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FEATURE ARTICLE

# Surface Nanoarchitecture for Bio-Applications: Self-Regulating Intelligent Interfaces

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**The surface nanoarchitecture provides spatially and temporally resolved stimuli response of the material, and offers defined control over the behavior of biomolecules and cells at the solid-liquid interface. Here, the focus is on metal-based systems that are interesting for biomedical applications. Intelligence of the surface is suggested to be achieved through its nanostructuring for stimuli responsive properties. Spatial and temporal cell performance at the surface, provided by the surface nanoarchitecture, offers advanced bio-applications of metal-based materials, such as implantation, lab-on-chip and organ-on-chip, biosensors, smart biomaterials and drug delivery systems. Spatial control is achieved by surface patterning. Temporal control is accessed through the application of stimuli responsive switchable surface chemistry, sensitive to external parameters: temperature, pH, light, electric and magnetic field, ionic strength, surrounding medium (hydrogels in water), multi-trigger response, and response to products of the cell metabolism. The key issue is the prospect in the formation of self-regulating "intelligent" surface cell interactions: biomimetic of natural systems.**

## 1. Introduction

Nanostructured surfaces that provide effective control over biomolecules/cell/tissue deposition, growth and release can be considered as self-regulated "intelligent" surfaces (**Figure 1**). Recently, significant efforts and challenges have been directed to produce active surfaces with pronounced stimuli sensitive and feedback properties to achieve self-regulation.[1] One of the successful examples are antifouling and self-healing coatings.[1] To achieve self-regulation of inorganic surfaces one needs to endow them active agents that are released on demand. An efficient, versatile and simple way towards formation of self-regulated surfaces is the formation of porous interfaces. The porous interfacial layer is a surface encapsulation system with stimuli responsive loading and release.[1,2] The approach of application of the porous interface for construction of self-regulated surfaces is schematically illustrated in Figure 1. In addition, such kind of the surfaces can provide control of biomolecules/microorganism/cell positioning, adhesion, spreading, growth and migration on surfaces which may have highest relevance in areas like implantation, lab-on-chip and organ-on-chip, biosensors, stem cell research.

Here we focus on titanium, magnesium, steel and their alloys as important metals for bioengineering, human implants. Implantable metal based devices such as orthopedic implants,[3a] vascular stents,[3b] and dental implants are widely used in medicine.[3c] Additionally, the porous metal interface can serve as a drug-delivery platform and stimuli responsive material (Figure 1). Noble metals being deposited on indium tin oxide (ITO) glass or silicon are discussed in the context of biosensors or lab-on-chip technology. Formation of metal based composites and hybrids provide complex morphology, regulated porosity, wettability and adhesion of self-regulated surfaces. Thus, fine tuning of elasticity, hydrophilic/hydrophobic properties, sorption capacity can be achieved. Moreover biocompatibility of metal based materials can be increased through, for example, formation of metal-hydroxyapatite composites or more complex composite metal (hydroxyapatite-carbon nanotubes).

Various design strategies have been suggested to direct the adhesion of cells to selected areas of a substrate.[4] One particularly versatile approach to control cell attachment and patterning is the physical or chemical adsorption of functional molecules such as extracellular matrix proteins and pH-, temperature-, electrically sensitive polymers to selected areas of a substrate. The development of patterning techniques coupled with functional surface chemistry has enabled the formation of surfaces with stringent control over the adsorption of biomolecules and cells in space. Furthermore, the development of switchable surfaces that are responsive to a particular signal and that switch between disparate properties, such as hydrophobic/hydrophilic, positive/negative charge or even swollen/condensed layers, has added a new dimension to biomolecule manipulation. Besides switchable hybrids examples can be found in ''smart" chemical (drug, vitamin, growth factors, biomolecules) delivery (Figure 1, bottom).

Elasticity, morphology, porosity, wettability of the components of the hybrid systems has to be considered in order to

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design bio-active surfaces.[5] By formation of hybrid systems initial “hard” metals can be changed to a soft elastic surface (Figure 1, right) by physical or chemical adsorption of organic polymer molecules/biomolecules. The inorganic-organic hybrid materials formed by using metal surfaces modified by polymers can adapt to the environment, change wettability and adhesion to the surface depending on the external conditions. Furthermore, the polymers deposited on a metal surface can convert chemical and biochemical signals into optical, electrical, thermal and mechanical signals, and so forth. A broad spectrum of available natural and synthetic polymers is available for formation of hybrid systems. These hybrid systems are perspective for construction of mimetic bio-interfaces, controlled drug-delivery and release systems, and stimuli responsive coatings.[1,2] Proteins and peptide chains, DNA, RNA, oligonucleotides, lipids and polysaccharides, as well as larger assemblies of these biomolecules, in particular living cells, can be used as building blocks for formation of the hybrids. The synthetic stimuli-responsive macromolecules are capable of conformational and chemical changes on receiving an external (T, pH, light, electric and magnetic field, ionic strength, surrounding medium (hydrogels in water) and multi-trigger response) or internal (cell metabolism) signals.[6]

## 2. Selected Materials

The bio-functional metal surfaces (**Figure 2**) for implantation are mostly composed of Ti, Mg, steel and their alloys. Metals should be biocompatible (Ti and its alloy) and preferably also biodegradable (Mg, Fe, Zn and Mo alloys).[7] For bio-electronic devices conductivity (noble metals) could be also a key factor. A porous metal matrix could be formed by surface anodization (Figure 2a), plasma or laser treatment (Figure 2b), chemical etching (Figure 2d), sol-gel route (Figure 2e,f), ultrasonic technique (**Figure 3**).

Biomimetic composite and hybrid materials are widely used in nature to sustain life and maintain biological function providing a specific morphology and functionality.[8] The issue of the composite is another effect to provide stimuli response of the surface. Hydroxyapatite-metal composites are an example of such materials. Hydroxyapatite is the major mineral phase in bone. Synthetic analogies can provide better integration of metallic surfaces with bone after implantation.[9] Thus, the stabilized composite of metal and apatite may improve biocompatibility and promote osteointegration. Moreover, there is a tendency to use carbon materials (carbon nanotubes, graphene layer) to further improve tensile strength, fracture toughness and mechanical properties of the surface.[10] For example, carbon nanotubes have excellent mechanical properties to strengthen and toughen hydroxyapatite. Studies of bioactive mineral–carbon composites show an expansion of potential applications to fields ranging from interdisciplinary science to practical engineering such as the fabrication of reinforced bone-implantable materials. The photocatalytically active mixed oxide composite systems can be formed by using, for example, TiO$_2$:In$_2$O$_3$.[11] Additional to the photocatalytically active composites with biocide activity are antifouling corrosion stable hydrophobic layered double hydroxides on metal surfaces (Figure 3e).[12]

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Hybrid materials (hybrids) are composites consisting of a combination of several compounds at the nanometer or molecular level. Commonly one of these compounds is inorganic and the other one–organic. Such submicron scale materials organization/combination can result in characteristics in between the original phases or even new properties. Here, described hybrids are composed of organic molecules connected to a metal matrix by:[13] (1) physical adsorption (via van der Waals or electrostatic interactions) (Figure 2h); (2) physical entrapment (loading of material inside pores of metal matrix) (Figure 1, below); and (3) covalent attachment (Figure 2g). The organics could be (i) biomolecules,[14a] for example, proteins and peptide chains, DNA, RNA, oligonucleotides, lipids and polysaccharides, cells, and drugs;[14b] (ii) stimuli responsive synthetic systems, for example, polyethylene glycol/oxide (PEG/PEO), polysiloxanes,[14c] poly(N-isopropylacrylamide)

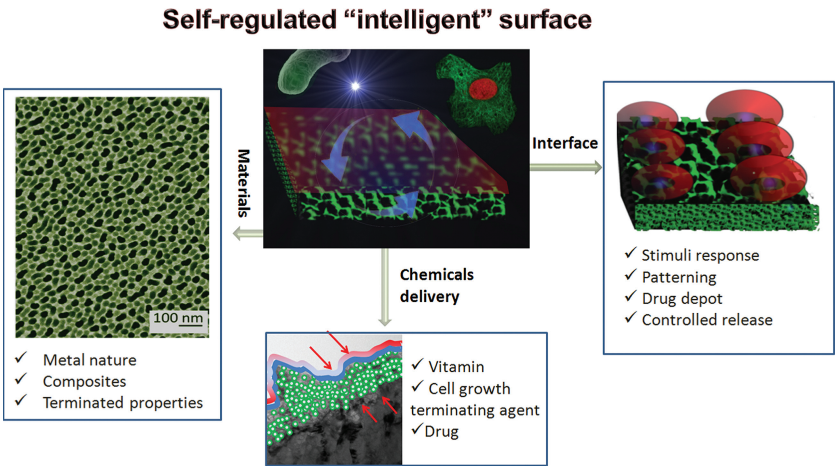

**Figure 1.** Design strategy for intelligent surfaces. Left: nanostructured metal surface (scanning electron microscope (SEM) image of a modified metal surface of potential implants), platform for optimization/modification of metal surfaces by formation of organic/inorganic composites. Right: formation of feedback coatings. Soft matter (micelles, hydrogels, polymer films) sensitive to external stimuli, for example, T, pH, light, electrical, magnetic field, grafted/adsorbed on the porous metal matrix. Bottom: generated porous nanonetwork well adhering to bulk metal as a universal encapsulation system suitable for multicomponent loading and time-resolved release. The metal surface capsules can be loaded with active agents, such as corrosion inhibitors, vitamins and drugs, enzymes, DNA fragments, or antibodies.

(PNIPAAM),[14d] copolymer systems,[14e] mixed polymers,[14f] micelles,[6] stimuli-responsive nanogels,[14g] and so forth.

### 2.1. Metals for Implant Biosurfaces and Fundamental Studies

At present, a wide range of materials are used in medical applications, including metals, ceramics, and polymers.[15] All bio-inspired materials for implants must fulfill the required specifications of biocompatibility, biofunctionality, biodurability and biosafety in short, medium and long term.[16]

The earliest records of the use of metallic implants in surgery go back to the 16th century.[17] Through the end of the 19th century, however, the attempts to introduce metal pieces into a human body were largely unsuccessfull focusing more on surgery-related infection.[18] Metal implants started to be widely developed after Lister's introduction of antiseptic surgical techniques in the 1880s,[19] sharply reducing the incidence of infection and making it possible to distinguish the tissue reaction to an implant from inflammation attributable to infection.

Titanium and its alloys, such as the dental alloy Ti-6Al-4V and others (e.g., Ti-5Al-2.5Fe, Ti-6Al-7Nb) are well known for implants because of their superior mechanical properties, hardness, corrosion resistance and biocompatibility.[20] After long term use, the alloys could release toxic components even from a corrosion resistant material.[21] The titanium alloys do not corrode in the body due to their passivation by a thin surface layer of metal oxide. However, metal ions could slowly diffuse through the oxide layer and accumulate in the tissue. For Ti-6Al-4V alloy, the ion release kinetics, as well as the presence of vanadium ions, may play a major role in influencing the osteoblast behavior. Co-Cr alloys are often used for stents.[22] For such alloys the chromium oxide layer, $Cr_2O_3$, provides good corrosion protection and the possibility of self-healing.[23] However, the problem related to toxicity of chromium ions is severe.

Nanostructuring of the implant surface can provide a change of the metal ion release profile. Metal ion release studies and surface analyses were performed by C. H. Ku et al.[24] on untreated and triton treated titanium alloys. In vitro experiments (from 72 h up to 4 weeks) show an accelerated peak of alkaline phosphatase activity on the control sample which to some extent can be evidence of Al ion release from the alloy. Osteonectin, osteopontin, and osteocalcin gene expression (at week 1) as well as total protein amount (until week 4) were not affected by the surface treatments.

An oxide layer on the surfaces of the implants is one of the main factors that are responsible for the biocompatibility of implants and the protection of their surface. After metal implantation the tissue is in contact with the native oxide layer and not with the metal or metal alloys themselves.[25] The metal oxide layer has a self-healing activity and can be recovered after its chemical or mechanical damage. In oxidizing media as in the human body fluid the metal oxide layer can rebuild in milliseconds after damaging. Moreover oxide surfaces have an important role in the formation of hybrids. The metal oxide surfaces can be used as apatite inducer providing apatite nucleation.[26] Porous metal oxide layers are commonly formed on the surfaces of alloy implants, passivating and fixing the implants to bone via bony ingrowths into the porous structure, promoting osseo/osetointegration.[27] A freshly formed oxide layer could provide chemical attachment of active chemicals through, for example, oxygen[28] bridges (Figure 2g): formation of hybrids and drug delivery systems.

