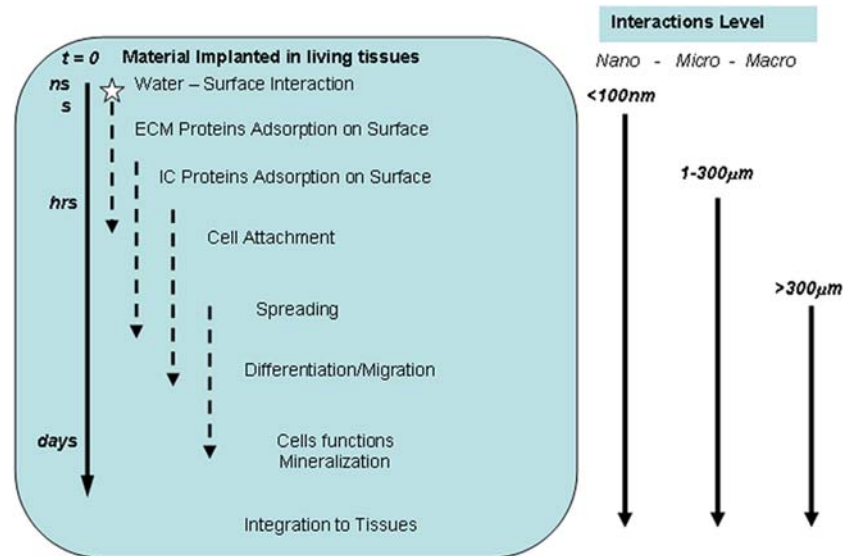
The time and size scales of interactions between materials and a mineralised tissue are shown schematically in Fig. 1. A biomaterials’ influence on living tissues and its interactions with cell functions is often studied using *in vitro* experiments designed to mimic *in vivo* experiments but in a simplified and more readily controllable manner [6]. Biocompatibility is thereby assessed *in vitro* by the observation of viability and bio-functionality of cells on a materials surface. Bioactivity or osseointegration experiments are usually performed *in vitro* by determining the apatite forming ability of a material soaked in simulated body fluid [7–9], which allows the rapid screening of materials for further development and optimisation for use in biomedical devices.

The focus of this review is on recent *in vitro* studies; bioactivity, cell and protein interactions with an emphasis on recent experiments performed on silica sol-gel and bio-inspired biodegradable poly(α -hydroxyacids)-silica composites designed for biomedical applications in contact with bone. Future studies and methodology are also considered.

Materials for medical applications

The improvements of biomaterials for use as hip replacements and scaffolds for tissue engineering have been made through the development and association of new alloys, ceramics and polymers, which can mimic the physical properties of tissues surrounding the implanted site [10, 11]. Now-a-days, a hip joint implant is always a carefully designed composite material comprising for example a femoral titanium core with high tensile strength and modulus, a femoral head made of a high strength and wear

Fig. 1 An example of biomaterial-tissue interactions, e.g., Implant–Bone interactions



resistant ceramic such as zirconia, an inorganic coating on the stem and a polyethylene acetabular cup with high strength and stiffness. All these attributes provide enhanced interface cohesion between the implant and the surrounding living tissue and decrease the risk of failure due to osteopenia via the stress shielding effect [12].

The latest generation of biomaterials are being developed to elicit specific biological responses from the host [4, 13]. Such materials are so-called bioactive materials [14]. Examples are the bioactive ceramics made by the sol-gel process that can trigger osseointegration and bone production due to their surface properties and the release of soluble degradation product (i.e., silica) thereby stimulating osteogenesis [15, 16]. Cell-material interactions are also controlled by the pore size and interconnections in the ceramic phase and by optimisation of the surface micro-roughness in terms of amplitude and organisation for the guidance and growth of cells and the circulation of nutrients [17–19]. As examples, porous bioactive sol-gel glass foams and poly-D,L-lactic acid filled with bioglass® have been prepared with macro pores of 100 µm diameter and above, that is considered the minimum spatial requirement for cell migration and transport [20–23]. In the latest advances, a favourable biological response can also be achieved with the delivery of bioactive (osteogenic) compounds such as bone morphogenic protein (BMP) growth factors entrapped in a porous ceramic scaffold or coated on a materials surface [24, 25].

Bio-inspired composites and the bone analogy

Bio-inspired materials combining the properties of a polymer and an inorganic phase are of great interest to

scientists working in biomedical science [26]. In vertebrate compact bone, the association of collagen fibres and calcium phosphate in a continuously renewing biological environment offers a multi-scale structured scaffold that can withstand acute mechanical forces and yet is flexible, Table 1 [27, 32–34]. The material also acts as an ion reservoir and has the ability to regenerate and heal itself if damaged during the individuals' life time [32]. Ultimately, all these properties should be found in biomaterials intended for hard tissue implantation.

Bio-mimetic materials made of collagen and calcium phosphate have been extensively studied [29, 35] as collagen is an ideal support for the regeneration of living tissue [36]. Addition of calcium phosphate based ceramics or glasses improves the mechanical properties of the collagen scaffold alone and increases the bioactivity of the material by forming a strongly bonded interface [37, 38]. However, the reproduction of the mechanical properties and the intricate structured organisation of bone have not yet been attained. Moreover, the high cost of pure type I collagen and the risk associated with the major source of extracted collagen (bovine spongiform encephalopathy) restrict its applications [36].

An approach to generating an analogue of collagen-calcium phosphate materials involves the use of synthetic biodegradable polymers such as poly(α -hydroxyacids) and bioactive inorganic fillers to produce materials that may be useful for hard tissue replacement devices and as scaffolds for tissue engineering [39]. Extensive knowledge has been gained in the last decade on the behaviour of these composites, however improvements are still required in understanding the physicochemical properties of the material as shown by the following example [40–43].

Table 1 Mechanical properties of natural and synthetic single phase and composite materials

Hard tissue	Young's Modulus, GPa	Tensile strength (at max. load), MPa	Ref.
Cortical bone	3–30	80–150	[27]
Cancellous bone	0.1–0.4	5–10	[27]
Collagen/CaP	2.82	50	[28]
PLLA	3.5–10	40–150	[29]
PCL M_w 10,000 g mol ⁻¹	0.11	10	[30]
PCL/SiO ₂ sol-gel M_w ~10,000 g mol ⁻¹	0.48	8.1	[31]
PCL/SiO ₂ sol-gel	0.1–0.51	20.8	[30]

Poly(α -hydroxyacid)-silica sol-gel composites. A useful model for understanding physicochemical-in vitro bioactivity relations

Material preparations and physico-chemical properties

Of particular interest as a model study of biodegradable and osteopductive/conductive biomaterial-living tissue interactions are poly(α -hydroxyacids)-silica sol-gel hybrids. Poly(α -hydroxyacids) polymers such as poly(ϵ -caprolactone) and poly(L-lactic acid), poly(glycolic acid) and co-polymers are well known biodegradable polymers that can be tailored and processed to control their degradation behaviour and physical properties, Table 1 [44–46]. However, they lack the bioactivity (osteoconduction) properties that would make them outstanding materials when implanted in a hard tissue. This can be achieved by the incorporation of a bioactive inorganic phase by blending, infiltration and more advantageously by using the sol-gel method whereby polymerisation of an inorganic precursor in the presence of the polymer is used to generate a composite material [47–49]. An interpenetrated hybrid network stabilised by hydrogen bonding interactions between the phases can be obtained by use of hydroxyl terminated poly(α -hydroxyacids) [49]. Triethoxysilane end capped poly(α -hydroxyacids) or coupling agents (e.g., hexamethyldiisocyanate) can be used to form covalent bonds between silica and the organic phases [31, 49]. These different types of hybrid sol-gel materials have been prepared to investigate the relationship (if any) between physico-chemical properties of the materials themselves and in vitro bioactivity.