Titanium implants with defined size and geometry of 3D micropores used in model experiments provide special terminated properties and chemistry.[29]

One of the key aspects in the mimic of the “intelligent” surfaces is the switchable stimuli response of the blocks used for the surface nanoarchitecture.[30] A representative example of metals with stimuli responsive properties is a shape memory alloy, nitinol. Nitinol is the equiatomic intermetallic compound Ni-Ti, containing to 60 wt.% of Ni and the rest Ti with less than 1 wt.% inclusions, for example, Co, Cr, Mn, Fe. Nitinol exhibits good biocompatibility and corrosion resistance in vivo. It belongs to a very special group of shape memory alloys: materials that can return to their original shape after having been plastically deformed.[31] The shape memory effect in nitinol is related to the nanocrystallographically reversible structural change: martensitic transformation. The martensitic transformation is induced by temperature changes. The transformation temperature[32] strongly depends on the alloy composition: an increase in Ni content above the stoichiometric composition results in a decrease in the transition temperature. The same effect can be achieved by adding cobalt as a substitute

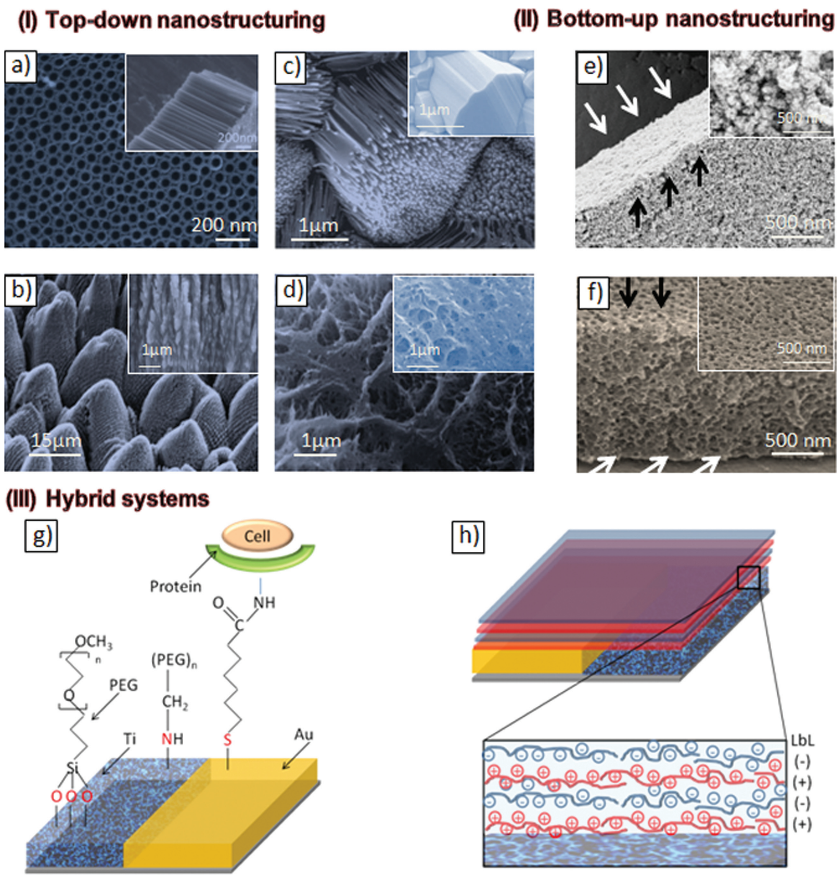

**Figure 2.** Materials for intelligent surfaces: nanostructured metal based surfaces formed by I) top-down and II) bottom-up nanostructuring. In particular, a) SEM images of titanium nanotube arrays formed by anodization. b) Titanium surface textured with femtosecond laser pulses. Reproduced with permission.[69] Copyright 2011, Wiley Periodicals, Inc. c) Titanium disk surfaces after plasma treatment. Reproduced with permission.[78] Copyright 2006, Materials Research Society. d) Surface characteristics of the inner pores of chemically etched titanium. e) Sol-gel titania film. f) Cross-section of gold-titania particle layer prepared by a sol-gel template route. Reproduced with permission.[84] Copyright 2009, American Chemical Society. (III) Examples of hybrid systems. A schematic representation of patterned (Ti/Au) surface modification with g) self-assembled monolayer through sulfur bridges on Au, and oxygen- amide- linking on Ti, and h) layer-by-layer (LbL). The possibility to guide cell adhesion is highlighted.

for Ni. The martensite transformation in nitinol brings about an abrupt change in Young's modulus and yield stress. This provides the rare ability to tailor the properties of nitinol to a specific application by slightly changing the alloy composition. One could image great prospects of such alloys for constructing the switchable stimuli responsive material and self-healing materials.

There are two classes of materials that have been proposed for biodegradable stents:[33,34a] (i) polymers from the lactic acid, glycolic and caprolactone families[34b,c] and (ii) metals, either Mg-based[34d,e] or Fe-based alloys.[35] In this article the focus is on metal based materials which have superior mechanical properties. The development of biodegradable metal implants is breaking the paradigm in which metallic biomaterials must be corrosion-resistant. It is a challenging topic to fundamentally understand the behavior of the implants since two main issues need to be understood: (i) corrosion kinetics to have effective clinical implantation, and (ii) pathway of the products of corrosion, for example, metal ions, nanoparticles, gas bubbles are travelling in the body. Nearly all patients benefited from the treatment with magnesium implants.[33] Although some patients experienced subcutaneous gas cavities caused by rapid implant corrosion, some patients had no pain, and almost no infections were observed during the postoperative follow up. Researchers and clinicians should be warned from all the historical reports that Mg is a special lightweight metal that needs specific knowledge, careful professional handling and experience-based design for its successful bio application. A recent patent claimed a stent with a base body consisting of a core of a biocorrosive alloy selected from the group composed of Mg, Fe, Zn and Mo, with a diffusion layer containing Mn and Se covering the core. Thus, several Mg alloys have been investigated, including the ones containing minor quantity (up to 10 wt.%) of other metals in composition,[36] for example, Al, Mn, Y, Zn, or more significant quantity of another metal, for example, Mg-Li (60:40) alloys. Pure Fe,[37a] and its alloy with Mn[37b] were also investigated as biodegradable ones. The potential can be expanded by the development of well based science behind the technology, since before taking the materials into applications, the understanding of the degradation mechanisms in vitro and in vivo must be fully developed. Thus, one of the issues regarding human health is the release of metal ions, nanoparticles from an alloy due to degradation of implanted materials through oxidation and/or hydrolysis or corrosion, which accelerates exposure of materials. The question of the safety of biodegradable metals placed in the body also requires specific attention. However, at the moment, the list of potential medical applications for biodegradable metals seems limited, but their potential is obvious.

There are investigations[38] which elucidate the biocompatibility and microstructure variation of steel alloys with Al and Mn, etc. in composition and favourable mechanical characteristics. A recast layer can be formed on the alloy surface, following electro-discharge machining. Nanocarbide was introduced on the recast layer following electro-discharging. The nanocarbide phase is important and has significant roles in forming a nanostructured oxide layer which further increases the alloy biocompatibility.

To provide models for confining proteins and cells, tissue behavior on surfaces, some well defined nanoarchitectured surfaces are needed. Metal deposited layers on glass or ITO conductive glass are in focus. Such layered systems are very convenient to manipulate with well defined variation of one parameter such as defined surface morphology, patterning,

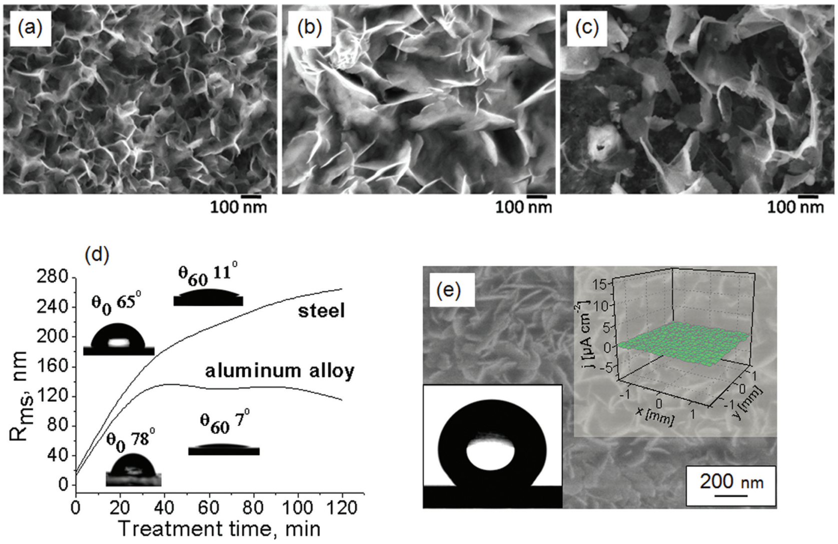

**Figure 3.** SEM images of a) aluminium, b) magnesium alloys and c) stainless steel after 40 min of sonochemical modification. d) Influence of sonication time on plate roughness, insets show the water contact angles of the surface before and after sonication. e) SEM image of a metal composite plate covered by layered double hydroxide laurate, insets show water contact angles onto this surface, and scanning vibrating electrode measurement of 3D current density map confirms the induced corrosion stability of the composite. Reproduced with permission.[12] Copyright 2010, Royal Society of Chemistry.

porosity, pore size and distance between them, surface conductivity, and so forth. They provide excellent models to follow and stimulate an attachment especially due to chemical bonding (on gold through sulphur bridges, on titanium, Al, Mg through oxygen bridges, amide linking, etc. (Figure 2g)). Well defined layered systems are very prospective for fundamental studies of biological objects, design of lab-on-chip and organ-on-chip, biosensors.

## 2.2. Methodology Used for Metal Surface Nanostructuring

In recent years, various processing routes have been developed for the synthesis of nanostructured metal-based systems such as anodization,[39a] plasma etching,[39b] sol-gel route,[39c] chemical reactions in liquid and gas,[39d] ultrasonic irradiation.[39e] All mentioned methods besides the sol-gel route are top-down (Figure 2a–d,3) methodologies of nanostructuring;[40] thus, an initially microscopic surface texture is modified to submicron architecture. The sol-gel approach (Figure 2e,f)[40] provides a microscopic system by sub-system organisation (using nanoblocks). Some examples of nanostructures include nanotubes, nanorods, nanowires, nanofibers, nanobelts, nanoribbons, nanowhiskers, nanoneedles, lamellar and hierarchical dendrites, and so forth.[41] Notwithstanding existing available techniques for metal surface nanostructuring, just a few were tested and totally understood for their effect on cell behavior.

### *2.2.1. Electrochemical Surface Modification*

Electrochemical modification of metals is one of the well known methods of metal nanostructuring. There are several competing possibilities to modify morphology and porosity of metal surfaces in electric fields. One can distinguish: (i) electropolishing pretreatment with formation of a surface with different roughness; and (ii) anodization aiming at formation of a porous interfacial layer.

Electrochemical modification of aluminum and titanium is the most popular example of the electrochemical nanofabrication approach. Other transition metals, also potentially interesting for implantation (e.g., Ta, Nb, Hf, Zr, W) can be effectively nanostructured by the electrochemical method. It is well known that during the anodization of a metal, there are two processes: field-enhanced oxidation of metal and field-enhanced oxide dissolution.[42] There ate two interfaces: solution/oxide and oxide/metal. Oxidation occurs at the metal/oxide interface when the oxygen containing ions ($O^{2-}$/$OH^-$) are transported from the solution to the oxide layer. At the same time, metal ions (e.g., $Al^{3+}$) migrate from the metal to the solution/oxide interface and dissolve into the solution. Since the electric field can enhance the migration of the metal ion, the latter process is called field-enhanced dissolution.

The metal oxide interfacial layer is responsible for bioapplication and cell integration with a metal. The anodic oxide interface involves generally an amorphous or a crystalline metal oxide layer.[43] During the Ti electrochemical modification formation of anatase and rutile was observed.[44] Anatase and rutile are able to induce various apatite structures in vitro. The type of electrolyte solution during oxidation[45] can affect metal nanostructuring and, thus, cell–material interactions. A commercially pure titanium substrate was nanostructured by an anodization process using different electrolyte solutions, for example, HF, $H_3PO_4$ and $H_2SO_4$.[45] Bioactive bulk $TiO_2$ films were produced in presence of different electrolytes. Rough surface morphology, high surface energy, thicker oxide layer and low values of contact angles were important factors for optimal cell/material interaction. It was shown that each of the surfaces prepared at different conditions exhibits different cell–material interactions. Colonization of the cells was shown with a distinctive cell-to-cell attachment in the HF anodized surface. Good cellular adherence with the extracellular matrix extensions in between the cells was observed for samples anodized in $H_3PO_4$ electrolyte. The $TiO_2$ layer grown in $H_2SO_4$ electrolyte did not show significant cell growth on the surface, and some cell death was also observed. Cell adhesion and differentiation, living cell density and proliferation were more pronounced on anodized surfaces in comparison with the initial ones. Similar to cell–material interaction, variations in mineral deposition behavior were also demonstrated for films grown in different electrolytes. Moreover, ions introduced in the interfacial layer during electrochemical modification in presence of different electrolytes,[46] have a significant impact on the functionality of the material. For example, nitrogen and carbon doping of

$TiO_2$ can shift titania absorption into the visible light spectrum, providing change of surface stimuli to electromagnetic irradiation.[47]

Since nanotube materials have been introduced by Zwilling et al.[48] they have attracted great attention also for bio-applications. Electrochemical surface modification is one of the most promising top-down synthetic approaches for fabrication of highly ordered one dimensional (1D) nanotube materials. The properties of nanotube modified surfaces depend on the diameters of the nanotubes, their length and wall thickness. Electrochemical growth of a nanotube on a metal surface is a relatively simple, low cost method of production of highly organized and uniform nanostructures with controllable dimensions and unique properties. Furthermore, interest in nanotubular titanium dioxide was significantly increased due to unique photocatalytic properties of the material. The photocatalytic materials exhibit antibacterial self-cleaning activity of the surfaces[49] or UV response of the interfacial layer for implantable drug delivery, stem cell-differentiation, and anti-cancer treatment. Additional to photocatalytic properties, charge transfer, molecular transport and loading capacity of nanotubes are important for bio-application. The tube formation is also an electrolyte sensitive process. The length of the nanotubes can be controlled by varying the anodization time. The diameter of the tubes is adjusted by changing the applied anodization voltage. The growth rate of the nanotubes is variable, for example, by ultrasonic excitation in the electrochemical bath.[50] The electrical field intensity at the pore bottom is much higher than that at the wall, and metals, for example, aluminum, can be consumed at a high rate near the bottom of the pore, allowing continuous growth of the pore depth. In contrast, for anodized titanium the final thickness of the porous oxide film does not increase with the anodizing time.[48] Titanium oxide can be etched at a high rate in HF solution even in the absence of an anodizing voltage. If the etching rate of the oxide in solution is comparable with that of the field-enhanced dissolution, the titanium oxide either in the wall or at the pore bottom can dissolve at a balanced rate resulting in a constant pore depth. Uniform long titania nanotube arrays of various pore sizes (ca. 20–100 nm), lengths, outer diameters (90–220 nm), and wall thicknesses (7–34 nm) have been reported[51] by tailoring electrochemical anodization conditions.[52] Moreover, dependent upon the titanium alloy used, metal composites can be readily introduced. It was mentioned above that in HF formation of a nonporous surface is possible, however HF can be suitable also for formation of nanotubes et al.[48] The nanotubular titania structuring can be achieved by using a mixture of $H_3PO_4$ and HF solutions with ultrasonic excitation.[53] Recently, Losic and co-workers[54] published a method of self-ordering electrochemical synthesis of titanium nanotubes with defined pore size and depth of the anodization (determine length of nanotubes). In particular, it was shown that nanotubes with desired nanotube diameters (70–180 nm) and length (5–200 μm) can be fabricated using anodization in $NH_4F$/ethyleneglycol electrolyte by controlling the anodization voltage and time (Figure 2a).