Physico-chemical properties

The physico-chemical properties of poly(α -hydroxyacid)-silica sol-gels such as mechanical and degradation properties are strongly affected by modifying the poly(α -hydroxyacid) to silica weight ratio, the polymer molecular weight and the materials processing parameters. Thermal analysis, transmission electron microscopy and X-ray

analysis (SAXS, XRD) have been used in conjunction with experimental design approaches to describe the effect of these parameters on composite structure and consequently on the properties of the biomaterials formed, i.e., mechanical properties, porosity, degradation rate and surface chemistry. The effect of varying the level of silica, water, catalyst, solvent and polymer molecular weight on the co-continuous organic and inorganic phase domains and polymer crystallinity have been reported [49–51]. For example, increasing the silica to polymer ratio in poly(ϵ -caprolactone)/silica hybrid sol-gel composites decreases the size of the organic domain and the crystallinity of the polymer [49]. The materials mechanical properties are consequently modified, and for another example, in layered poly(ϵ -caprolactone)/silica hybrids, stiffness is improved at low (5%) silica concentration, but further loading leads to a levelling off and then decrease in the desired mechanical properties [52]. The mechanical performance of polymer composites is generally related to the mechanical properties of its counterparts and also the interfacial strength between the phases. Mechanical properties measured using classical and dynamic methods, typical of poly(ϵ -caprolactone)-silica sol-gel materials are reported in Table 1. They compare well with those of natural cancellous bone and can be modified by varying the molecular weight of the poly(α -hydroxyacid) and the silica level [53]. In most conventionally filled polymer systems, the modulus increases linearly with the filler volume fraction, and if the interface between the two components is good, the external load will be transferred from the polymer matrix to the mineral phase through the interface and the mechanical performance of the composite increase. In the case of a co-continuous poly(ϵ -caprolactone)-silica hybrid sol-gel the mechanical behaviour is complex owing to the ‘‘poor’’ organic and inorganic interface caused in part by the presence of Si–OH groups [54]. Highly cross-linked poly(ϵ -caprolactone)-silica hybrids with few unreacted Si–OH should show improved mechanical properties [55]. An important point which has not yet been addressed is the retention of the mechanical properties of such biodegradable composites upon ageing and degradation. A report has

shown limited in vitro degradation with a faster weight loss for a poly(ϵ -caprolactone)-silica composite compared to pure poly(ϵ -caprolactone) [54]. Although the nature of the products released into the medium is not stated, it is thought that the poly(ϵ -caprolactone) degrades first [54]. The fast degradation of the composites compared to the pure polyester is most probably due to the decrease in crystallinity of the poly(ϵ -caprolactone) from its interaction with the silica phase, therefore allowing for a faster diffusion of water and acid degradation products away from the material thus leading to chemical degradation of the polymer [56]. Release of soluble silica from poly(α -hydroxyacids)-silica has been studied as it is important for the osseoprotective property of composites in a similar manner to the silica containing bioactive ceramics and glasses [57–59]. Again, it has been shown that processing parameters including those that affect the condensation of the silica network and the amount of cross-linking influence the rate of soluble silica release into solution [49, 55].

It must be noted that nitrogen adsorption measurements on poly(ϵ -caprolactone)-silica sol-gels indicate that the composites have no meso- or micro-porosity (value of N_2 adsorbed at 0.9 relative pressure between 2.0 and $4.5 \times 10^{-4} \text{ cm}^{-3} \text{ g}^{-1}$), and therefore porosity effects on mechanical and degradative properties of the composite are not known [60, 61]. Porosity could be obtained using a highly cross-linked composite that shows elastomeric-like behaviour when swollen [55, 62]. Finally, the surface chemistry of poly(ϵ -caprolactone)-silica sol-gel composites has not yet been fully characterised. Wettability studies have shown that increasing silica content increases the hydrophilicity of the surface [54]. Increasing the bonds between the organic and inorganic phases has an opposite effect and decreases the hydrophilicity of the material with the consequence that the in vitro bioactivity of the composite is strongly diminished [51, 55].

In vitro bioactivity

Formation of an apatite-like layer, indicating the osteoconductivity of a material occurs not only inside the body but also in vitro, when the materials are soaked in solutions emulating human plasma [8, 63]. In vitro apatite forming ability methods are far from reproducing the phenomena involved in bone formation. However, they can be used as a rapid screening of a materials in vitro bioactivity to determine if a material is worth testing further [7]. In vitro osseointegration or the apatite forming ability of silica gel and poly(α -hydroxyacid)-silica sol-gel composites on their surfaces in simulated body fluid have been assessed [7, 54, 55, 64]. What has been found is that an increase of the sintering temperature delayed the formation of apatite layers on gel surfaces [65]. This was proposed to be due

to the surface chemistry of the sol-gel; with a decrease of Si–OH chemical groups leading to a consequent decrease of nucleation sites for calcium phosphate precipitation. A recent study suggests that the silicate trimer (Si_3O_9) on silica sol-gel surfaces is the active site for the earliest heterogeneous nucleation of calcium phosphate [66]. In the case of the poly(α -hydroxyacid)-silica sol-gel composites, increasing the amount of silica in the composite speeded up the observation of the first Ca and P containing precipitates on a materials surface [64]. Also, Si–OH groups on a materials surface are dependent on the release of soluble silica species from the bulk of the material and the formation of a silica sol-gel like layer on the materials surface. This requirement is supported by the lower bioactivity of composites with a high amount of bonding between the organic and inorganic phases and associated lower soluble silica release [55].

The development of new degradable and bioactive materials such as poly(α -hydroxyacid)-silica sol-gel composites is very complex. Their structure and physicochemical properties must be well understood as in the biological medium, they will succumb to mechanical constraints and degradation with associated changes in mechanical strength, porosity, topography and surface chemistry that occur on precipitation of an apatite layer. Moreover, in a living tissue cells interact with the material and the best physicochemical properties are of little use if the material is not biocompatible. In vivo, the biocompatibility of a material is determined by the host reaction; a sequence of inflammatory and healing reactions to the implantable materials [5]. In vitro, the sequence of events in vivo can be approximated by seeding cells on a materials surface and studying their interactions.

Cell–surface interactions

Any attempt to engineer biomaterials having functional surfaces must make allowance for the highly precocious ability of biological systems to recognise specific features. Surfaces of medical devices are rarely flat at the molecular level, having an imposed topography from the processes followed in the fabrication of the biomaterials used. These topographical features, such as roughness, may or may not be formed intentionally. Micron sized topography has been shown to play an essential role in determining cell adhesion and surface-bound characteristics. Biocompatibility of surfaces is closely related to the response of cells in contact with the surface and in particular their adhesion [67]. Implant coatings or modifications are currently of particular interest, with the aim being to control cell attachment and spreading by means of tailored topography and chemistry.

Cells are complex, self-sustaining units that interact and communicate intracellularly via receptors located on their outer walls. Specific binding of antibodies or antigens to these receptors creates a receptor response, which starts a chain reaction of events within the cell leading to an appropriate trigger response. One such class of cell receptors called ‘integrins’ bind specifically to an arginine-glycine–aspartic acid (RGD) tripeptide found in cell adhesive proteins such as fibronectin, vitronectin and laminin, which in turn can attach to solid surfaces [68, 69]. Integrins are a superfamily of more than 20 transmembrane heterodimers [70] formed by non-covalent association of α and β subunits, where the diameter of the receptor on the cell membrane is between 8 and 12 nm [71].

Cells sense their surroundings using protrusions termed ‘*lamellipodia*’ in epithial cells and fibroblasts, or ‘*pseudopodia*’ in amoebae and neutrophils. These are micron sized sheet-like structures composed of an actin filament mesh which lies parallel to the surface [3] but which can be pushed back across the cell if attachment to the surface is not possible. On the ends of these, smaller hair-like protrusions termed ‘*filopodia*’, composed of long, thin actin filament bundles [72] act as feelers that ‘sense’ the extracellular matrix and substrate surface. When the *filopodia* find a suitable binding site, such as the lock and key integrin binding sites discussed earlier, a feedback signalling pathway within the cell allows more integrin receptors to be localised in that region of the cell. By progressively sending out *lamellipodia* along the leading cell edge focal contacts are made which transmit strong propulsive traction stress allowing the cell to move across a surface [73, 74]. The attachment and separation of the cell from the surface is controlled by an integrin feedback loop, mediating the leading and trailing cell boundary [75].

Cells crawl across a surface allowing them to spread and proliferate. Non-adhesion of cells is fatal; likewise, if cells adhere too tightly to a surface they cannot proliferate and also die. A situation between these extremes is therefore the most desirable situation for healthy, confluent cell cultures.

Surface chemistry

The surface chemistry of an implanted material is important and can be altered to induce cell adhesion and spreading, Table 2. Control of cell signalling by surface chemistry has been directly demonstrated—an extremely important break-through highlighting the importance of protein adhesion control for cell binding [94]. There is great interest in this field with a number of review articles published in recent years [3, 95–98].

Material surfaces can be modified by a variety of different methods, such as the application of a surface chemical gradient, self-assembled films, surface-active bulk additive and surface chemical reaction [99]. Chemical surface modifications can be achieved fairly easily at a number of different levels, where the most common is to make use of chemical self-assembled monolayers (SAMs) which are surface coatings that form highly ordered structures on specific substrates [100, 101]. SAMs utilise thiols that are arranged in a close packed array on a gold surface [83, 102, 103], similarly behaviour is observed with silanes on a silica substrate [99]. By changing the terminal group on the SAM layer any functional group can be investigated [104].

Self-assembled monolayers with different functional end groups have been used to study how different cell lines interact with a range of chemically functionalised surfaces. The surface chemistry of these materials modulate focal adhesion composition and signalling of cells [105] and hence can control the phenotype and function of a cell [79] whereby the level of adhesion influences cell proliferation and differentiation [80] and modulates other cell signalling pathways, Table 2 [106–108]. The substrate can also influence fibrillar adhesions from the cell due to its surface properties and composition [109]. Surfaces have been modified with various functional groups such as methyl-, hydroxyl, amino- and carboxyl-, all of which can be found on natural biological surfaces. The methyl- and hydroxyl-surfaces represent neutral hydrophobic and hydrophilic functionalities, respectively, whilst amino- and carboxyl-surfaces exhibit negatively and positively charged characteristics, respectively. The differing responses of cell lines to differing surface chemistry is thought to be due to the changes induced on the pre-adsorbed protein layer which mediates cell attachment and will be discussed below. Different cell types as well as the same cells of different phenotypes bind to different domains of the integrin presented by the surface-bound ECM proteins [110].