By using electrochemical treatment of nitiol,[55] the problem of uncontrollable release of Ni can be solved. Electropolishing pretreatment and photoelectrocatalytic Fenton's oxidation of nitiol in presence $Fe^{2+}/H_2O_2$ has been studied. By using this method, a titania layer with a Ni-free zone near the surface and a graded interfacial structure between the titania layer and the NiTi substrate can be formed. These layers successfully suppress Ni ion release from the alloy, although this film has a porous structure on the nanometer scale.

Thus, electrochemical modification of metal surfaces can help to avoid introduction and release of toxic compounds from the implant surface and to improve biocompatibility of the metal by controllable formation of an oxide layer. The chemically active oxide layer can be a source of oxygen bridges, amide-linkage for formation of stable hybrids. The porous network of the metal oxide layer can be used as a drug depot for controlled local drug delivery and release on demand.

### 2.2.2. Plasma Etch Technologies

The plasma etching is widely used for continuous cleaning of surfaces by physical and/or chemical processes.[56] (i) Dry plasma etching-substrates are immersed in a reactive gas (plasma). The layer to be etched is removed by chemical reactions and/or physical means (ion bombardment). (ii) Wet plasma etching-substrates are immersed in a reactive solution. One of the advantages of plasma etching is that nanostructuring with small extension of the surfaces can be achieved. High energy provides both thermodynamically and kinetically stable surfaces.

In all, etching in a discharge environment is achieved by providing active species, which react with the substrate forming volatile compounds. In plasma etching, the reactive species are ions and activated neutrals, created in an electrical discharge. The process usually takes place inside a reactor under well controlled environmental parameters like, for example, pressure, temperature and feed gas flow. The following processes are observed: chemical etching, ion enhanced etching, physical etching, trenching, sidewall passivation and mask erosion. It is still a challenge to describe in detail a rather complex system as plasma etching.[57]

Examples of chemical reactions, which are relevant for plasma etching with the reaction of etch agent with the surface,[58] are $CF_4$, $BCl_3$, HCl, $Cl_2$ for Al; $CF_4$, $Cl_2$ for Ti and its alloys; $SF_6$, $CF_4$, $NF_3$, $Cl_2$, $O_2$ for W and $O_2$, $SO_2$, $CF_4$ for polymers. Thus the proper etching gas composition is important. For example, aluminum is not etched by fluorine, because $AlF_3$ is not volatile, chlorine is used. $AlCl_3$, the etch product, is highly corrosive to the remaining aluminum film, and must be removed quickly after plasma etching. Native aluminum oxide is an etch resistant barrier, which is removed by $H_2$ plasma reduction and/or by sputtering by bombardment with Ar at high energies. A bombardment mixture which includes the additional components Ar, $O_2$ usually enhances the etch rate of the surface[59] and can provide the following increase of possible nanostructures. This was the proof for the concept of ion enhanced chemical etching,[58–60] which is the foundation of plasma etching. It is extremely interesting that by using plasma etching it is possible to introduce some elements at the atomic level on the metal surface.

Wet etching has several important methodological advantages: simple equipment, high throughput (batch process), high selectivity.[61] For uncoated metals, a high-voltage (>1000 V) pulsed discharge method of preparation of thin anodic films on valve metals under conditions of extremely high rates of the film growth was also studied, for example, for titanium[62a,b] and for aluminium[62c] The peculiarities of this method have been demonstrated with the anodic oxidation of titanium in sulfuric acid solutions, $H_3PO_4$. Ammonium pentaborate aqueous solution was used as the electrolyte for aluminium hot pulsed discharge modification, since alumina films prepared by the conventional anodization methods in that electrolyte are well-studied. Thus, the previously discussed electrolytes usually suitable for anodization can be also used for electric discharges. A light flash at the electrode was generated upon pulsed anodization of metals. The emission spectra consist of narrow lines assigned to electronically excited O, H and metal atoms, indicating that hot plasma is created at the electrode surface.

The plasma technique is also widely used for the deposition of nanocomposite coatings and hybrid formation.[63] Moreover, plasma treatment was effectively used for further regulation of pore diameters after anodization to slow down release of encapsulated materials.[64]

### *2.2.3. Laser-Induced Surface Modification*

The development of laser-induced modification[65] of metal surfaces leads to formation of micrometer, sub-micrometer, and nano-scale surface textures. The laser technique can be used for removal of organic molecules from the surface.[66]

A porous metal interface can be formed by laser-induced melting or sintering of metal particles on the metal surfaces.[67] By using a laser, porous layers can be formed on metallic constructions of different shape. The laser technologies have been used to first pattern grooves and ridges onto silicon and titanium.[68] A single-step ultrafast-laser texturing process has been developed. The surface structured by using this method is illustrated in (Figure 2b).[69] The textured titanium samples remained completely hydrophilic with no measurable contact angle even after several weeks in normal atmosphere. An increase in mesenchymal stem cell number was observed on laser-modified surfaces in comparison with an untreated control titanium surface. Extensive formation of cellular bridges by stromal cells between pillars shows the favorable response of differentiated cells to the surface and the promotion of their attachment.

Biocompatibility of the micropatterned NiTi surface produced by femtosecond laser was studied.[70] It was shown that grooves and ripples covered by nanoparticles were formed on the sample surfaces. The crystal structure was not changed by laser treatment. However, the cell culture test proved that the micro-patterns were beneficial to improve the biocompatibility of NiTi alloys: the growth of osteoblasts oriented along the grooves, a large amount of synapses and filopodia were formed due to ripples, holes and nanoparticles on the alloy surface, and the proliferation rate and alkaline phosphatase content of cells were increased after laser treatment.[70]

Besides formation of nanostructured porous surfaces, effective polishing can be achieved by, for example, excimer laser titanium surface treatment.[66]

After the irradiation of a material by short laser pulses of frequencies close to the ablation threshold, periodic structures frequently appear in the form called laser-induced periodic surface structures.[71–73]

### *2.2.4. Chemical Etching*

The mechanism of chemical etching consists of three elementary steps: (i) adsorption of reactive species on the surface; (ii) etch product formation (chemical reaction); (iii) etch product desorption. In general, just simple thermodynamic calculation and comparison of red/ox potentials suggest suitable reagents for chemical etching.[74] Most etching chemical agents (oxidation agent) for particular metal are well known.[74] However, it is still a challenge to predict the surface morphology and chemistry after chemical etching.[75a] It is even more difficult to optimize parameters during modification, for example, concentration, duration of modification, activation steps. Thus, there is still a great area of material science and engineering with hope to have unique functional surfaces.

The alkali-treated titanium[75b] without heat treatment leads to formation of an unstable reactive surface layer capable of bone-bonding. In Takemoto et al.,[76] treatment of titanium in a dilute hydrochloric acid (HCl) leads to the formation of a porous layer known as titanate gel layer.[77] Comparison between three types[78] of surface treatment was demonstrated: (a) alkali and heat treatment (Figure 2d, inset); (b) alkali, hot water, and heat treatment; and (c) alkali, dilute HCl, hot water, and heat treatment (Figure 2d). The porous bioactive titanium implant prepared by treatment had the highest osteoinductivity, with induction of a large amount of bone formation within 3 months.

Chemical treatment of titanium with a mixture of $H_2SO_4$/$H_2O_2$ can uniquely generate sponge-like networks of nanopits within the surface layer of titanium-based metals.[79] The mixture of $H_2SO_4$/$H_2O_2$ firstly triggers the surface etching and its oxidation in a controlled manner.

It has been reported[80] that the titanate gel layer can also be produced using $H_2O_2$ treatment on the surface of pure titanium. It was shown that the surface of Ti–6Al–4V alloy was modified using various concentrations of $H_2O_2$ and subsequently heat treated. A porous anatase titania layer was formed on the surface of Ti–6Al–4V alloy upon treatment with 15 wt% and 25 wt% of $H_2O_2$. In vitro characterization revealed that the treatment of Ti–6Al–4V alloy significantly improved the bioactivity of the material.[81]

### *2.2.5. Sol-Gel Route*

The sol-gel method is one of the suitable ways of preparing films of metal oxides or crystalline materials.[40] For preparing crystalline materials, this method includes crystallization and/or transformation processes. The precursors generally consist of fine particles which are amorphous or crude crystallites. Precursors with various microstructures can be prepared by the hydrolysis and sol-gel conditions, yielding various degrees of crystallization and transformation. The various microstructures enable to control the transition behavior of precursors and produce a desirable structure. The addition of seeds to precursors or using double alkoxides as raw material are ways of controlling the microstructure of precursors, so as to obtain ceramics with desirable properties by the sol-gel method.[82] The titania layer can be also formed by the sol-gel route (Figure 2e).

The sol-gel route (often combined with templating self-organized supramolecular assemblies of small molecules, surfactants, and block copolymers) is used for synthesis of porous materials of various sizes, morphology and highly ordered 3D porous structures.[83] A 3D template composed of self assembled amphiphilic diblock copolymer nanoparticles with diameter below 100 nm provides well ordered film formation (Figure 2f).[84] For example, Janus and core/shell nanoparticles could be self-assembled and well-ordered on the substrate using a vertical immersion method.

The future studies could be directed towards the formation of special structures specifically designed for enhancing sensing properties such as vertically aligned, flower-like, and hierarchical dendrites with the loading of nanoparticles, biocides, drugs, and so forth. Special attention should be paid to the operating temperature for a given metal-oxide and surface configuration, such that the balance between biocompatibility, stimuli response and power consumption can be optimized.

### *2.2.6. Bio-Inspired Ultrasound Assisted Methodology*

There is a perspective bio-inspired approach to fabricate synthetic porous materials via intensive ultrasonication.[85] Ultrasound of high intensity triggers the formation of cavitation bubbles in liquid in a controlled way. Cavitation happens when a rapid change in pressure occurs. In nature, cavitation occurs in fast streams, produced in some shrimps and in the xylem of plants. The cavitation erosion of metal surfaces is known for a long time causing erosion of ocean propellers. Simultaneously, it is also known that surfaces and particles are often exposed to ultrasound in an ultrasonic bath for cleaning. Effects of ultrasound on the surface depend on its intensity, duration of the process, conditions of the sonoreactor (solvent, additives, sonotrode position, etc.)

The most pronounced effects of ultrasound on liquid–solid systems are mechanical and chemical, and these effects are attributed to symmetric and asymmetric cavitation bubble collapses.[86] The symmetric bubble collapse in a liquid medium causes shock waves with high pressures in addition to "hot spots" (high local temperatures in the range of thousands atm and K). The pressure on a material may be focused on defects or grain boundaries. Thus, solid metals with a defined microstructure can provide defined nanostructuring. Moreover, the bubble collapse causes a hot spot, the pressure pulse is also converted into heat, and this may cause local melting. A crystalline surface may locally melt and become amorphous after cooling. Shock waves also potentially create microscopic turbulences.[87] This phenomenon increases the transfer of mass across the solid, therefore, increasing the intrinsic mass-transfer coefficient. Ultrasonically generated active species, for example, radical species such as $OH\cdot$ and $H\cdot$, for water sonolysis,[88] provide effective surface etching, possibly creating a nanostructured surface, such as metal based surface sponges well adhering to the bulk metal.[2a,5e] By asymmetric bubble collapse close to the surface, solvent microjets are formed perpendicular to the solid surface.[89] These microjets have an estimated speed of 100 m $s^{-1}$ and lead to pitting and erosion of the surface. Moreover, the impact of microjets in the metal surface leads to an enhancement in free radical-assisted heterogeneous red/ox reactions (secondary cavity-assisted processes).

The ultrasound-driven modification of metals in aqueous solutions results in the modification of an outer surface (roughness, surface area, surface chemistry) the inner structure of the metals (crystallinity, amorphization, phase separation) (Figure 3).[12,90] The modification of metals by ultrasound depends on the nature of the metal. We have shown that metals with relatively high reactivities (Zn, Al, Mg) could be oxidized during the sonochemical process. The control of the oxidation process allows the formation of mesoporous metals that consist of a metal skeleton stabilised with an oxide layer.[90b] Phase segregation via ultrasonication was also observed in alloys.[90c,d] Beside formation of metal oxide phase, the ratio of the intermetallic phases in the Al/Ni, Al/Fe can be ultrasonically changed. It was shown that the ultrasound-induced red/ox reactions in metal alloys can be applied for design of effective and stable catalysts.[90c,d] Selective oxidation of one component of the alloy (Al) is followed by hydrogen release and reduction of the second component (Ni).[90c]

The ultrasonically formed surface with defined mesoporous metal sponges[2a,5e,90e] can serve as a platform for the construction of surface-attached capsules for the storage of active components and their stimuli controlled release. Even more prospective is the possibility of sonochemical formation of hybrid structures with regulated releases of encapsulated agent.

## 2.3. Composite and Hybrid Materials

It was already mentioned above that together with individual metals their alloys are used for implant formation (Section 2.1). The alloys, in comparison with the individual metals, have better mechanical and biodegradation properties. To achieve other advanced functionalities, biomimetic composite and hybrid materials are suggested as very prospective materials. In focus for bio-application are (i) natural, and (ii) engineered composite materials (composites) which are made from two or more constituent materials with significantly different physical or chemical properties. Natural and engineered materials can be combined to form co-composites. Hybrid materials are composites consisting of several compounds at the nanometer or molecular level. Commonly, one of these compounds is inorganic and the other one organic. Such sub-micrometer scale materials organization/combination can result in characteristics in between the original phases or even new properties. Here we focus on hydroxyapatite (HA), carbon based materials, and metal/polymer hybrids.

### *2.3.1. Biomimetic Composites with Hydroxyapatite*

High priority nanocomposites for bio-application are bioactive calcium phosphate materials, for example, brushite, hydroxyapatite, fluoridated hydroxyapatite, and so forth. The hydroxyapatites have received considerable attention as materials for implants and bone augmentation procedures, since they chemically bond directly to bones[91] and in the formation of a strong bone implant interface. Nanostructured bio-metals can provide better integration with synthetic and natural hydroxyapatite. Moreover, the combination of the high mechanical strength of metals with the osteoconductive properties of calcium phosphates make hydroxyapatite coatings on titanium implants widely used in orthopedic surgery.

The cellular responses depend upon the physical and chemical characteristics of the surface and upon its chemical composition, crystallinity and particle size.[92] The effect of hydroxyapatite surface roughness and generally surface texture on cellular response is a developed area from a practical and fundamental point of view. The oxide layer on a metal is important to provide different nanocomposites. A chemical treatment of the titanium surface would inhibit the negative effect of titanium processing on amorphous layer formation and subsequently hydroxyapatite precipitation. The plasma-spray process, electrophoretic deposition, sputter deposition, and sol–gel methods were used for forming titanium-hydroxyapatite (HA) composites.[93] Additionally, the biomimetic approach was used. It has four main advantages:[94] (i) it is a low-temperature process applicable to any heat-sensitive substrate, including polymers; (ii) it forms bone-like apatite crystals having high bioactivity and good resorption characteristics; (iii) it is evenly deposited on, or even into, porous or complex implant geometries; and (iv) it can incorporate bone growth-stimulating factors.

Hydroxyapatite being deposited on metal surface can be a protective layer which regulats the kinetics of biodegradation of metals. Thus the hydroxyapatite layer can slow down degradation of magnesium-based implants.[95] The method for formation of a dense, strong, and thick calcium-phosphate coating on titanium and porous tantalum implants using a two-step biomimetic procedure was elaborated.

Apatite deposition on surfaces of titanium specimens is a general phenomenon related to surface contact. Some chemical species[96] were released from the titanium surfaces, and, as a result, the properties of the simulated body fluid between the two contact surfaces and/or the properties of the contact surfaces themselves were altered during soaking so as to favour apatite deposition. Oxide-covered titanium is simple in terms of composition. The species that may be released are $H^+$ or $OH^-$ ions and the ions related to titanium.[97] Obviously, the accumulation of $OH^-$ ions on the surface would lead to a more negatively charged surface that is believed necessary for apatite nucleation.[98] The release of titanium hydroxide and $OH^-$ ions from the titanium surfaces and their accumulation inside the confined space between the two contact surfaces were suggested to be responsible for the apatite deposition.

The titanium treated in NaOH can form hydroxycarbonated apatite after exposition to simulated body fluid.[99] The hydroxycarbonated apatite layer, however, was inhomogeneous and non-uniform even after 20 days in a synthetic body fluid (SBF). Acid etching of titanium in HCl under inert atmosphere leads to the formation of a uniform micro-roughened surface that provides improved conditions for in situ hydroxycarbonated apatite formation. After alkali treatment in NaOH the apatite nucleation was homogeneous and the thickness of the precipitated hydroxycarbonated apatite layer increased continuously with time. The treatment of titanium by a two step HCl and NaOH treatment is a suitable method provided the titanium surface with bone-bonding ability. The process of apatite formation on chemically treated titanium is supposed to be similar to that on bioactive glasses.

Composite with hydroxyapatite can provide geometric control of loading and release of material from the surface. For example, the metal based porous layer can be loaded with an active agent and then hydroxyapatite can provide either control of release or be loaded with other agents.

#### *2.3.2. Composites with Carbon-Based Materials: Carbon Nanotubes, Graphene-Based Materials*

The carbon-based materials have a great potential for bio-applications: formation of implant materials (improved mechanics), and biosensors (fast electron transfer kinetics).

Carbon nanotubes are attractive materials in materials science and engineering due to their advantageous mechanical properties including high tensile strength, high resilience, flexibility and other unique structural, electrical and physicochemical properties.[100] Currently, carbon nanotubes are used in several biomedical applications including cancer therapy, treatment of the central nervous system, and tissue engineering applications including bone tissue engineering, sensing cellular behavior, augmenting cellular behavior, cell tracking, and labeling.[101] Furthermore, the composite with carbon nanotubes acts as an excellent reinforcement material with enhanced strength, toughness and flexural strength for major load bearing applications. Moreover, the bioactivity of hydroxyapatite is not affected by the incorporation of carbon nanotubes.[102]

#### *2.3.3. Hybrid Systems*

There are two types of interactions between metals and organic molecules in hybrids: (i) chemical bonding, for example, self-assembled monolayers (SAMs); and (ii) physical interaction, for example, electrostatic layer-by-layer (LbL). In an intelligent system, the hybrids could regulate: (i) the surface-cell interface, (ii) the terminating properties (elasticity, hydrophilicity); (iii) drug loading and release; and (iv) stimuli response.

The 1980s showed an explosion of interest in SAMs-densely packed, oriented monolayer films of long-chain surfactants that spontaneously chemisorb from solution onto solid surfaces.[103] SAMs are ideal models for the study of fundamental processes at organic surfaces, such as wetting, adsorption, adhesion, chemical reactivity and, as result, surface bio-applications.[104] They also can be considered as models for explanation of physical principles that govern the assembly of other supramolecular structures, such as membranes and micelles. Potential applications of SAMs are in the traditional areas of surface chemistry-adhesion, wetting, corrosion and lubrication. The SAMs, often made from amphiphilic hydrocarbon molecules, are expected to function as a barrier to prevent the uncontrollable leaching of drugs from the metal substrate.[105] Three categories of molecules are mostly used for this purpose: fatty acids with carboxylic end groups that form electrostatic interactions with metal substrates, alkylthiols that are used for gold, copper or steel substrate by metal-sulfur bonding, alkyl phosphonates and alkylsilanes that react with the metal oxide from the metal, and metal alloy substrates.

The multicomponent nanonetwork formed by polyelectrolyte multilayers using the LbL technique could offer new opportunities for formation of stimuli response interfaces with self-regulation and self-healing properties.[106] The LbL deposition procedure involves the step-wise electrostatic assembly of oppositely charged species (e.g., polyelectrolytes and inhibitors or others: proteins, nanoparticles) on the substrate surface with nanometer scale precision, and allows the formation of a coating with multiple functionality. The coating properties can be controlled by the number of deposition cycles and the types of polyelectrolytes used. Polyelectrolytes exhibit very good adhesion to the substrate surface and are able to seal surface defects, be sensitive to external stimuli[107] (pH, T, ionic strength) and show self-healing properties. The conformation of polyelectrolytes is mostly dependent on their nature and adsorption conditions and much less dependent on the substrate and charge density of the substrate surface. Polyelectrolyte coatings are expected to cover many kinds of surfaces including non-ionic and a polar substrates. Polyelectrolyte multilayers offer a broad range of applications in the fields of nonlinear optics, light emission, sensing, separation, bioadhesion, biocatalytic activity, corrosion protection, drug delivery, and specific bio-applications based on surface modifications.

Metal hybrids with conducting polymers could be also used for formation of self-healing surfaces sensitive to electric fields. Electrically conducting polymers, for example, biocompatible polypyrrole, have been the subject of continuous research and development due to their potential applications in many technological areas including bio-applications.[108]

Comparing different nanoarchitectures one should consider the dynamics of stimuli response and the amplitude of changes of the materials' properties, reversibility of the changes, and the intensity of the external signal that could trigger the changes. Reconstructable surfaces fall into several categories: (i) grafted polymer thin films (here referred to as polymer brushes); (ii) thin films of polymer networks; (iii) self-assembled multilayered thin films, and (iv) block-copolymer self-organisation on different surfaces depending on their physico-chemical characteristics.

Some of the methods described above have great advantage because of the possibility of single step formation of composites and hybrids. For example, the formation of a metal/polymer interpenetrating hybrid nanocomposite was demonstrated for aluminium with ultrasonic high intensity treatment.[109] An initial sonochemically induced increase in the hydrophilicity can aid in the formation superhydrophobic surfaces by chemical modification of the ultrasonically formed structure. As an example, sodium laurate[110] was attached to the surface with ultrasonic assistance forming a layered double-hydroxide laurate with a water contact angle of 163°, Figure 3e. In the case of unmodified aluminium, the double-hydroxide layer exhibits very poor adhesion properties. In contrast, adhesion of the double-hydroxide layer on a sonicated surface is very strong.

Effective magnesium/polypyrrole hybrid systems were formed by the sonochemical methodology to provide an example of multi-stimuli-responsive systems: (i) sensitive to pH due to magnesium biocorrosion, and (ii) electric fields due to the conductive nature of polypyrrole.

## 3. Active Cell-Surface Interfaces

The cell adhesion, proliferation, differentiation or apoptosis on a solid surface, including metal implants, mainly depends on its surface physico-chemical properties including elasticity, morphology and roughness, texture, porosity, and wettability.[111] After implantation the implant surface is in contact with body fluids and interacts with a number of proteins and different cell types.[112] In addition, implant surface-specific cellular responses are the result of a complex biological system that includes protein adsorption, receptor-ligand binding, and signal transduction.[113] The implant should present a surface that will induce osseointegration. The methodology to control surface-cell interactions is based on physicochemical, morphological and biochemical approaches.[114] According to the physicochemical approach, surface energy, surface charge, and surface composition were altered in order to improve the bone-implant interface. Glow discharge was used to increase the surface free energy in order to increase tissue adhesion.[115] However, increased surface energy does not lead to selectively increased adhesion of particular cells and tissues and bone-implant interfacial strength.[116] Also, both positively and negatively charged surfaces were observed to promote bone formation.[117] Alterations in surface morphology and roughness were used to regulate cell and tissue response of implants. The mechanical interlocking surfaces with grooves and pits can induce "contact guidance", whereby the direction of the cell movement is affected by the morphology of the substrate.[118]

The aim of biochemical surface modification is to immobilize proteins, enzymes or peptides on biomaterials for the purpose of inducing specific cell and tissue responses or to control the tissue-implant interface with molecules delivered directly to the interface. One approach to biochemical surface modification applies cell adhesion molecules.[119] In accordance with a second approach to control cell-biomaterial interactions, one uses biomolecules having demonstrated osteotropic effects.[120]

For biomolecules delivery to the tissue-implant interface, it is essential that local cell populations interact with the biomolecules in order to initiate cellular events, and that concentrations of biomolecules are greater than threshold levels for cellular activity.[121]

To control exposure and concentration, retention and/or release of biomolecules from implants, the surfaces can be altered using different methods, including adsorption and covalent immobilization.[122]

### 3.1. Terminated Properties

In the past few years, interest in regulating bioactivity has shifted to exploration of nanostructured biomaterials.[123] Several reports showed that nanometer-scale surface features can influence cellular attachment, differentiation, and alignment.[124] A simple surface treatment can generate multifunctional nanostructured interfacial layer that could selectively control cell growth. Ideally the surfaces promote the growth of certain cells and inhibit the undesirable growth of other cells without the addition of any exogenous biological or pharmacological agents.

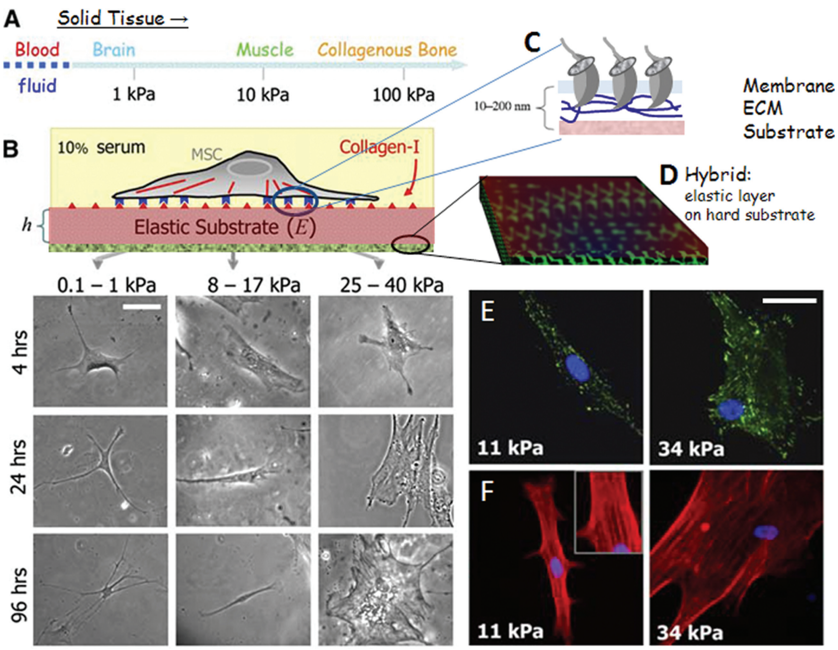

**Figure 4.** Tissue elasticity and differentiation of naive mesenchymal stem cells (MSCs). A) Solid tissues exhibit a range of stiffness, as quantified by the elastic modulus, (*E*–elasticity of micro-environment). B) In vitro gel system allows for control of *E* through crosslinking, control of cell adhesion by covalent attachment of collagen-I, and control of thickness. MSCs of a standard expression phenotype are initially small and round but develop increasingly branched, spindle, or polygonal shapes when grown on matrices, respectively, in the range typical of Ebrain (0.1–1 kPa), Emuscle (8–17 kPa), or stiff crosslinked-collagen matrices (25–40 kPa). Adhesions grow and cytoskeletal organization increases with substrate stiffness. C) Extra cellular matrix (ECM); D) Hybrids for elasticity regulation. E) Paxillin-labeled adhesions grow from undetectable diffuse "contacts" on myogenic gels (11 kPa). On the stiffest, osteogenic gels (34 kPa), the adhesions are long and thin and slightly more peripheral than they appear on glass. (F) F-actin organization shows a similar trend, from diffused on soft gels to progressively organized on stiffer substrates (as stress fibers). Scale bar is 20 μm. Reproduced with permission.[127] Copyright 2006, Elsevier.