An extension of such chemical modification allows part of the SAM to be selectively replaced by a second molecule having a different functionality, permitting the surface to be chemically patterned, Fig. 2 [93]. The chemical patterning of surfaces allows cell movement and growth to be controlled. Hydrophobic regions hinder cell attachment and spreading but neighbouring hydrophilic regions allow cells to attach and spread [83, 111–115].

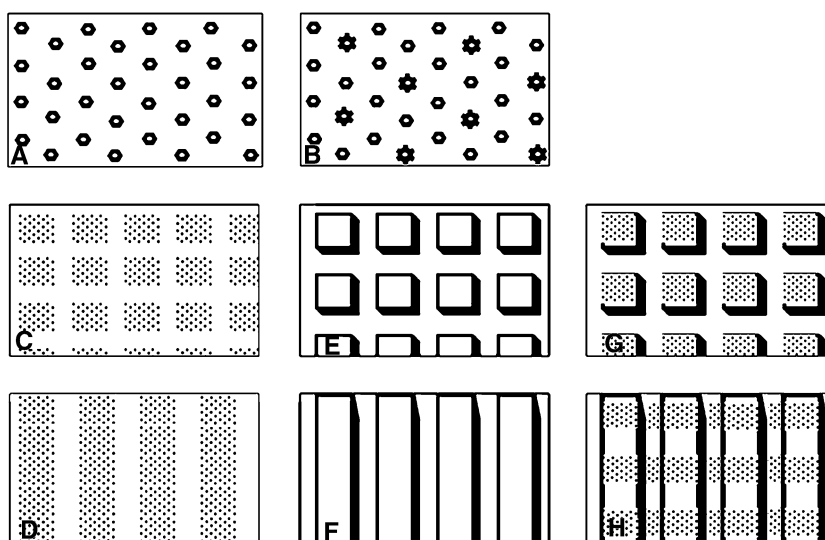
Progressively, more complex surfaces are being explored with amino acids being bound to surfaces. By using a range of amino acids the effects of surface charge and wettability have been examined with respect to the motility of osteoblasts. A correlation between increased cell spreading and an increase in surface wettability have been observed [89].

Table 2 Surface chemistry: functional groups giving varied cell responses

Functional group	Cells studied	Type of study
CH ₃ ^{a,b}	Endothelial cells	Adhesion [76]
	Human fibroblast cells	Adhesion [77, 78]
	Mesenchymal stem cells	Adhesion, proliferation, mRNA expression [79]
	Erythroleukemia cells	Attachment [80]
CH ₃ ^{a,c}	Myoblast cells	Proliferation and differentiation [80]
	Neutrophile cells	Adhesion [81]
	Mammalian endothelial cells	Adhesion [82]
	Osteoblasts	Adhesion, mineralisation [83–85]
OH ^{a,d}	Endothelial cells:	Adhesion [76]
	Human fibroblast cells	Adhesion [77]
	Mesenchymal stem cells	Adhesion, proliferation, mRNA expression [79]
	Erythroleukemia cells	Attachment [86]
OH ^{a,e}	Myoblast cells	Proliferation and differentiation [80]
	Mammalian endothelial cells	Adhesion [82]
	Osteoblasts	Adhesion, mineralisation [84, 85]
CH ₂ =CH(CH ₂) ₉ OH NH ₂ ^{a,f}	Neutrophiles	Adhesion [81]
	Endothelial cells	Adhesion [87]
	Human fibroblast cells	Adhesion [77]
	Erythroleukemia cells	Attachment [86]
	Mesenchymal stem cells	Adhesion, proliferation, mRNA expression [79]
	Myoblasts	Proliferation and differentiation [80]
	Osteoblasts	Adhesion, mineralisation [84, 85]
NH ₂ ^{a,g}	Myoblasts	Proliferation and differentiation [80]
	Osteoblasts	Adhesion, mineralisation [84, 85]
COOH ^{a,h}	Human fibroblasts	Adhesion [77, 78]
	Mesenchymal stem cells	Adhesion, proliferation, mRNA expression [79]
	Erythroleukemia cells	Attachment [86]
COOH ^{a,i}	Myoblasts	Proliferation and differentiation [80]
	Mammalian endothelial cells	Adhesion [82]
	Osteoblasts	Adhesion, mineralisation [83–85]
COOH ^{a,j} [COCH ₃ , CO ₂ CH ₃ , CONH ₂ , CO ₂ NH ₂ , CN, PEG ^k , CF ₃ , F, Br, Cl, SH, CH=CH ₂] ^a	Fibroblasts	Adhesion [88]
	Endothelial cells	Adhesion [76,87]
	Human fibroblasts	Adhesion [77]
	Mesenchymal stem cells	Adhesion, proliferation, mRNA expression [79]
	Mammalian endothelial cells	Adhesion [82]
Phosphorylcholine	Neutrophiles	Adhesion [81]
Amino acids	Osteoblasts	Spreading [89]
Polypeptide		
RADS	Human epidermoid carcinoma cells	Adhesion and spreading [90]
RGDS, RDGS	Rat calvarial osteoblasts	Calcification and mRNA expression of ECM proteins [91]
RGD	Osteoblasts and melanocytes, ^l Fibroblast ^m	Spreading and morphology and cytoskeletal organisation [92]
		Spreading and morphology and cytoskeletal organisation [80]
RGDG ₁₃ PHSRN	Rat calviara osteoblast	Adhesion, spreading [93]

^a SAM (Self-Assembled Monolayers), ^bOctadecyldimethylchlorosilane, ^cHS(CH₂)₁₁-CH₃, ^d10-undecenyldimethylchlorosilane and hydroboration, ^eHS(CH₂)₁₂-OH, ^f3-aminopropyldimethyl ethoxysilane and 11-bromoundecyldimethylchlorosilane, azidation and reduction, ^gHS(CH₂)₁₂-NH₂, ^h10-undecenyldimethylchlorosilane and oxidation, ⁱHS(CH₂)₁₀-COOH, ^jPEO and polyacrylic acid films, ^kpolyethylene glycol oligomers, ^lpolystyrene-blockpoly[2-vinylpyridine (HAuCl₄)0.5] and RGDfk peptide grafting, ^mpolyacrylic & polyacrylamide with grafting of RGD on NH₂ groups

Fig. 2 Surface chemical and topographic patterning examples (a) isolated single molecule grafted pattern (i.e., RGD), (b) Isolated molecules grafted pattern (i.e., RGD-PHRSN), (c) island molecules pattern, (d) line molecules pattern, (e) pillar topographic pattern, (f) grooves topographic pattern, (g) and (h) mixed chemical and topographic patterns



A more direct method to influence cell attachment has been to functionalise surfaces directly with the RGD tripeptide, which binds specifically to integrin receptors [116]. This approach has shown that simply the presence of such surface groups affects cell adhesion and also that the spacing between the groups (packing density) is important. As polyethylene glycol (PEG) based materials are well known to be biologically inert and resistant to cell attachment [117], these have been used as base materials to investigate the effect of the spacing of surface-bound peptides in relation to cell adhesion and metabolic activity. Spacings of 58–73 nm have been found to give effective responses for focal adhesion whilst larger spacings restrict cell attachment [118], possibly due to insufficient clustering of integrin binding sites [119]. An array with 4 nm spacings between an RGD sequence and its synergistic ligand [PHSRN (Pro-His-Ser-Arg-Asn)] has also been shown to induce cell adhesion [93].

Topography

For many years, it has been recognised that cell attachment and growth on a surface can be guided by micron sized features. However, it is unknown at present if cells actually perceive nano-sized topographical effects directly or only act on them as a consequence of changes imparted onto the pre-adsorbed protein layer. Many studies have been conducted to determine the effect of topography on cell behaviour, by investigating how different cell types react to varying substrates [120, 121]. Topography can influence behaviour [122] such as adhesion [123–125], cell morphology [126], migration [127–129], orientation [130], focal adhesion [131] and differentiation [128, 132, 133]. Different types of substratum topography have been shown

to influence cell behaviour depending on both scale and feature type, for example micro- and nanometre scale ridges and grooves [134–137] and if there are randomly or evenly distributed features or artefacts such as pits or spikes [138–141]. Fibres present in the ECM have sub-micron dimensions and mineral structures found in vivo have nanometre topographical features, both of which impact on cell behaviour. The actual means by which small features affect cells are largely unknown but it is thought that the proteins mediating cell attachment are altered by such surface parameters.