The mechanism of the selective cell growth is based on surface structuring regulating the terminated properties or their active chemical local controllable delivery without negative effect on the surrounding environment.

A significant challenge in implantology is the design of biomaterials[125] that actively promote functional regeneration of the host tissue, and avoiding undesirable tissue responses. This requires selective control of interactions at the tissue/implant interface, a series of complex events that depend on synergistic parameters[126] including surface chemistry, elasticity, topography (morphology and porosity), and energy. To date, efforts have focused on defining how microtexture influences the molecular and cellular events of tissue repair.

### 3.1.1. Elasticity

In specific examples, it is highlighted that microenvironments appear as very important factors in stem cell lineage specification. The stem cell specification is difficult to adequately characterize or to control with soft tissues. Naive mesenchymal stem cells (MSCs) were shown to specify the lineage and commit to phenotypes with extreme sensitivity to tissue level elasticity (**Figure 4**).[127] Soft matrices that mimic brain are neurogenic. Stiffer matrices that mimic muscle are myogenic. Comparatively, rigid matrices that mimic collagenous bone are osteogenic. During an initial week in culture, reprogramming of these lineages is possible with addition of soluble induction factors. After several weeks in culture, the cells commit to the lineage specified by matrix elasticity, consistent with the elasticity-insensitive commitment of differentiated cell types. Inhibition of nonmuscle myosin II blocks all elasticity directed lineage specifications–without strongly perturbing many other aspects of cell functions. The results have significant implications for understanding physical effects of the in vivo microenvironment and also for therapeutic uses of stem cells.

### 3.1.2. Morphology

The major role of surface roughness of Ti and its alloys in cell response is elucidated in the literature.[128] Osteoblast-like cells attach more readily to a rough Ti surface. Synthesis of extracellular matrix and subsequent mineralization were found to be enhanced on rough or porous Ti.[129]

Surface nanostructuring could be used for regulation of osteoblast response and bone growth.[130] The ability to control the lateral spacing of these structures is important.[131] Micrometer scale features and optimized lateral spacing enhance cell attachment, spreading, and differentiation by providing higher surface energy and improved hydrophilicity of smooth titanium surfaces. The superimposed sub-micrometer scale features appear to enhance production of local factors that modulate differentiation and other cellular responses.[132] Certain patterns of the surfaces have also been able to control cell growth, being more preferable to adhesion of osteoblasts than to that of other cell types.[133]

In addition, the importance of nanometer scale roughness on bone cell function has been shown by creating ceramic substrates of the same crystallinity, crystal phase, and chemistry, altering only in degree of nanometer surface features.[134] Nanoscale roughness on these nanophase ceramics improved both osteoblastic and osteoclastic responses, whilst simultaneously inhibiting fibroblast function.

A key tenet of bone tissue engineering is the development of scaffold materials that can stimulate stem cell differentiation in the absence of chemical treatment.[135] Nanoscale disorder to stimulate human mesenchymal stem cells leads to formation of bone mineral in vitro in the absence of osteogenic supplements. This approach has similar efficiency to that of cells cultured with osteogenic media. In addition, the current studies show that topographically treated mesenchymal stem

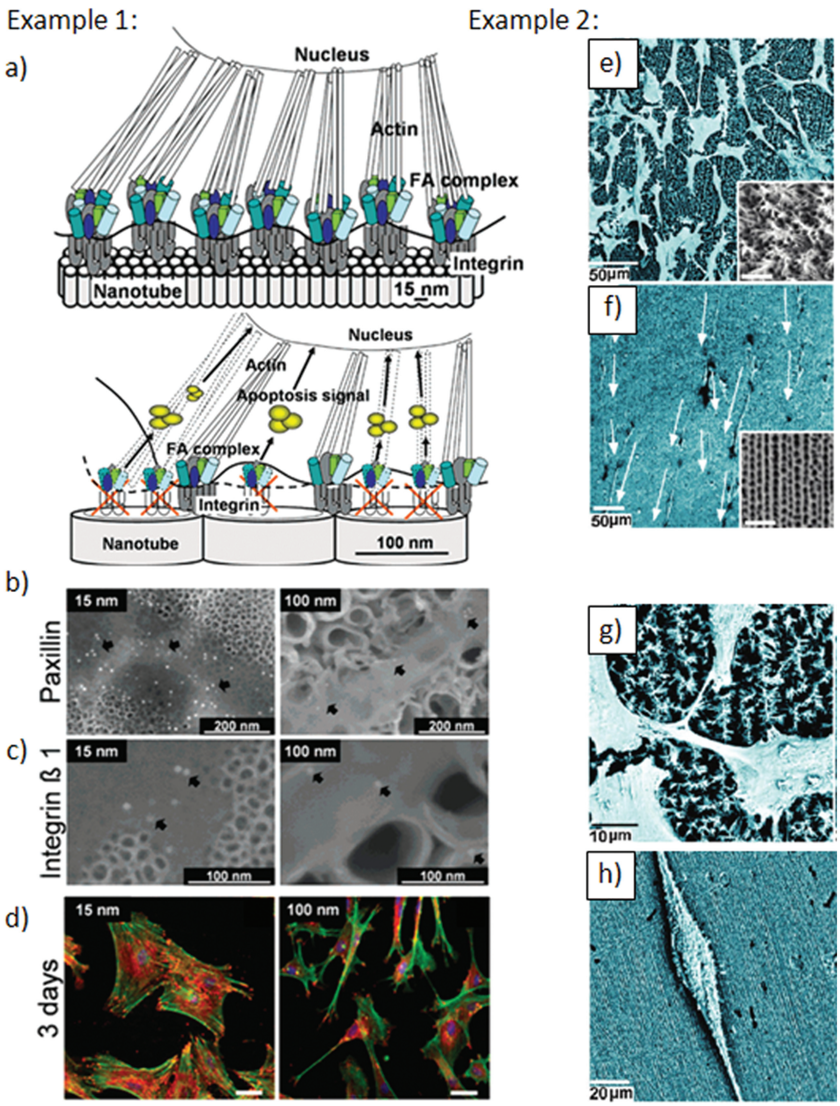

**Figure 5.** Example 1: focal contact formation, differentiation, and apoptosis of mesenchymal stem cells on 15 and 100 nm nanotubes. a) Nanoscale spacing directs cell fate: hypothetical model showing the lateral spacing of focal contacts on nanotubes of different diameters. A spacing of 15 nm seems optimal for integrin assembly into focal contacts, thus inducing assembly of actin filaments and signaling to the nucleus. Nanotubes larger than 70 nm diameter do not support focal contact formation and cell signaling, thus leading to apoptosis (anoikis). b) Analysis of focal contacts by SEM using immunogold staining with paxillin, and c) β1-integrin antibodies reveal dense packing of paxillin in focal contacts on 15 nm tubes, while labeling was sparse on 100 nm tubes. d) At 3 days, cells are well spread on 15 nm tubes (d, right), but develop a migratory morphology on 100 nm tubes with few focal contacts and stress fibers (d, left). Reproduced with permission.[138] Copyright 2007, American Chemical Society. Example 2: morphological changes of neuroblastoma cells grown on anodized aluminum (AAO). e) Polygonal morphology of cells on pores with nanobrushes showing randomly oriented cells with extensive cell to cell interconnection. Inset shows underlying surface with partially collapsed brushes. f) Elevated linear morphology of cells with orientation on which follows orientation of pores (lines). Inset shows underlying surface with aligned pores. SEM images of single neuroblastoma cells grown on different pore morphologies of AAO: g) pores with nanobrushes show an extensive branching and interconnection of cells; h) aligned pores show a linear morphology of neuroblastoma cells directed by aligned pores. Reproduced with permission.[140] Copyright 2010, American Chemical Society.

cells have a distinct differentiation profile compared with those treated with osteogenic media, which has implications for cell therapies.

### 3.1.3. Porosity

The mesoporous surfaces promote[136] both early and longer-term osteogenic events in cell cultures and, therefore, imparting bioactive properties to the materials. It is possible (1) to modulate the formation of nanopores in a titanium alloy (Ti6Al4V), and (2) to control the growth of common cell types on the implant metal. Osteogenic cells were evaluated because they are critical for the successful integration of implants in bone. Fibroblastic cell can form a fibrous capsule, weaken the bone/implant interface, and represent a major complication for permanent implants, ultimately requiring their replacement. Smooth muscle cells were investigated because their hypertrophy and proliferation are widely known to contribute to the restenosis of blood vessels after corrective surgery.[137] In all cases, culture intervals corresponding to initial stages of cell colonization were examined, as these are critical for biomaterial integration at any site of implantation.

$TiO_2$ nanotubes with different diameter were studied to direct cell vitality (**Figure 5**).[138] Self-assembled layers of vertically oriented $TiO_2$ nanotubes with defined diameters between 15 and 100 nm can regulate adhesion, spreading, growth, and differentiation of mesenchymal stem cells. The nanotubes with a spacing <30 nm and a diameter ≈15 nm provided an effective length scale for accelerated integrin clustering/focal contact formation and strongly enhanced cellular activities compared to smooth $TiO_2$ surfaces. Cell adhesion and spreading were severely impaired on nanotube layers with a tube diameter > 50 nm, resulting in dramatically reduced cellular activity and a high extent of programmed cell death. Thus, on a $TiO_2$ nanotube surface, a lateral spacing geometry with openings of 30–50 nm represents a critical borderline for cell fate.

In current trends of diagnostics with microchip biosensors prospects of porous material, nanoporous material, for example, anodized aluminium oxides and ultrasonically modified metal were demonstrated.[139a] In the work of the Losic group,[139b] a microchip biosensor based on nanoporous alumina coated with gold was suggested for detection of circulating tumour cells. Moreover, porous anodic aluminum oxide with mesoporous gradients consisting of a spatial distribution of pore diameters was fabricated using a non-uniform anodization process to design a surface with multi-functional gradients (structural, optical, wetting). This is important for application in microfluidics, diagnostics, drug delivery, and cell manipulation.[140] The pore gradient surface and neuroblastoma cell exhibit potential for pore topography directed cell growth and hence possess biological relevance (Figure 5e,f). Pore gradients fabricated by this method open opportunities to design cell culture platforms with desired structural, chemical, and growth factor controlled cell growth and differentiation.

#### *3.1.4. Hydrophilic/Hydrophobic Properties*

A number of studies provide the evidence that enhancing the hydrophilic properties of the surface enhances cell spreading and cell adhesion. On the other hand, some authors reported that cells have greater adhesion and proliferation on hydrophobic surfaces or on surfaces with intermediate contact angles. Materials able to integrate with bone, for example, titanium and hydroxyapatite, are hydrophilic.[141,142] On the other hand, many polymers used for tissue engineering are hydrophobic in their native state, and require surface modification or wetting procedures before cell seeding.

For instance, Webster and colleagues demonstrated that aqueous contact angles were three times smaller (i.e., more wettable) when the alumina grain size was decreased from 167 to 24 nm.[143] They also reported that the adsorption of vitronectin, which stimulates osteoblast adhesion, increased on nanophase ceramics with greater wettability. Moreover, when vitronectin adsorbed on nanophase ceramics, it was unfolded to a larger extent that on conventional ceramics, which exposed larger numbers of osteoblast adhesive epitopes to the absorbed proteins.

Increased wettability of hybrids has been achieved through the use of nanophase ceramics in order to increase bone cell function. Specifically, Kay and colleagues demonstrated that nanosized titania particles embedded in poly(lactic-*co*-glycolic acid) promoted osteoblast adhesion compared with conventionally sized titania (100 nm) in poly(lactic-*co*-glycolic acid).[144]

In a recent study, Cai et al.[145] investigated the influence of titanium films with micro- and nanoscale topography (by varying the deposition rate and the film thickness) on protein adsorption and cell growth. They studied their chemical composition, surface topography and wettability and found that the films shared the same surface chemistry but exhibited very different topographies. Interestingly, they found no statistically significant differences in protein adsorption, however, albumin and fibrinogen adsorption only were measured. Also, no statistically significant influences of surface roughness on osteoblast proliferation and cell viability were detected in their study.