The question of whether cells perceive nano-scale topography has been probed by using an array of chemical and nanometre sized topographical surface modifications, the height of which have been systematically varied [142]. Chemical patterning gives rise to a small topographical alteration due to the differing height not only of the adsorbed chemical layer but also due to the adhering protein layer (discussed in more detail below). Cells have been found to align with a chemical ‘cue’ over topographical grooves less than 500 nm deep, but for deeper grooves (e.g., 5 μm) the vast majority of cells (80%) were found to orient themselves irrespective of the surface chemistry. It is relatively well established that features of diameter 10–100 μm typically get a positive response from cells [100]. However, not all cell types respond to surface topography in the same fashion. For example, PLGA surfaces with modified micro and nano topographies can inhibit the proliferation and migration of epithelial cells, and yet are found to promote proliferation and the directional migration of osteoblasts [143].

Tissue engineering has developed an understanding of how cells react differently when surrounded by a matrix rather than observing only a pseudo 2-dimensional substratum as under typical culture conditions [144]. In vitro

and *in vivo*, cells interact differently within a 2D and a 3D scaffold due to the possibility in the latter for circulation of ECM proteins. Also cell–cell interactions and vascularisation are strongly dependent on porosity. Consequently the determination of optimum implant porosity is not straightforward, especially since it has been demonstrated that depending on the materials used and the interconnectivity of pores present, a range of behaviours can be observed for different cell types. This effect has been connected with changes of porosity, which affect the internal curvature of the pores, i.e., the topography [17]. Comparison of porous materials (hydroxyapatite ceramic rods) with identical macropore size but different surface micro-roughness has shown that micro-topography enhances cell adhesion [145].

Cell adhesion relating to protein adsorption

In a physiological environment cell adhesion always follows protein adsorption. Although cell–surface interactions are understood to be affected by underlying surface chemistry, structural information on surface-bound protein conformations and geometries and their effect on cell adhesion are yet to be elucidated. There are several proposed mechanisms by which cells are thought to ‘observe’ surface characteristics; cells may reach the surface by protrusions through the protein layer or by consuming pre-adsorbed proteins [99]. This may be possible in the later stages of cell adhesion, although it is more likely that adsorbed proteins themselves convey the underlying chemistry through their specific geometry and conformation [84].

Proteins adsorb in differing quantities, densities, conformations, and orientations depending on the chemical and physical characteristics of the surface [146–150]. Although surface–protein interactions are not well understood, surface chemistry has been shown to play a fundamental role in protein adsorption [147–149]. Moreover, the properties of protein over-layers can be altered by the underlying surface chemistry of a material and this behaviour has exciting implications for controlled biocompatibility.

Protein adsorption

On implantation a material is conditioned in several stages by biological fluids and components therein [96]. Shortly after the initial hydration layer surrounds the material, blood proteins and other macromolecules (e.g., lipids and sugars) arrive at the surface. Since blood contains many hundreds of different proteins competition for the surface

ensues. After a complex process of adsorption, desorption and surface rearrangement, wherein the protein layer composition changes dramatically over time, equilibrium is reached at the interface. It is generally accepted that the more abundant small proteins will adsorb first due to their rapid transport to the surface. Over time these are then replaced by larger proteins with a greater affinity towards the surface. The ‘*surface enrichment*’ of a protein from the ECM such as fibrinogen was first observed by Vroman and Adams and is generally termed the ‘Vroman effect’ [151]. The protein layer may then subsequently mediate cell attachment and progressively the material is integrated into the biological system [5].

Fundamentally, biomaterial responses are governed by the interaction of protein molecules on surfaces, involving both binding in the initial stage and subsequent unfolding. Residues pointing outwards into solution are available for surface interaction whereas those in the core of the protein are not. Unfolding, or denaturing of the protein would allow the internal amino acid residues to become accessible to the external environment thereby making them available to take part in external interactions. Protein deformation may be induced by interaction with a surface and is affected by several factors including electrostatic forces and entropic effects, hydrophobic interactions and conformational changes [152]. Dehydration of hydrophobic regions both on the substratum and on the external protein surface is favourable, which may cause the protein to deform to move its hydrophobic sections away from the aqueous environment. Bonding between adsorbed neighbouring protein molecules can allow hydrophobic regions to remain shielded from the aqueous phase, due to the increase in flexibility of the polypeptide backbone brought about by loss of secondary structure. Van der Waals interactions, electrostatic interactions and hydrogen bonds can also form between proteins, provided they are enthalpically favourable.

Model surfaces with varying functionalities have been used to assess protein adsorption rates and conformational stability upon adsorption. Proteins adsorb rapidly and become deformed to a greater extent on surfaces with decreasing wettability, minimising hydrophobic contact with the aqueous phase as described above [149]. Likewise charged protein regions can interact with oppositely charged surfaces [153], although electrostatic effects are much weaker than hydrophobic effects when dealing with proteins adsorbing from an aqueous phase, due to complications arising from the charges being shielded by water molecules [154] and small ions [155, 156].

Protein–surface interactions are very important factors when considering the adsorbed protein state. Initially protein molecules will adsorb giving the kinetic adsorption product, however, if the interaction is very high a

deformation of the protein to afford an energetically more favourable thermodynamic adsorption product occurs. This process, sometimes termed '*relaxation*' occurs either directly on adsorption or some time thereafter, probably involving the protein spreading to increase its interaction with the surface and/or to decrease its interaction with the aqueous phase. Similarly, differing protein–surface interactions could force protein molecules to adopt specific orientations. As a result the protein may lose the specific structure required for activity, or functional sites may become obscured due to conformational/orientational rearrangements that hinder protein function.

A large number of investigations examining bound proteins have used techniques that give limited information: fluorescence [157, 158], atomic force microscopy [159–161], mass spectrometry [162], zeta potential [163], nuclear magnetic resonance (NMR) [164], ellipsometry [165, 166], circular dichroism [167] and antibody or platelet binding [168, 169]. Most of the analytical tools used give results where generalised information on the adsorbed protein is obtained or conformational changes are implied but have not been measured or quantified. Examination of the ease of protein removal from surfaces has been suggested by authors to provide evidence of protein structural change, although such studies only show that conformational and/or orientation change could be occurring [170].

Surface chemistry

It has been shown that protein adsorption characteristics can be controlled by changing substrate surface parameters [150, 171–173]. Surface chemistry plays an important role in determining adsorbed protein conformation as well as the rates of adsorption and the amount of protein adsorbed through interaction between the functional groups on the substratum and those of the protein itself. The chemical nature of the surface can be modified to induce greater protein–surface affinity by either electrostatic or hydrophobic interactions. The outer shell of adsorbing proteins is therefore an important factor in determining how proteins bind, but generally hydrophobic bonding is a major contributor and induces rapid adsorption compared to that observed on hydrophilic surfaces [174, 175].

Although it is well known that the extent of protein adhesion can be directed by surface chemical patterning, Fig. 3, the degree of control over the conformation and orientation of adsorbed proteins is not well detailed. It has been suggested that protein–surface interactions induce deformation of an adsorbing protein molecule but many of the techniques used to examine such ultrathin layers of adsorbed proteins only imply conformational/orientational

change rather than providing an absolute measure of protein structure.

One of the main concerns of using such chemically patterned surfaces (as described earlier) is that the boundaries of each chemical area may in fact impose an additional topographical effect. Since protein molecules are likely to adsorb preferentially onto only one of the chemical areas the height of the protein layer will consequently confer a defined topographical edge. These layers may only be on the order of nanometres but at present it is unknown whether adhering cells respond to the defined chemical edge (possibly inferred from the conformation/orientation of the protein molecules) or to this topographical edge [175].

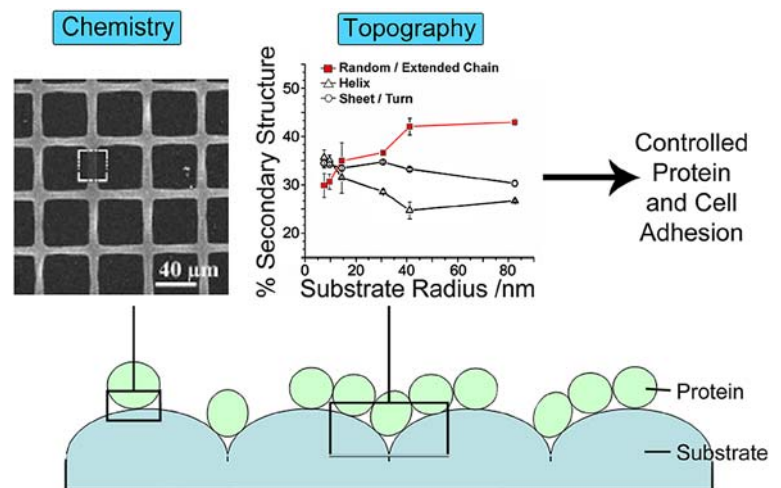
Topography

Micron-sized features are far too large for individual protein molecules to observe, however, if surface features are produced on the same length scale as the protein molecules themselves then the architecture of the surface may be used to manipulate protein shape and form upon adsorption. Can protein molecules mould around surface curvature? Can packing density and/or arrangement be controlled by surface features? These questions are a topic of much interest at present as the answers may allow one to predict or even control the structure and therefore the activity of surface-bound proteins.

Topography on the order of nanometres (up to a few hundred) has been shown, to some extent, to impact on the conformation of bound proteins as experiments using the surface curvature of varying sized colloidal substrates has shown [175–177]. These topographical changes have been found to alter the activity of adsorbed protein molecules [177, 178], a finding which supports a change in protein conformation having occurred. The investigation of topographical effects is somewhat sparse at present, with only a small number of reports covering a narrow size range of surface features.

Conformational assessment of surface-bound proteins has shown that lysozyme and human carbonic anhydrase, two small globular proteins, display native-like secondary structures when bound to spherical substrates with radii <15–20 nm but exhibit a loss of ordered structure upon binding to larger substrates [176–178]. A detailed structural investigation has shown that the globular protein albumin retains native-like conformation upon binding to <15 nm particles although is denatured to a larger extent when adsorbed onto larger particles [177]. In contrast adsorption of fibrinogen onto particles of different radius followed an opposite trend indicating that protein shape influences its interaction and therefore the proteins adsorbed conformation and orientation [146, 177].

Fig. 3 Schematic of protein–surface interactions: Chemistry—adsorption onto biotinylated stripes which appear white, whilst adsorption is hindered on square oligo-ethylene-glycol regions, the white box shows an intentionally bleached area [reprinted from 176 with permission from RSC] Topography—albumin adsorption onto hydrophilic silica spheres of varying dimensions as a model of surface curvature



The extent to which surface chemistry and topography contributes to the control of conformation and, by inference, the activity of surface-bound proteins is not fully understood. Detailed investigations must be conducted examining a series of proteins interacting with a range of surfaces having defined chemistry and nano-topography. Adsorption characteristics and surface-bound conformations can then be assessed eventually leading to predictive modelling of protein–surface interactions enabling the design of advanced functional materials.

Conclusion: evolving material surfaces

The field of biomaterials is becoming increasingly complex from both materials science and surface science directions. One must have an appreciation for the intricacy of the interface between the living tissue and a biomaterial, with interactions occurring on the nano, micro and macro length scales. Medical implant design is now involved with not only the bulk physicochemical properties of the material used that dictate its mechanical and physical properties, but also with surface coatings having defined chemistry and topography in order to direct a desired cellular response, Fig. 4. The site of implantation and the application of the biomaterial dictate the appropriate response which can include rapid healing, hard tissue replacement, or conversely be biologically inert. New biomaterials are also under investigation for in vitro applications such as tissue engineering or controlled growth of cells that are particularly resistant to cell culture [179, 180].

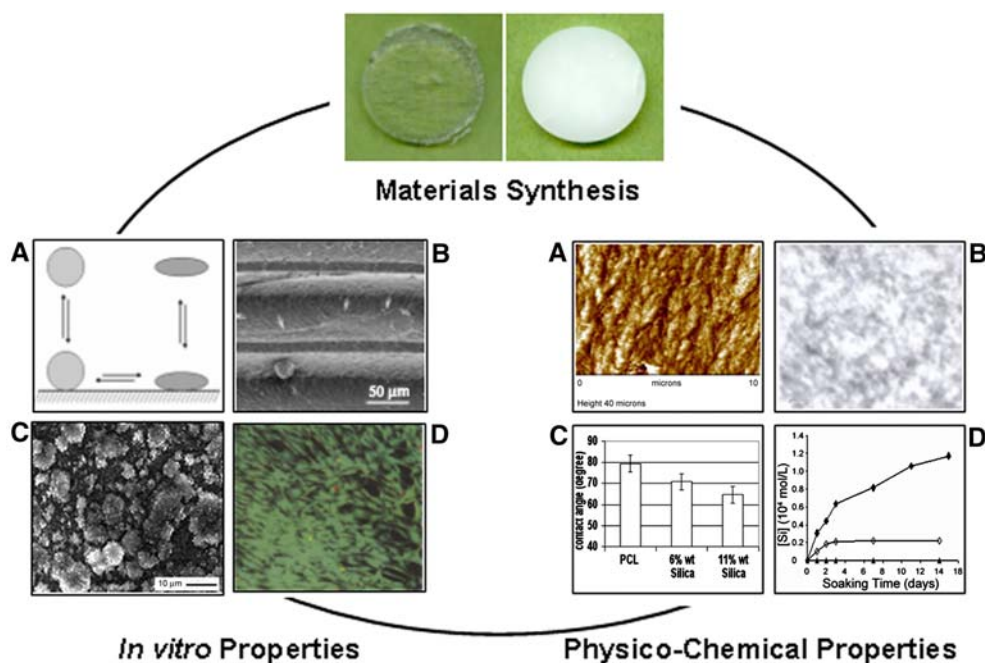
From the very first contact with the extra cellular matrix upon implantation and throughout the entire lifespan of a biomedical device, the material and the surrounding biological host evolve together according to their interactions

and therefore, materials should be developed for use in specific applications. However, in all living tissues the primary interactions between the extra cellular matrix and a biomaterials surface condition the future stages; hydration, protein adsorption, cell attachment, spreading, proliferation, etc. Consequently the effect of surface chemistry and nano-topography on the adsorption of extra cellular matrix proteins is of paramount importance to the development of the next generation of bioactive biomaterials [181]. By first understanding these fundamental factors we may then be able to control the implant interface and design materials to a predetermined specification.

Figure 4 presents a general approach for the understanding and optimisation of materials–biological tissue interactions. Understanding and correlating the effect of materials processing with the physicochemical and in vitro properties of the materials so formed should permit the development of more efficient biomedical devices, fit for purpose.

Host–implant material interactions begin nanoseconds after first contact and from then on are in a state of flux due to protein adsorption, cell adhesion, degradation of the material both physically (stress fractures, etc) and chemically (decomposition and dissolution). The development of materials that are compatible with living tissues depends largely on understanding the different scale of interactions that are present and the processes occurring at the material–biological host interface. Even the smallest of length scales (nanometres) and the shortest of timescales (nanoseconds) are of great importance to the development of the implant interface. It is these key factors which must be understood—however, this presents a problem. How can we accurately measure such small interactions and how do we interpret the data if we can obtain it? Also, how useful are in vitro experiments for the development of materials intended for use in vivo?

Fig. 4 Materials synthesis, silica and poly(ϵ -caprolactone)-silica sol-gels; Physicochemical properties, (a) microtopography: AFM micrograph [Reprinted from ref. [179] with permission from Elsevier], (b) bulk structure: TEM micrograph, (c) surface chemistry: wettability data, (d) in vitro properties: release of soluble silica, (a) proteins adhesion, (b) cells attachment [Reprinted from ref. [180] with permission from Elsevier], (c) in vitro bioactivity: calcium phosphate layer & (d) biocompatibility: viability assay



The future for biomaterials is exciting. The many questions concerning the conformational form and control thereof of bound proteins and how this may impact on cell adhesion in the first instance and later on cell signalling can be answered by systematic investigations using model materials. The investigation of useful biomaterials is truly becoming more diverse, bringing together knowledge from a variety of disciplines.