"Hot" specific examples here are bio-inspired self-repairing slippery surfaces with pressure-stable omniphobicity (SLIPS)[146] for, e.g., antifluidic drag, antibiofouling. Thus, most state-of-the-art liquid repellent surfaces are modeled after lotus leaves, which, due to their rough, waxy surface and contact angle characteristics, are known to exhibit superhydrophobicity and self-cleaning, as water droplets remove contaminants from their surfaces when they roll-off. The SLIPS technology, inspired by the Nepenthes pitcher plant, provides unique capabilities that are unmatched by any other liquid-repellent surface technologies. SLIPS can be optimized for extreme temperature and pressure conditions, rapid self-healing, biocompatibility, and chemical inertness, and so forth. In particular, the biocompatibility of SLIPS and its ability to repel biological fluids allow its application in antifouling coatings for medical devices and instruments, and even for marine vessels. Its pressure resistance is well-suited for deep-sea exploration. It is anticipated that SLIPS can serve as omniphobic materials capable of meeting emerging needs in biomedical fluid handling, fuel transport, anti-fouling, anti-icing, self-cleaning windows and many more areas that are beyond the reach of current technologies.

### 3.2. Surface Drug Depot

The nanostructured surfaces can be used for regulated drug delivery. There are four main strategies to design a surface drug depot: (i) the porous metal interface can accumulate pharmaceutical compounds,[5e,139] (ii) immobilization drugs in a porous scaffold[109,147] (iii) an organic component, for example, micelles, of a metal-organic hybrid can immobilize drugs,[139] and (iv) combine the above strategies to provide multicomponent drug delivery systems sensitive to different stimuli.

In the group of D. Losic[148,54] nanotube arrays (on plates, wires) are suggested as a drug career. Several advanced strategies are suggested for drug loading: (i) chemical bonding of active substances to the wall of titanium nanotubes; (ii) loading of tubes with micelles which contain hydrophobic drugs; (iii) step-wise loading with a certain amount of drug in well ordered 1D nanotube arrays; and (iv) multicomponent loading in anodized metal. Besides release control through pore size regulation, or temporally resolved release due to spatially resolved loading in 1D tubes magnetic fields suggested to control release can be highlighted for the application of intelligent surfaces. Thus magnetic field responsive drug delivery systems using titania nanotube arrays loaded with polymer micelles can serve as drug-carriers due to the special inner tube nanoarchitecture with magnetic nanoparticles loaded at the bottom of the nanotubes **Figure 6**.

Mesoporous metal sponges are effective bases for the construction of surface-attached capsules for the storage of active components and their stimuli controlled release.[139] Sonochemically formed metal-polyelectrolyte capsules **Figure 7**[5e] loaded with active chemicals introduce the possibility of providing metal surfaces with important properties, including high biocide activity, anti-friction properties, and the ability to release active components to stimulate activities of attached cells, such as in stem-cell research. Different ultrasonic intensities result in the formation of structures with different features that determine their subsequent use for active surface construction. The pores of the sonochemically formed metal surface are loaded with biocide and then closed using a complexation reaction between the active incorporated agent and the polyelectrolyte.[149] Thus, smart surface capsules formed by the walls of the metal pores and polyelectrolytes for the prolonged storage of the active component were constructed. The metal walls provide the high stability and adhesion of the capsules to the metal plate; the polyelectrolytes are responsible for the loading and release of the active species on demand. Our system offers an elegant approach to trigger biocide release by adjusting the

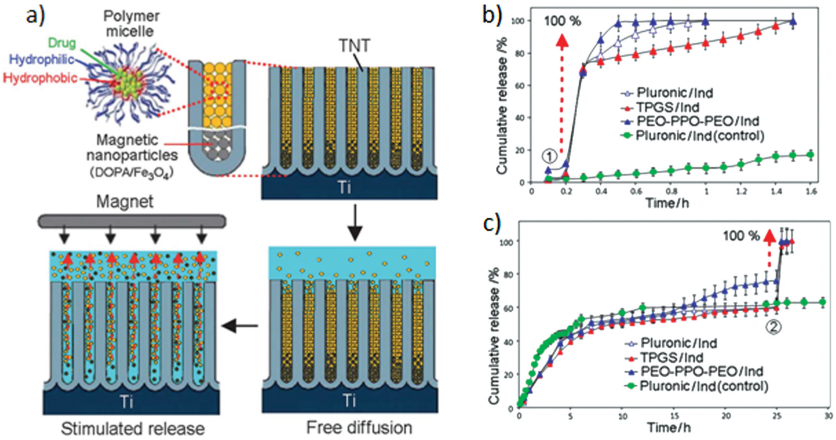

**Figure 6.** a) Scheme of the magnetic stimuli-responsive drug release from titanium nanotubes which integrate polymer micelles as drug carriers loaded with poorly soluble drugs and magnetic nanoparticles. The release is achieved by applying an external magnetic field. b,c) Release profiles of magnetic field triggered release of drug (indomethacin (Ind)) encapsulated in polymer micelles (TPGS, Pluronic and PEO-PPO-PEO). Non-triggered release with Pluronic-Ind was used as a control. Reproduced with permission.[54] Copyright 2012, Royal Society of Chemistry.

mobility of the pH sensitive complex. Moreover, it allows the release of the biocide in a controlled way, which is of high priority (regulated local release). . The possibility of bacteria deactivation due to silver release from sonochemically formed surface capsules was also shown.[2a]

Ultrasonic assisted surface immobilization of magnesium/polypyrrole capsules allows formation of stimuli response systems for release of active chemicals.[109] The hybrid materials can respond to several stimuli. Due to magnesium, the hybrid is sensitive to pH change. Polypyrrole is sensitive to electric field. In this case, no external coating is needed, since ultrasonic induced particle collisions allow melting of metal based capsules in the metal substrate, in particular, Ni patterns on ITO.

Mesoporous silica, titania or $CaCO_3$ based capsules can be homogeneously distributed on the surface by using the sol-gel approach or through formation of polymer hybrid coatings.[147] Micrometer-sized polyelectrolyte capsules made with the LbL technique are not suitable for deposition in sol-gel coating due to their instability in the coating. However nanosized polymer micelles are also promising candidates.

### 3.3. Patterning

The development of a number of high-resolution patterning techniques coupled with functional surface chemistry has enabled the formation of surfaces that offer stringent control over the adsorption of biomolecules and cells in space.[150] Specific strategies for the surface patterning of biomolecules include photolithography, laser ablation, robotic spotting, electron beam lithography, microcontact printing, templating, imprint lithography, soft-lithography, microfluidics, microelectronics, magnetic forces, and so forth.[151] Each of the techniques has its inherent advantages and limitations, for example, electron beam lithography enables high-resolution periodic patterns but is time-consuming.[152]

Patterned surfaces that contain (i) a well-defined surface geometry/topology, can be obtained with additional (ii) localized chemical functionalities being useful in a large field of applications,[153] for example, tissue engineering, cell behavior investigations, co-cultivating of different cells types, artificial growth of neuron networks, and so on. Such surface is urgent for a fundamental understanding between surface cell interactions with precise variation of selected trigger parameters.

A specific example of an attempt to control human mesenchymal cell differentiation using nanoscale symmetry and disorder is the work of M. J. Dalby and co-workers.[154] The nanotopographies had well defined holes and periodic distances between them. In particular, five different patterns were used, all with either absolute or average center-center spacing of 300 nm: (1) square array; (2) hexagonal array; (3) disordered square array with dots displaced randomly by up to 50 nm on both axes from their position in a true square; (4) disordered square array with dots displaced randomly by up to 20 nm on both axes from their position in a true square; (5) pits placed randomly over a 150 μm by 150 μm field, repeated to fill a 1 cm$^2$ area. It would seem likely that one of the initial events in substrate induced stem cell differentiation is the ability of the cells to form mature febrile-like adhesions. The nanotopographies modulated the ability of the osteoprogenitor cells to form very long (>8 μm) fibrillar-like adhesions, with square arrays and hexagonal arrays resulting in shorter adhesions and disordered square arrays with dots displaced randomly by up to 50 nm resulting in longer adhesion. It was also shown that the type of patterning is important for the initial stages of the tissue growth; however after formation of a dense layer of tissue on the substrate the cell growth follows the classical type.

The principle of chemical surface special functionalization for bio-applications can be demonstrated by using polyethylene glycol (PEG) (Figure 2g), polyethylene oxide (PEO), and so on, and selective surface grafting for, e.g., antifouling applications.[155] Cell manipulation on a surface can be achieved through proteins. Synthetic polymers can be combined with protein, for example, photolithographic techniques were used to create PEG hydrogel scaffolds which can be modified with various biomolecules such as peptides, growth factors, and other signaling molecules;[156] this system can be applied in basic science to investigate various aspects of cell locomotion to advance our knowledge in wound healing, inflammation, embryogenesis, and tumor cell metastasis.

Surface manipulation using biomolecules currently permits spatial control over the biomolecule position via patterned surface (i) chemistry or (ii) topography. It is expected that for self-regulated intelligent systems deposition of biomolecules is a strong instrument of nanoarchitecture control for stimuli responsive surfaces.

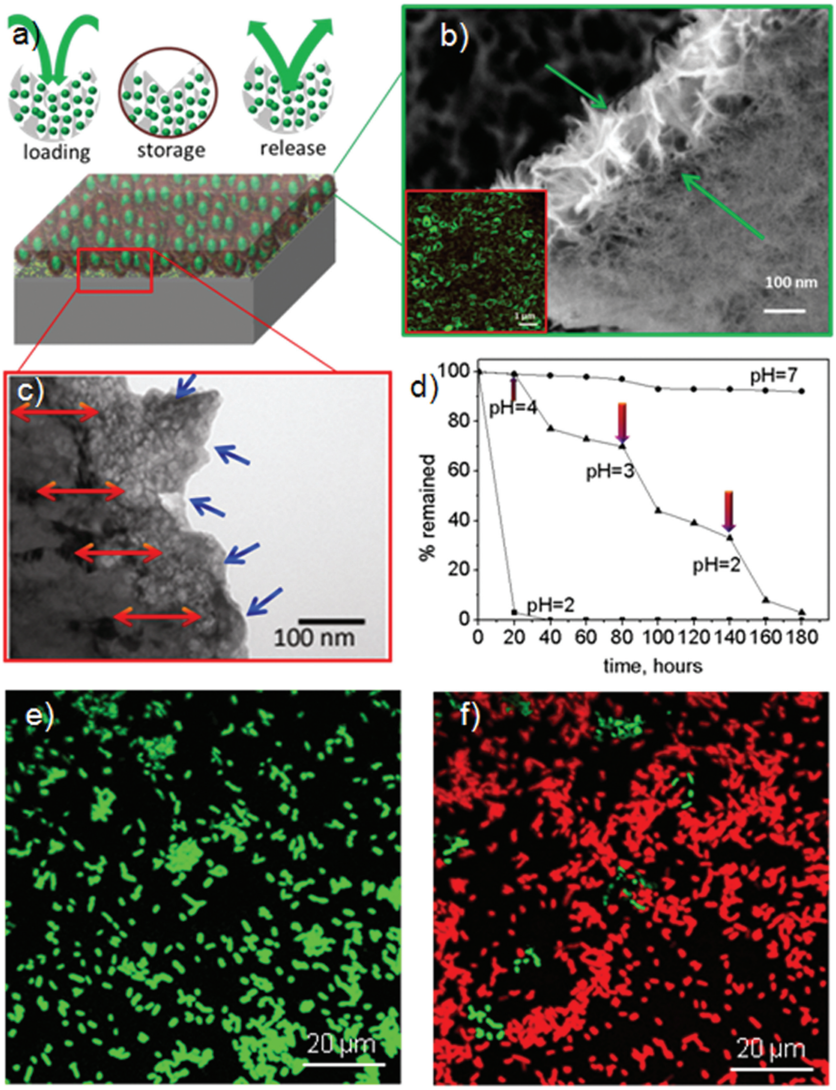

**Figure 7.** a) Schematic illustration of upload, storage and release of active component and general view of capsules generated at the metal surface. b) SEM image of the cross section of an aluminum sponge-like layer and luminescence confocal image of the surface capsules loaded with doxorubicin. c) Transmission electron microscopy image of aluminum with surface capsule layer for chemical storage (blue arrows show the loading direction, red arrows show the interface between bulk metal and capsules' layer). d) Doxorubicin release under different pH. e,f) Confocal images show the inactivation of *E. coli* due to 8-hydroxyquinoline release from the surface capsule layer: e) before release stimulation and f) after release. Inactivated bacteria are red and alive bacteria are green. Reproduced with permission.[5e] Copyright 2012, Wiley-VCH.

### 3.4. Stimuli Sensitive Response

Stimuli responsive behavior, which is intrinsic to natural systems, is becoming a key requirement for advanced artificial materials and devices, presenting a substantial scientific and engineering challenge (Figure 6–10). Intelligent surfaces are able to control the behavior of biomolecules (proteins, peptides, lipids, polysaccharides, cells, etc.) in both space and time. External stimuli (pH, T, ionic strength, humidity, electric or magnetic potential) alter surface properties. In particular, surfaces in response to external stimuli can exhibit (i) changes in adhesion, growth and desorption of cells, (ii) adhesion, mobility and desorption of biomolecules (iii) loading/release of active chemicals (drugs, antifouling and anticorrosion agents). Moreover, the adsorption/desorption/morphology of biomolecules (DNA, proteins, etc.) and cells (bacteria, fibroblast and osteoblast, etc.) onto the surface may be self-regulated by their metabolism (biomimetic of natural system). The possible environmental conditions for this purpose are limited due to the biomedical setting of drug delivery as application. Synthetic materials capable of responses to external or internal stimuli represent most exciting and emerging areas of fundamental scientific interest. In most trends, stimuli responsive changes of a system are inherent to an organic component of a hybrid. However, biodegradation of metal components of a hybrid can also provide release of an active chemical.

#### *3.4.1. Thermo-Response*

Temperature-responsive polymers and hydrogels can be utilized for the preparation of so-called (i) cell adhesion switchable surface, and (ii) "smart" drug delivery systems (**Figure** 8,9a).[155,157] Temperature-responsive systems exhibit a volume phase transition at a certain temperature, which causes a sudden change in solvation. Polymers, which become insoluble upon heating, have a so-called lower critical solution temperature (LCST). Systems, which become soluble upon heating, may have an upper critical solution temperature (UCST). There are also systems, which exhibit both LCST and UCST behavior, but that is usually not occurring within the setting of the intended biomedical applications. Most applications use the change from, for example, room temperature to body temperature in order to induce a change in the physical properties for gelation, especially in topical applications and in injectable biodegradable scaffolds. In vitro applications in cell culture are also using the stimulated swelling and collapsing of hydrogels with their change in surface properties.

Typical LCST polymers are based on *N*-isopropylacrylamide (NIPAM),[158] *N*,*N*-diethylacrylamide,[159] methylvinylether,[160] and *N*-vinylcaprolactam[161] as monomers. A typical UCST system is based on a combination of acrylamide and acrylic acid,[162] PEO-*b*-poly(propylene oxide) block copolymers (PEO-*b*-PPO), PEO-*b*-PPO-b-PEO and PEG-*b*- poly lactic-*co*-glycolic acid-b-PEG.[163]

Thermoresponsive poly(*N*-isopropylacrylamide) (PNIPAM) microgel films[164] allow controlled detachment of adsorbed cells at a temperature change. Cell response occurs on the time scale of several minutes, is reversible, and allows harvesting of cells in a mild fashion.

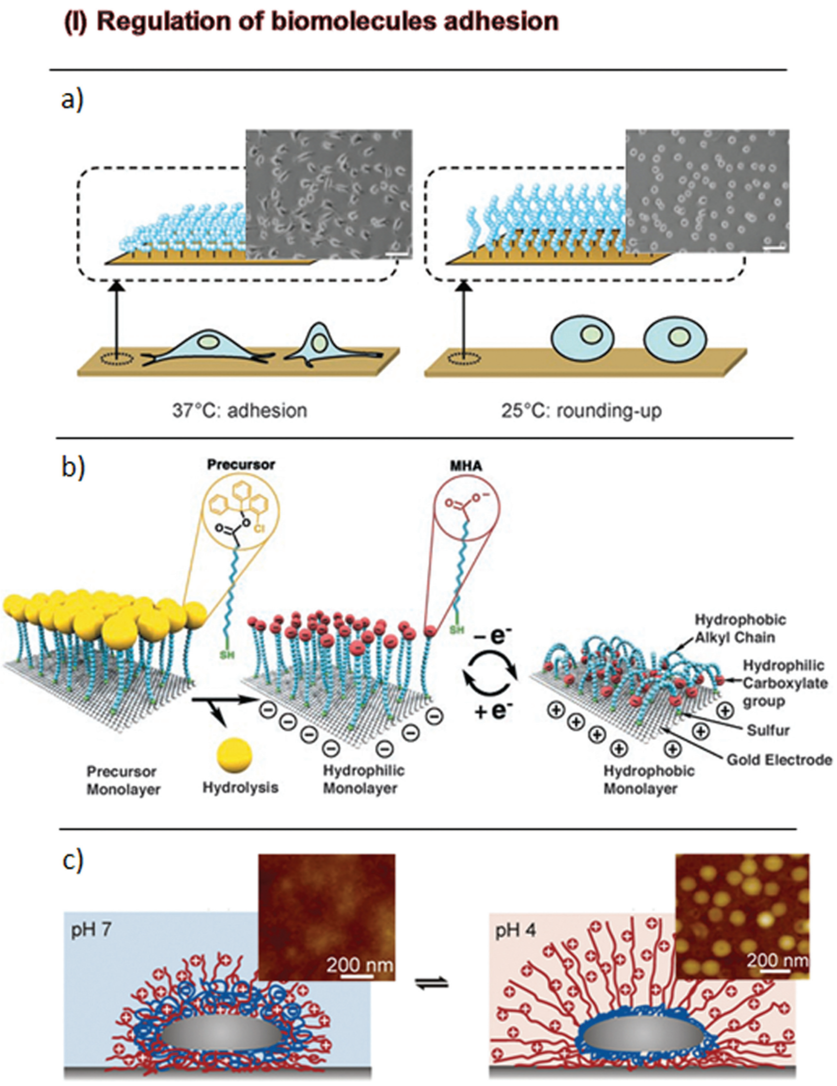

**Figure 8.** Part I: Stimuli for regulation of biomolecule adhesion. a) Thermo-response and phase-contrast microscopy images of L929 mouse fibroblasts behavior of polymer grafted gold substrates coatings at different temperatures. Reproduced with permission.[155] Copyright 2008, Wiley-VCH. b) Electrochemical transition between straight (hydrophilic) and bent (hydrophobic) molecular conformations. Reproduced with permission.[176] Copyright 2003, American Association for the Advancement of Science. c) Surface immobilization of pH-responsive triblock terpolymer micelles on a porous aluminum surface formed by ultrasonication: schematic representation of adsorbed micelles on the porous surface depicting their pH-dependent morphology. Insets show atomic force microscopy images of a sponge-like metal surface before and after adsorption of micelles. Reproduced with permission.[2a] Copyright 2012, Wiley-VCH.

The fact that microgels are attached non-covalently allows using them on a broad variety of (charged) surfaces and is a major advantage as compared to approaches relying on covalent attachment of active films. PNIPAM derivatives undergo a sharp coil to globule transition in water at 32 °C, changing from a hydrophilic state below this temperature to a hydrophobic state. Cell cultures, which prefer more hydrophobic surfaces, on such responsive PNIPAM surfaces can be directly associated with hydrophilic and hydrophobic surface properties. Cells are able to attach to PNIPAM at physiological conditions because of hydrophobicity of PNIPAM modified surface. The cells can be detached due to increased hydrophilicity at low temperature.

Novel smart substrates advantageously combine some features of PNIPAM surfaces (i.e., stimuli response) and PEG surfaces (i.e., bio-repellence at room temperature). Zareie et al.[165] reported on a temperature-responsive SAM based on the synthesis of an oligo(ethylene glycol) (OEG) molecule modified with a phenyl-methyl-trithiocarbonyl-propionic acid 2-dithiopyridyl-ethyl ester that exhibited a LCST of 37 °C. The widespread, successful application of OEG molecules for formation of low-fouling layers makes this molecule an attractive starting point for generating surfaces with switchable low-fouling properties (Figure 8a).[155] Thermoresponsive OEG–Au surfaces allow efficient control over cell-adhesion within a convenient and easily achievable temperature range (25–37 °C).

Thermo responsive LBL films can provide an effective base for formation of hybrid systems for drug delivery. Temperature treatment turns out to be a unique tool to precisely tune the permeability of polyelectrolyte multilayers or also complex micelles containing LbL.[157]

Nanoparticles incorporated in LBL films can absorb electromagnetic irradiation and, thus, provide drug delivery systems with thermo responsive properties.[166]

### 3.4.2. pH-Response

pH variation is well known and a fundamentally investigated stimulus to switch either (i) cell adhesion[2a] or (ii) sorption capacity of delivery systems[167] (Figure 8,9c). In the extracellular tissue tumour tissue has extracellularly a pH of 6.5–7.2, thus slightly lower than the physiological pH of 7.4.[168] After cellular uptake of the drug, release can be achieved in the lysosomes at pH of 4.5–5.0. Hydrolytic enzymes, such as cathepsin B, are also frequently utilized for drug release. The nanometer size of the drug conjugates or micellar structures allow passive targeting of the pharmaceutical compounds.[169] An ideal “intelligent” surface has internal regulation adapted to a particular application.

pH responsive compounds have ionizable functional groups capable of donating or accepting protons upon pH changes. Electrostatic repulsion between generated charges causes alterations of the hydrophobic volume along a polymer chain. The following mechanisms of response of polyelectrolyte multilayers (Figure 2h, 8c) to external stimuli as pH change can be proposed: (i) the polyelectrolytes have pH-buffering activity and can stabilize the pH in the metal surface; (ii) the active chemical can be released pH dependently from the

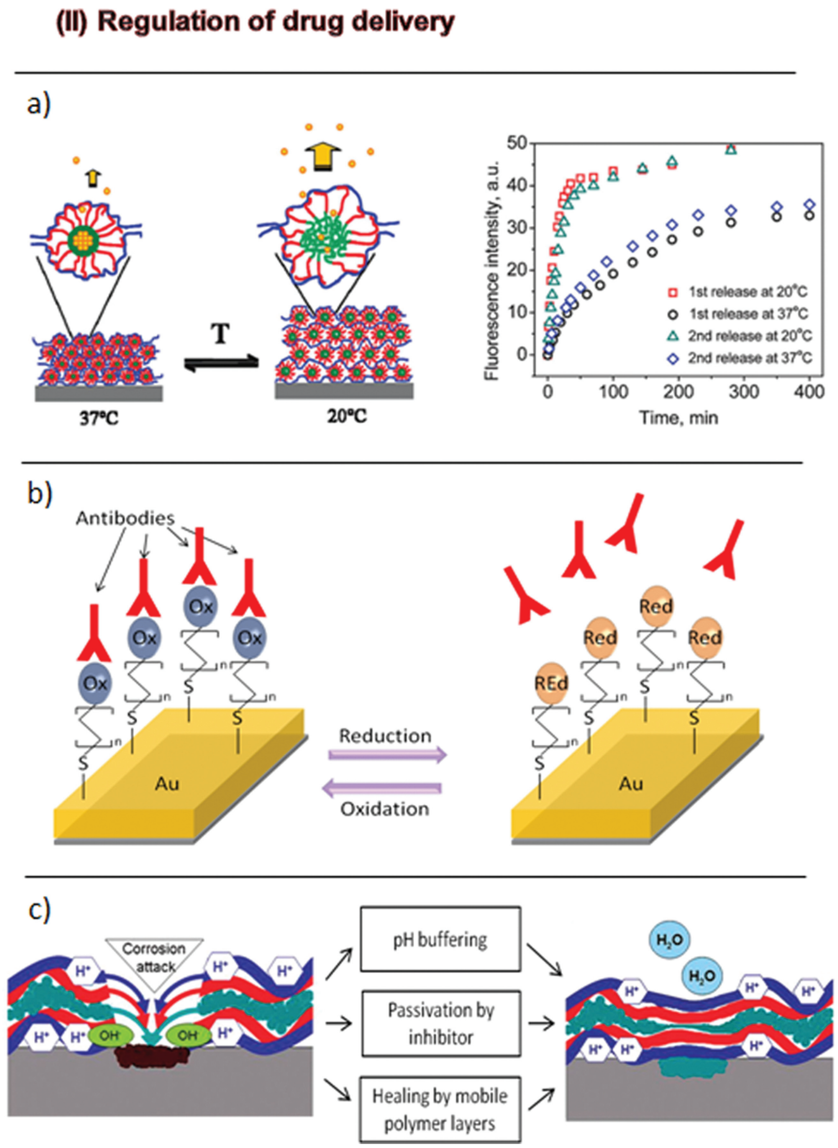

**Figure 9.** Part II: Stimuli for regulation of drug delivery. a) Reversible temperature-triggered swelling of stimuli-responsive block copolymer micelle layers. Release kinetics of pyrene from micelle layers of pH 5.0 buffer solution at 20 °C and 37 °C. Reproduced with permission.[157] Copyright 2009, American Chemical Society. b) Antibodies have a high affinity for benzoquinone but not for its reduced form, hydroquinone. They can thus be reversibly attached to an electrode and released in solution through the oxidation and reduction. Adapted with permission.[177b] Copyright 2010, Nature Publishing Group. c) Schematic mechanism of self-healing effect based on LbL hybrid. Reproduced with permission.[167a] Copyright 2008, Wiley-VCH.

polyelectrolyte multilayers; (iii) polyelectrolytes forming the coating are relatively mobile and have the tendency to seal the mechanical cracks of the coating.[167]

Recently, Peterson et al.[170] demonstrated pH-controlled release of proteins from polyelectrolyte-modified anodized titanium surfaces for implant applications. A polyelectrolyte coating of poly-L-histidine and poly(methacrylic acid) was used for sustained release of negatively charged species (morphogenetic proteins such as BMP-2) under physiological conditions. This complex demonstrated pH-dependent release, with maximum release at pH 5–6, but low levels of sustained release at pH 7–8. Smaller initial burst release and higher amounts of sustained release were observed when lower molecular weight poly(methacrylic acid) was used.

For certain designs of cell surface interactions, it is preferable to have surface contact with developed morphology metal based surfaces, not with polymer layers, or a stable matrix is required. In particular, two types of encapsulation systems based on surface metal sponges have been suggested.[5e] The simultaneous activation/modification of the metal surface and incorporation of active chemicals by ultrasound are suggested in Type I. The metal capsules can be formed and loaded by ultrasound treatment of the metal surface in presence of an active component. As a model (Figure 7) encapsulation of a fluorescent antibiotic (doxorubicin) was presented. The pH dependent release from such capsules is shown in **Figure 10**d. At pH = 7 the encapsulation system is very stable and release from the capsules is negligible. At pH 4-2 we monitored a step-wise release of chemisorbed doxorubicin. At pH lower than 2 the release continues due to degradation of the metal oxide and dissolution of aluminum. Thus, the generation of a pH responsive encapsulation system on a metal surface can be performed in a single-step mode.