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References

- C. C. PERRY, in "Chemistry of advanced materials: An overview." In *Biomaterials*, edited by L. V. INTERRANTE and M. J. HAMPDEN-SMITH (Wiley-VCH, New York, US, 1998), p. 499
- D. D. ATEH, P. VADGAMA and H. A. NAVSARIA, "Handbook of Nanostructured Biomaterials and Their Applications in Nanobiotechnology," Vol. 1, Chapter 12, *Biocompatibility of Materials*, edited by H. S. Nalwa (American Scientific Publishers, 2005), p. 411
- K. ANSELME, *Biomaterials* **21** (2000) 667
- B. D. RATNER and S. J. BRYANT *Annl. Rev. Biomed. Eng.* **6** (2004) 41
- H. A. CURRIE, S. V. PATWARDHAN, C. C. PERRY, P. ROACH and N. J. SHIRTCLIFFE, "Natural and artificial hybrid biomaterials." In: *Hybrid Materials—Synthesis, Characterisation and Applications*, edited by G. KICKELBICK (Wiley-VCH, Weinheim, 2007), Chapter 7, pp. 255–300
- C. J. KIRKPATRICK, K. PETERS, M. I. HERMANN, F. BITTINGER, V. KRUMP-KONVALINKOVA, S. FUCHS and R. E. UNGER, *ITBM-RBM* **26** (2005) 192
- D. EGLIN, S. ALI, C. C. PERRY, *J. Biomed. Mater. Res.* **69** (2004) 718
- T. KOKUBO, H. KUSHITANI, S. SAKKA, T. KITSUGI and T. YAMAMURO, *J. Biomed. Mater. Res.* **27** (1990) 721
- K. TSURU, M. KOKUBO, S. HAYAKAWA, C. OHTSUKI and A. OSAKA, *J. Ceram. Soc. Jpn* **109** (2001) 412
- K. S. KATTI, *Colloids Surfaces B: Biointerfaces* **39** (2004) 133
- K. J. L. BURG, S. PORTER and J. F. KELLAM, *Biomaterials* **21** (2000) 2347
- A. J. TONINO, C. L. DAVIDSON, P. J. KLOPPER and L. A. LINCLAU, *J. Bone and Joint Surgery* **58** (1976) 107
- L. L. HENCH and J. M. POLAK, *Science* **295** (2002) 1014
- J. A. HUBBELL, *Current Opin. Biotech.* **10** (1999) 123
- T. KOKUBO, Eds. DUCHEYNE, T. KOKUBO and VAN BLITTERSWIJK, *Pub. Read Healthcare Commun.* (1992) 31
- I. D. XYNOS, A. J. EDGAR, L. D. K. BUTTERY, L. L. HENCH and J. M. POLAK, *J. Biomed. Mat. Res.* **55** (2001) 151
- V. KARAGEORGIO and D. KAPLAN, *Biomaterials* **26** (2005) 5474
- U. MEYER, A. BUCHTER, H. P. WIESMANN, U. Joos and D. B. JONES *Euro Cells Mater.* **5** (2005) 39
- M. J. DALBY, L. DI SILVIO, G. W. DAVIES and W. BONFIELD, *J. Mat. Sci. Mater. Med.* **12** (2000) 805
- S. F. HULBERT, F. A. YOUNG, R. S. MATHEWS, J. J. KLAWITTER, C. D. TALBERT and F.H. HULBERT, *J. Biomed. Mat. Res.* **4** (1970) 433
- J. R. JONES, L. M. EHRENFRIED and L. L. HENCH, *Biomaterials* **27** (2006) 964
- J. J. BLAKER, V. MAQUET, R. JEROME, A. R. BOCCACCINI and S. N. NAZHAT, *Acta Biomaterialia* **1** (2005) 643
- S. VERRIER, J. J. BLAKER, V. MAQUET, L. L. HENCH, A. R. BOCCACCINI, *Biomaterials* **25** (2004) 3031
- Y. KUBOKI, H. TAKITA, D. KOBAYASHI, E. TSUGURA, M. INOUE, M. MURUTA, N. NAGAI and Y. DOHI, *J. Biomed. Mater. Res.* **39** (1998) 190
- Y. LIU, K. DEGROOT and E. B. HUNZIKER, *Bone* **36** (2005) 745
- C. SANCHEZ, H. ARRIBART and M. M. GIRAUD GUILLE, *Nat. Mater.* **4** (2005) 277
- S. WEINER and D. H. WAGNER, *Annl. Rev. Mater. Sci.* **28** (1998) 271

28. M. KIKUCHI, T. IKOMA, S. ITOH, H. N. MATSUMOTO, Y. KOYAMA, K. TAKAKUDA, K. SHINOMIYA and J. TANAKA, *Composit. Sci. Technol.* **64** (2004) 819
29. P. TORMALA, T. POHJONEN and P. ROKKANEN, *Proc. Inst. Mech. Engrs.* **212** (1998) 101
30. I. ENGELBERG and J. KOHN, *Biomaterials* **12** (1991) 292
31. D. EGLIN, Thesis, Nottingham Trent Univeristy (2002)
32. J. D. CURRY, *The Mechanical Adaptation of Bones* (University Press Princeton, US, 1984)
33. M.-M. GIRAUD GUILLE, L. BESSEAU and R. MARTIN, *J. Biomech.* **361** (2003) 1571
34. M. J. GLIMCHER, *Metabolic Bone Disease and Related Disorders*, edited by L.V. AVIOLI and S. M. KRANE (Academic press, London, UK, 1998), p. 23
35. A. C. LAWSON and J. T. CZERNUSZKA, *Proc. Inst. Mech. Engrs.* **212** (1998) 413
36. C. H. LEE, A. SINGLA and Y. LEE, *Intl. J. Pharm.* **221** (2001) 1
37. M. VALLET-REGÍ and J. M. GONZÁLEZ-CALBET, *Progress Solid State Chem.* **32** (2004) 1
38. K. de GROOT, in *Bioceramics of Calcium-Phosphate*, edited by K. de GROOT (CRC Press, Boca Raton, 1983) p. 99
39. J. YAO, S. RADIN, P. S. LEBOY and P. DUCHEYNE, *Biomaterials* **26** (2005) 1935
40. J. F. MANO, R. A. SOUSA, L. F. BOESEL, N. M. NEVES and R. L. REIS, *Composit. Sci. Technol.* **64** (2004) 789
41. P. FABBRI, B. SINGH, Y. LETERRIER, J. A. E. MANSON, M. MESSORI and F. PILATI, *Surface Coat. Technol.*, online November (2005)
42. S. RAMAKRISHNA, J. MAYER, E. WINTERMANTEL, K. W. LEONG, *Composit. Sci. Technol.* **61** (2001) 1189
43. A. G. STAMBOULIS, A. R. BOCCACCINI and L. L. HENCH, *Adv. Eng. Mater.* **4** (2002) 105
44. I. ENGELBERG and J. KOHN, *Biomaterials* **12** (1991) 292
45. A.-C. ALBERTSSON and I. K. VARMA, *Biomacromolecules* **4** (2003) 1466
46. S. A. M. ALI, S. P. ZHONG, P. J. DOHERTY and D. F. WILLIAMS, *Biomaterials* **14** (1993) 648
47. K. YOSHINAGA, K. KONDO and A. KONDO, *Colloid Polym. Sci.* **275** (1997) 220
48. M. AHOLA, J. RICH, P. KORTESUO, J. KIESVAARA, J. SEPPALA and A. YLI-URPO, *Intl. J. Pharm.* **181** (1999) 181
49. D. TIAN, P. H. DUBOIS and R. JEROME, *Polymer* **37** (1996) 3983
50. D. TIAN, S. BLACHER and R. JEROME, *Polymer* **40** (1999) 951
51. D. TIAN P. H. DUBOIS and R. JEROME, *Polym. Chem.* **35** (1997) 2295
52. B. LEPOITTEVIN, M. DEVALCKENAERE, M. ALEXANDRE, N. PANTOUSTIER, D. KUBIES, C. CALBERG, R. JEROME and P. DUBOIS, *Polymer* **43** (2002) 4017
53. D. TIAN, S. BLACHER, Ph. DUBOIS and R. JÉRÔME, *Polymer* **39** (1998) 855
54. S.-H. RHEE, *Biomaterials* **24** (2003) 1721
55. D. EGLIN, S. A. ALI and C. C. PERRY, *J. Bioactive Biocompat. Polym.* **20** (2005) 437
56. C. ELDSÄTER, B. ERLANDSSON, R. RENSTAD, A.-C. ALBERTSSON and S. KARLSSON, *Polymer* **41** (2000) 1297
57. S. I. ANDERSON, S. DOWNES, C. C. PERRY and A. M. CABALLERO, *J. Mater. Sci. Mater. Med.* **9** (1998) 731
58. S. RADIN, S. FALAIZE, M. H. LEE, P. DUCHEYNE and J. KORVENTAUSTA, *Biomaterials* **23** (2002) 3113
59. M. JOKINEN, A. ROSLING, T. PELTOLA and A. YLI-URPO, *Biomaterials* **24** (2003) 5173
60. L. B. WU and J. D. DING, *Biomaterials* **25** (2004) 5821
61. S. I. JEONG, S. H. KIM, Y. H. KIM, Y. JUNG, J. H. KWON, B.-S. KIM and Y. M. LEE, *J. Biomat. Sci. Polymer Ed.* **15** (2004) 645
62. H. R. KRICHELDORF and H. HACHMANN THIESSEN, *Polymer* **46** (2005) 12103
63. A. RÁMILA and M. VALLET-REGÍ, *Biomaterials* **22** (2001) 2301
64. D. EGLIN, S. ALI and C. C. PERRY, *Polym. Int.* **52** (2003) 1807
65. S.-B. CHO, K. NAKANISHI, T. KOKUBO, N. SOGA, Ch. OHTSUKI, T. NAKAMURA, T. KITSUGI and T. YAMAMURO, *J. Am. Ceram. Soc.* **78** (1995) 1769
66. N. SAHAI and M. ANSEAU, *Biomaterials* **26** (2005) 5763
67. J.-P. KAISER and A. BRUININK, *J. Mater. Sci. Mater. Med.* **15** (2004) 429
68. M. C. SIEBERS, P. J. TER BRUGGE, X. F. WALBOOMERS and J. A. JANSEN, *Biomaterials* **27** (2005) 137
69. C. BOKEL and N. H. BROWN, *Develop. Cell* **3** (2002) 311
70. A. Van der FLIER and A. SONNERBERG, *Cell Tissue Res.* **305** (2001) 285
71. J.-P. XIONG, T. STEHLE, R. ZHANG, A. JOACHIMIAK, M. FRECH, S. L. GOODMAN and M. AMIN ARNAOUT, *Science* **296** (2002) 151
72. A. MOGILNER and G. OSTER, *Biophys. J.* **71** (1996) 3030
73. K. A. BENINGO, M. DEMBO, I. KAVERINA, J. V. SMALL and Y. L. WANG, *J. Cell Biol.* **153** (2001) 881
74. B. ZIMMERMAN, M. ARNOLD, J. ULMER, J. BLÜMMEL, A. BESSER, J. P. SPATZ and B. GEIGER, *IEE Proc. Nanobio-technol.* **151** (2004) 62
75. S. LI, J.-L. GUAN and S. CHIEN, *Annu. Rev. Biomed. Eng.* **7** (2005) 105
76. S. MARGEL, E. A. VOGLER, L. FIRMENT, T. WATT S. HAYNIE and D. Y. SOGAH, *J. Biomed. Mater. Res.* **27** (1993) 1463
77. K. B. MCCLARY, T. UGAROVA and D. W. GRAINGER, *J. Biomed. Mater. Res.* **50** (2000) 428
78. N. FAUCHEUX, R. SCHWEISS, K. LUTZOW, C. WERNER and T. GROTH, *Biomaterials* **25** (2004) 2721
79. J. M. CURRAN, R. CHEN and J. A. HUNT, *Biomaterials* **26** (2005) 7057
80. M. A. LAN, C. A. GERSBACH, K. E. MICHAEL, B. G. KESELOWSKY and A. J. GARCÍA, *Biomaterials* **26** (2005) 4523
81. V. A. TEGOULIA, W. S. RAO, A. T. KALAMBUR, J. R. RABOLT and S. L. COOPER, *Langmuir* **17** (2001) 4396
82. M. MRKSICH, L. E. DIKE, J. TIEN, D. E. INGBER and G. M. WHITESIDES, *Exp. Cell Res.* **235** (1997) 305
83. C. A. SCOTCHFORD, C. P. GILMORE, E. COPPER, G. J. LEGGETT and S. DOWNES, *J. Biomed. Mater. Res.* **59** (2002) 84
84. B. G. KESELOWSKY, D. M. COLLARD and A. J. GARCÍA, *Proc. Natl. Acad. Sci USA* **102** (2005) 5953
85. B. G. KESELOWSKY, D. M. COLLARD and A. J. GARCÍA, *Biomaterials* **25** (2004) 5947
86. M. H. LEE, P. DUCHEYNE, L. LYNCH, D. BOETTIGER and R. J. COMPOSTO, *Biomaterials* **27** (2006) 1907
87. B. J. SPARGO, M. A. TESTOFF, T. B. NIELSEN, D. A. STENGER, J. J. HICKMAN and A. S. RUDOLF, *Proc. Natl. Acad. Sci.* **91** (1994) 11070
88. E. SARDELLA, R. GRISTINA, G. CECONE, D. GILLI-LAND, A. PAPADOPOULOU-BOURAOUI, F. ROSSI, G. S. SENESI, L. DETOMASO, P. FAVIA and R. D'AGOSTINO, *Surface Coat. Technol.* **200** (2005) 51
89. R. E. RAWSTERNE, S. J. TODD, J. E. GOUGH, D. FARRAR, F. RUTTEN, M. R. ALEXANDER and R. V. ULIJN, Personal Communication 2005

90. S. G. ZHANG, L. YAN, M. ALTMANN, M. LAESSLE, H. NUGENT, F. FRANKEL, D. A. LAUFFENBURGER, G. M. WHITESIDES and A. RICH, *Biomaterials* **20** (1999) 1213
91. K. C. DEE, T. T. ANDERSEN and R. BIZIOS, *Biomaterials* **20** (1999) 221
92. M. ARNOLD, E. A. CAVALCANTI-ADAM and B. RUBNER, *Langmuir* **20** (2004) 1362
93. D. S. W. BENOIT and K. S. ANSETH, *Biomaterials* **26** (2005) 5209
94. B. G. KESELOWSKY, D. M. COLLARD and A. J. GARCIA, *Proc. Natl. Acad. Sci.* **102** (2005) 5953
95. B. KASEMO, *Current Opin. Solid State Mater. Sci.* **3** (1998) 451
96. B. KASEMO, *J. Gold Adv. Dental Res.* **13** (1999) 8
97. M. M. STEVENS and J. H. GEORGE, *Science* **310** (2005) 1135
98. D. A. PULEO and A. NANCI, *Biomaterials* **20** (1999) 2311
99. B.D. RATNER and A.S. HOFFMAN, *Biomaterials Science: An Introduction to Materials in Medicine* (Academic Press Inc. London, 2004), p. 105
100. M. MRKSICH, *Current Opin. Chem. Biol.* **6** (2002) 794
101. W. L. MURPHY, K. O. MERCURIUS, S. KOIDE and M. MRKSICH, *Langmuir* **20** (2004) 1026
102. C. GRUNWALD, W. ECK, N. OPITZ, J. KUHLMANN and C. WÖLL, *Phys. Chem. Chem. Phys.* **6** (2004) 4358
103. M. MRKSICH, *Chem. Soc. Rev.* **29** (2000) 267
104. H. WANG, S. CHEN, L. LI and S. JIANG, *Langmuir* **21** (2005) 2633
105. B. G. KESELOWSKY, D. M. COLLARD and A. J. GARCÍA, *Biomaterials* **26** (2005) 5947
106. N. KOTOBUKI, K. IOKU, D. KAWAGOE, H. FUJIMORI, S. GOTO and H. OHGUSHI, *Biomaterials* **26** (2005) 779
107. W. L. MURPHY, S. HSIONG, T. P. RICHARDSON, C. A. SIMMONS and D. J. MOONEY, *Biomaterials* **26** (2005) 303
108. K. UEMATSU, K. HATTORI, Y. ISHIMOTO, J. YAMAUCHI, T. HABATA, Y. TAKAKURA, H. OHGUSHI, T. FUKUCHI and M. SATO, *Biomaterials* **26** (2005) 4273
109. N. FAUCHEUX, R. TZONEVA, M.-D. NAGEL and T. GROTH, *Biomaterials* **27** (2006) 234
110. K. E. MICHAEL, V. N. VERNEKAR, B. G. KESELOWSKY, J. CARSON MEREDITH, R. A. LATOUR and A. J. GARCÍA, *Langmuir* **19** (2003) 8033
111. S. FERRETTI, S. PAYNTER, D. A. RUSSELL, K. E. SAPSFORD and D. J. RICHARDSON, *Trends Anal. Chem.* **19** (2000) 530
112. P. M. St. JOHN, L. KAM, S. W. TURNER, H. G. CRAIGHEAD, M. ISSACSON, J. N. TURNER and W. SHAIN, *J. Neurosci. Methods* **75** (1997) 171
113. H. ZREIQAT and C. R. HOWLETT, *J. Biomed. Mater. Res.* **47** (1999) 360
114. H. ZREIQAT, P. EVANS and C. R. HOWLETT, *J. Biomed. Mater. Res.* **44** (1999) 389
115. K. E. HEALY, C. H. THOMAS, A. REZANIA, J. E. KIM, P. J. MCKEOWN, B. LOM and P. E. HOCKBERGER, *Biomaterials* **17** (1996) 195
116. V. D. BHAT, G. A. TRUSKEY and M. REICHERT, *J. Biomed. Mater. Res.* **40** (1998) 57
117. D. L. ELBERT and J. A. HUBBELL, *Biomacromolecules* **2** (2001) 430
118. M. ARNOLD, E. A. CAVALCANTI-ADAM, R. GLASS, J. BLÜMMEL, W. ECK, M. KANTLEHNER, H. KESSLER and J. P. SPATZ, *Chem. Phys. Chem.* **5** (2004) 383
119. N. Q. BALABAN, U. S. SCHWARZ, D. RIVELIN, P. GO-ICHBERG, G. TZUR, L. SABANAY, D. MAHALU, S. SAFRAN, A. BERSHADSKY, L. ADDADI and B. GEIGER, *Nat. Cell Biol.* **3** (2001) 466
120. R. G. FLEMMING, C. J. MURPHY, G. A. ABRAMS, S. L. GOODMAN and P. F. NEALEY, *Biomaterials* **20** (1999) 573
121. G. A. ABRAMS, A. I. TEIXEIRA, P. F. NEALEY and C. J. MURPHY, "Effects of substratum topography on cell behavior." In: *Biomimetic Materials and Design*, edited by A. K. DILLOW, A. M. LOWMAN, A. M. MARCEL (Dekker, Inc., New York, 2002), Chapter 4, pp. 91–137
122. E. K. F. YIM and K. W. LEONG, *Nanomed. Nanotechnol. Biol. Med.* **1** (2005) 10
123. H.-H. HUANG, C.-T. HO, T.-H. LEE, T.-L. LEE, K.-K. LIAO and F.-L. CHEN, *Biomol. Eng.* **21** (2004) 93
124. K. ANSELME and M. BIGERELLE, *Acta Biomater.* **1** (2005) 211
125. Y. W. FAN, F. Z. CUI, S. P. HOU, Q. Y. XU, L. N. CHEN and I.-S. LEE, *J. Neurosci. Methods* **120** (2002) 17
126. C. S. CHEN, M. MRKSICH, S. HUANG, G. M. WHITESIDES and D. E. INGBER, *Science* **276** (1997) 1425
127. J. TAN and W. M. SALTZMAN, *Biomaterials* **23** (2002) 3215
128. E. K. F. YIM, R. M. REANO, S. W. PANG, A. F. YEE, C. S. CHEN and K. W. LEONG, *Biomaterials* **26** (2005) 5405
129. K. D. CHESMEL, C. C. CLARK, C. T. BRIGHTON and J. BLACK, *J. Biomed. Mater. Res.* **29** (1995) 1101
130. N. M. DOWELL-MESFIN, M.-A. ABDUL-KARIM, A. M. P. TURNER, S. SCHANZ, H. G. CRAIGHEAD, B. ROYSAM, J. N. TURNER and W. SHAIN, *J. Neural. Eng.* **1** (2004) 78
131. A. DIENER, B. NEBE, F. LÜTHEN, P. BECKER, U. BECK, H. GEORG NEUMANN and J. RYCHLY, *Biomaterials* **26** (2005) 383
132. F. HAQ, Y. L. RAO, C. KEITH, Y. ZHAO and G. ZHANG, *J. Biomed. Nanotech.* **1** (2005) 313
133. O. ZINGER, G. ZHAO, Z. SCHWARTZ, J. SIMPSON, M. WEILAND, D. LANDOLT and B. BOYAN, *Biomaterials* **26** (2005) 1837
134. J. Y. LIM, J. C. HANSEN, C. A. SIEDLECKI, R. W. HEN-GTEBECK, J. CHENG, N. WINOGRAD and H. J. DONAHUE, *Biomacromolecules* **6** (2005) 3319
135. E. T. den BRABER, J. E. de RUIJTER, H. T. J. SMITS, L. A. GINSEL, A. F. von RECUM and J. A. JANSEN, *Biomaterials* **20** (1996) 1093
136. P. F. NEALEY, *Biomaterials* **20** (1999) 573
137. P. CLARK, P. CONNOLLY, A. S. G. CURTIS, J. A. T. DOW and C. D. W. WILKINSON, *J. Cell Sci.* **99** (1991) 73
138. E. MARTINES, K. MCGHEE, C. WILKINSON and A. CURTIS, *IEEE Trans. Nanobiosci.* **3** (2004) 90
139. B. MÜLLER, M. RIEDEL, R. MICHEL, S. M. De PAUL ROLF HOFER, D. HEGER and D. GRÜTZMACHER, *J. Vac. Sci. Technol. B* **19** (2001) 1715
140. Y. WAN, Y. WANG, Z. LUI, X. QU, B. HAN, J. BEI and S. WANG, *Biomaterials* **26** (2005) 4453
141. A. S. G. CURTIS, B. CASEY, J. O. GALLAGHER, D. PASQUI, M. A. WOOD and C. D. W. WILKINSON, *Biophys. Chem.* **94** (2001) 275
142. S. BRITLAND, C. PERRIDGE, M. DEYNER, H. MORGAN, A. CURTIS and C. WILKINSON, *Exp. Biol. Online—EBO* (1996) 1:2
143. G. R. H. OWEN, J. JACKSON, B. CHEHROUDI, H. BUR and D. M. BRUNETTE, *Biomaterials* **26** (2005) 7447
144. M. M. STEVENS, M. MAYER, D. G. ANDERSON, D. B. WEIBEL, G. M. WHITESIDES and R. LANGER, *Biomaterials* **26** (2005) 7636
145. H. YUAN, K. KURASHINA, J. D. De BRUIJN, Y. LI, K. De GROOT and X. ZHANG, *Biomaterials* **20** (1999) 1799
146. P. ROACH, D. FARRAR and C. C. PERRY, *J. Am Chem. Soc.* **127** (2005) 8168

147. M. TABORELLI, L. ENG, P. DESCOUTS, J. P. RANIERI, R. BELLAMKONDA and P. AEBISCHER, *J. Biomed. Mater. Res.* **29** (1995) 707
148. T. J. LENK, T. A. HORBETT, B. D. RATNER and K. K. CHITTUR, *Langmuir* **7** (1991) 1755
149. W. G. PITT and S. L. COOPER, *J. Biomed. Mater. Res.* **22** (1988) 359
150. G. B. SIGAL, M. MRKSICH and G. M. WHITESIDES, *J. Am. Chem. Soc.* **120** (1998) 3464
151. L. VROMAN and A. L. ADAMS, *J. Biomed. Mater. Res.* **3** (1969) 43
152. J. LYKLEMA, "Physical chemistry of biological interfaces." In: *Surface and Interfacial Aspects of Biomedical Polymers*, edited by J.D. ANDARDE (Plenum Press, New York, 1985), p. 1
153. C. GERGELY, S. BAHİ, B. SZALONTAI, H. FLORES, P. SCHAAF, J.-C. VOEGEL and F. J. G. CUISINIER, *Langmuir* **20** (2004) 5575
154. D. HANEIN, B. GEIGER and L. ADDADI, *Langmuir* **9** (1993) 1058
155. C. A. HAYNES and W. NORDE, *Colloids Surfaces B* **2** (1994) 517
156. J. L. BRASH and T. A. HORBETT, *Proteins at Interfaces II: Fundamentals and Applications* (ACS, Washington D.C., 1995), p. 1
157. C. YONGLI, Z. XIUFANG, G. YANDAO, Z. NANMING and Z. TINGYING and S. XINQI, *J. Colloid Int. Sci.* **214** (1999) 38
158. M. PROKOPOWICZ, J. L-TUKASIAK, B. BANECKI and A. PRZYJAZNY, *Biomacromolecules* **6** (2005) 39
159. A. AGNIHOTRI and C. A. SIEDLECKI, *Langmuir* **20** (2004) 8846
160. K. L. MARCHIN and C. L. BERRIE, *Langmuir* **19** (2003) 9883
161. P. CACCIAFFESTA, A. D. L. HUMPHRIS, K. D. JANDT and M. J. MILES, *Langmuir* **16** (2000) 8167
162. N. XIA and D. G. CASTNER, *J. Biomed. Mater. Res.* **67** (2003) 179
163. C. WERNER, R. ZIMMERMANN and T. KRATZMÜLLER, *Colloids Surfaces A Physicochem. Eng. Aspect* **192** (2001) 205
164. M. LUNDQVIST, I. SETHSON and B.-H. JONSSON, *Langmuir*, **21** (2005) 5974
165. B. W. MORRISSEY, L. E. SMITH, C. A. FENSTERMAKER, R. R. STROMBERG and W. H. GRANT, *NBS Special Publ. (US)* **415** (1975) 83
166. P. TENGVALL, I. LUNDSTRÖM and B. LEIDBERG, *Biomaterials* **19** (1998) 407
167. C. YONGLI, Z. XIUFANG, G. YANDAO, Z. NANMING, Z. TINGYING and S. XINQI, *J. Colloid Int. Sci.* **214** (1999) 38
168. J. M. GRUNKEMEIER and T. A. HORBETT, *J. Mol. Rec.* **9** (1996) 247
169. V. BALASUBRAMANIAN, N. K. GRUSIN, R. W. BUCHER, V. T. TURITTO and S. M. SLACK, *J. Biomed. Mater. Res.* **44** (1999) 253
170. R. J. RAPOZA and T. A. HORBETT, *J. Colloid Int. Sci.* **136** (1990) 480
171. S. SRIVASTAVA, A. VERMA, B. L. FRANKAMP and V. M. ROTELLO, *Adv. Mater.* **17** (2005) 617
172. T. C. TA and M. T. MCDERMOTT, *Anal. Chem.* **72** (2000) 2627
173. G. K. IWAMOTO, L. C. WINTERTON, R. S. STOKER, R. A. Van WAGENEN, J. D. ANDRADE and D. F. MOSHER, *J. Colloid Int. Sci.* **106** (1985) 459
174. C. GRUNWALD, W. ECK, N. OPITZ, J. KUHLMANN and C. WÖLLA, *Phys. Chem. Chem. Phys.* **6** (2004) 4358
175. A. A. VERTEGAL, R. W. SIEGEL and J. S. DORDICK, *Langmuir* **20** (2004) 6800
176. M. LUNDQVIST, I. SETHSON and B.-H. JONSSON, *Langmuir*, **20** (2004) 10639
177. P. ROACH, D. FARRAR and C. C. PERRY, *J. Am. Chem. Soc.* **128** (2006) 3939
178. A. KONDO, F. MURAKAMI, M. KAWAGOE and K. HIGASHITANI, *Appl. Microbiol. Biotechnol.* **39** (1993) 726
179. G. MARELETTA, G. CIAPETTI, C. SATRIANO, S. PAGANI and N. BALDINI, *Biomaterials* **26** (2005) 4793
180. G. R. H. OWEN, J. JACKSON, B. CHEHROUDI, H. BURT and D.M. BRUNETTE, *Biomaterials* **26** (2005) 7447
181. B. WÓJCIAK-STOTHARD, A. CURTIS, W. MONAGHAN, K. MACDONALD and C. WILKINSON, *Exp. Cell Res.* **223** (1996) 426