According to the Type II capsules, the surface sponges can be used for construction of metal-polyelectrolyte capsules for storage of active chemicals and their release on demand. The pores of metal sponges can be simply sealed by polyelectrolyte complexes.[149] The polyelectrolyte complexes are a universal encapsulation/carrier system and provide safe storage of active chemicals and their release in response to external stimuli, here pH. In this case, one does not need a specific interaction (chemisorption) between metal surface and active chemical. The upload/ release of the active chemical in Type II capsules can be controlled by the formation of a complex between chemical and polyelectrolyte. The complex has one or two pH windows (pH regions of complex instability) and provides an easy way of upload/release manipulation. The surface capsules loaded with 8-hydroxyquinoline (8-HQ) provide long-term antiseptic activity of the metal surface (Figure 7d,e). The metal surface contaminated by *E. Coli* bacteria before and after biocide release is shown in Figure 7e,f, correspondingly. Live and dead bacteria visualization proofs high biocide activity of the metal surface with 8-HQ-loaded capsules.

Recently, bacterial detachment from the surface initiated regulated by bacteria themselves was shown.[2a] Thus, on a metal surface a special type of pH responsive micelles[6b,c] was

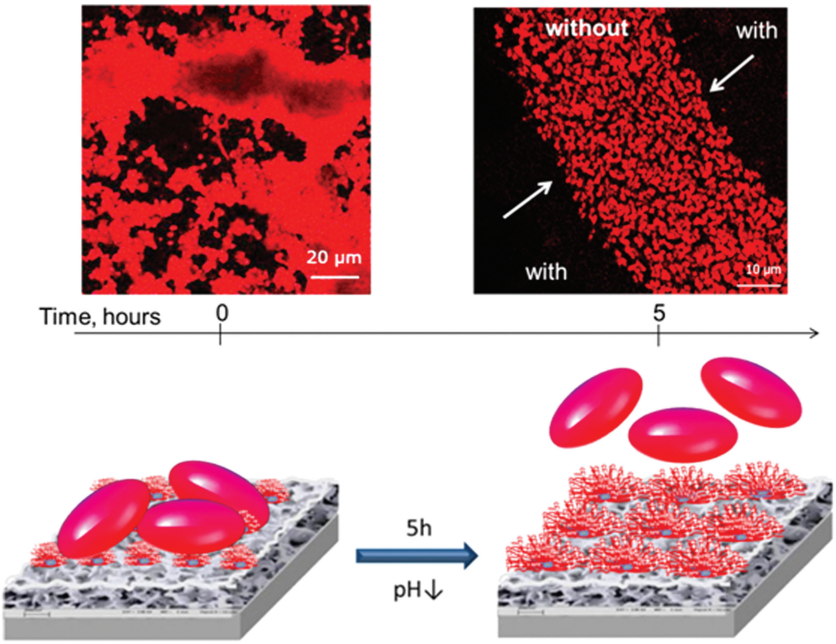

**Figure 10.** Synergetic surface activity: variation of cell adhesion through patterning with pH-responsive triblock terpolymer micelles and self-cleaning by antiseptic/disinfectant properties of porous Ag/Al surface. As model cells, *L. lactis 411* bacteria were used to present the idea of self-controllable intelligent surfaces. Reproduced with permission.[2a] Copyright 2012, Wiley-VCH.

immobilised. The micelles change their corona sizes depending on pH. Increase of micelle's corona size results in effective bacteria detachment from the surface at low pH.

Hydrogels change the orientation depending on pH, which is a significant prospective for both (i) control of biomolecules' surface behavior, and (ii) drug delivery targeting. A novel chemo-mechanical hybrid actuation system that enables pH-responsive reversible motion of microstructures in liquid has been demonstrated recently.[171] Inspired by biological actuators, the system is a composite of passive structural skeletal elements, put in motion by a poly(acrylic acid-*co*-acrylamide) hydrogel muscle that swells and contracts in response to chemical signals. The dynamics and mechanism of actuation were studied using electrochemically generated pH gradients that allow visualization of the hydrogel volume-phase transition and translation into movements of the microstructures.

### 3.4.3. Electro-Magnetic Irradiation

An electromagnetic field initiated by light could be an effective stimulus for (i) biomolecule detachment as well as (ii) drug delivery. The mechanism of surface sensitivity to light is based on light adsorption around adsorbed centers; for example, semiconductors such as $TiO_2$ absorbed effectively UV light, doped $TiO_2$ or its composites can be adapted also to the visible region, noble metal nanoparticles absorb IR light.[172]

Titanium covered with a native oxide layer has been selected to focus on intelligent metal surfaces. Moreover, it was mentioned that the layer can be modified to have different crystallinity, morphology, porosity, and so on, which can have a critical role also for the photocatalytic activity inherent to $TiO_2$. The latter can be used to impart antiviral, antibacterial, and fungicidal properties, and effectively inhibit biofilm formation on the surface of $TiO_2$ implants. Titanium dioxide is known to be activated upon exposure to ultraviolet light, where irradiation of the surface promotes electrons from the valence band to the conduction band leaving a positively charged hole. As electrons and holes migrate, potent oxidative species, such as OH· and $O_2{\cdot}^-$, are produced.[173] The quantity of each species can be regulated by, for example, modification of the surface with metallic, bimetallic particles or formation of mixed oxide composites which can have a significant role to increase antibacterial activity selectively either to gram negative bacteria or gram positive ones.[173b] In particular, the nanostructured photocatalysts $TiO_2$, $TiO_2$:$In_2O_3$, $TiO_2$/Ag, and $TiO_2$/Ag/Ni prepared as thin film on ceramic substrates by spaying oxide sols with subsequent silver photodeposition and electroless nickel deposition were screened for their antibacterial efficiency against *P. fluorescens* and *L. lactis*.[173b] The photocatalysts show higher activity against *P. fluorescens* than *L. lactis* that can be explained in terms of different morphologies of gram positive and gram negative cell envelopes. Gram positive bacteria were more sensitive to $O_2{\cdot}^-$. Probably, active species initiate different deactivation mechanisms of OH· due to cell wall degradation/mineralisation, and $O_2{\cdot}^-$ affects the cell nucleus.

For the preparation of hybrid systems sensitive to UV-light mesoporous $TiO_2$ can be added to, for example, an LbL surface layer. The LbL method in this system can locally enable switching to open/close state by UV-irradiation. It was shown that short-term mild irradiation results in reversible permeability changes due to local variations in pH due to photocatalytic processes occurring at the titania surface.[147c,d] The irreversible decomposition of the LbL film is also possible after long term strong UV irradiation due to photodecomposition of polyelectrolytes.

Different sensitivity of different microorganisms, for example, bacteria and bacteriophages,[173c] provide effective instrumentation to manipulate their live cycles. In particular it was shown that the kinetic activity of the deactivation of bacteriophages dramatically exceeds that of bacterial deactivation. This allows to suggest a procedure of photocatalytic lysogenic bacteria detection including the following steps: (a) daily obtaining lysogenic bacteria cultures by standard methods; (b) cultivation of lysogenic bacteria in a sterile physiological solution and addition of the nanodispersed titania photocatalyst to the resultant suspension; (c) UV-irradiation of the mixed suspension; (d) seeding of the irradiated culture by a standard two-layer method with the subsequent crops incubated for some days; (e) calculation of the number of negative colonies and concentration of phages (plaque forming unit per mL). This is a strong example of providing the stimuli to affect the metabolism of microorganisms, which is of high priority for intelligent surface design and biomimetic of natural systems.

Laser-induced remote release is based on localized heating of the polymeric component in hybrids in the vicinity of metal nanoparticles absorbing laser light.[147b,d,174] Thus, the polyelectrolyte multilayer becomes mobile above the glass temperature $T_g$ of the polyelectrolyte complex. It should be noted that excessive heating is undesirable for living cells, but it is desirable for cancer treatment. Besides noble nanoparticles, to make surface light switchable special molecules can be suggested. For example, azobenzene molecules, which undergo transitions from cis- to trans-configuration upon exposure to light close the pores in a cooperative way thus entrapping molecules inside, e.g., a porous surface.

One more attractive possibility of laser-induced cell detachment on gold nanoparticle functionalized surfaces was demonstrated recently.[175] It is interesting that the selective cell detachment from nanoengineered gold nanoparticle surfaces, triggered by laser irradiation, occurs in a nonthermal manner. It was shown that detachment is attributed to a photochemical mechanism due to production of reactive oxygen species under illumination of gold nanoparticles by green laser light. It was also demonstrated that cells migrate from unirradiated areas leading to their reattachment and surface recovery which is important for controlled spatial organization of cells in self-healing and tissue engineering.

### 3.4.4. Electric or Magnetic Field, Electrochemical Activation

Electric fields can be also used to protonate/deporotonate SAM of acid-terminated thiols. Lahann et al.[176] developed a surface coating on Au that transduced conformational changes in a low-density SAM, initiated by a voltage bias into wettability changes (Figure 8b). The self-assembled surfaces offer exciting opportunities when applied to the manipulation of biomolecules.

The uptake and release of antibodies from modified electrodes (Figure 9b) were described by Sivan and co-workers.[177] In these experiments, antibodies were selected such that they recognize and bind strongly to n-decanethiolbenzoquinones, but have a lower affinity for their reduced form (hydroquinones). This enabled the electrochemical control of their release: antibodies were attached to a benzoquinone monolayer self-assembled on a gold electrode, and subsequently released and re-attached on successive electrochemical reduction and oxidation.

Polypyrrole finds vast applications in biosensors, controlled drug delivery, gas sensors, artificial muscles, and so on.[178] For biological applications, polypyrrole has advantages as it is biocompatible, stable and conductive.[179] Thus, besides using metals to provide sensitivity to electrical potential, biocompatible polypyrrole can be used for the same purpose. Its conductive nature allows an applied current to be switched on and off reversibly, to facilitate the controlled delivery of drugs or the release of other chemicals. In addition, due to its stability, it can protect the capsules used in controlled release systems from degradation before reaching the target site. These properties of polypyrrole help to overcome the present problem of achieving efficient low molecular weight active component delivery. Thus, it was shown that hybrid Mg-polypyrrole composites immobilized onto ITO provide release of encapsulated active agent under electric currents.[109]

Magnetic field effects are very similar to the effects of lasers since both represent various components of electromagnetic fields. In the case of magnetic fields, magnetic nanoparticles serve as absorbing centers producing localized heating centers and inducing release. Recently, the group of Losic[54] demonstrated a very prospective way of magnetic-responsive delivery of drug-carriers using titania nanotube arrays (Figure 6). It was shown that a rapid (1–1.5 h) triggered release of drug encapsulated polymer micelles as drug-carriers with 100% release can be achieved at desirable times of the drug release process. The release of polymer micelles from titania nanotubes is proven to be generic by exploring three types of amphiphilic micelles, and indomethacin, a hydrophobic drug entrapped inside the lipophilic core of micelles. This strategy is particularly valuable for drug-releasing implants in orthopaedics and bone surgery, where on-demand release is needed under conditions (infections) where no time delay can be afforded for drugs to be administered by conventional therapy.

### 3.4.5. Solvent and Ionic Strength

The permeability of polyelectrolyte complexes or micelles on surfaces can also be changed by adding an organic solvent or salt to the aqueous suspension.[180] The decrease in the dielectric constant of the medium increases the strength of the electrostatic interactions between the polyion chains and, thus, leads to a collapse of the polymers. As a result, the LbL are much more permeable than the initial films. It can be noted that solvent used during film manufacture also plays an important role.[181] However, contrary opinions exist about the reversibility of the solvent treatment. For the influence of salt on the properties of polyelectrolyte complexes two concentration ranges have to be distinguished. At very low ionic strength, the complex does not change, but its permeability increases due to a partial breakage of ionic bonds. At higher salt concentrations, the electrostatic interactions between the oppositely charged polyions are sufficiently weakened to enable their rearrangements. Although addition of salt increases the permeability of the polyelectrolyte layer, an opposite effect can be achieved if one polymer in the pair possesses hydrophobic groups, for example poly(styrenesulfonate sodium salt) (PSS). Upon increase of ionic strength, the electrostatic interaction between the charged groups decreases.[182] Moreover, low salt concentrations can even inhibit fusion of layers upon heating.

Altogether a change of ionic strength can be a suitable stimulus due to the difference in ionic strength at environmental conditions and, for instance, in the human body. Thus, for example, the system can be designed in a way to have release of drug after material implanting into the body.

### 3.4.6. Multi-Trigger Response

One advanced trend for stimuli responsive surfaces is the development of a system that can respond to several external and/or internal stimuli in an intelligent way and mimic natural systems. A double-responsive system can be achieved (i) with a hybrid system whose components are sensitive to different stimuli, or (ii) with special nano-organized assembly into micelles, hydrogel. A background idea is to combine in one system the nanoblocks responsive to different stimuli. An example of a hybrid system can be surface organized magnesium-polypyrrole hybrids.[109] An active chemical release from such a hybrid is achievable by pH-change (due to magnesium biodegradation) or an electric field (due to the conductivity of polypyrrole). It can be noted that functionalization by magnetic and metal nanoparticles produces capsules which response to multiple stimuli. Double- or multi-responsive systems can be based just on the polymer architecture. Random copolymers are used to tailor the transition point depending on two independent parameters, for example, pH and temperature. In contrast block copolymers tend to self-assemble reversibly and form micelles depending on the environmental conditions. The micelles are then either stabilised through strong non-covalent interaction (e.g., ionic) or fixed through subsequent crosslinking. In both cases, one obtains a nano-object, which can be utilized as a micellar responsive drug delivery system, but it can also mimic biological entities like vesicles.[183]

Summarizing: (i) noble metal nanoparticles and nanorods can provide surface plasmon resonance in the visible region of the electromagnetic spectrum; (ii) magnetic nanoparticles can guide a system, (iii) semiconductor particles, (iv) quantum dots, (v) carbon nanostructures, (vi) biomolecules, antibodies and targeting molecules, (vii) stimuli micelles, etc., and their combination can provide intelligent systems with multi-trigger response.

### *3.4.7. Self-Regulated Processes: Biomimetic of Natural Systems*

The last example (Figure 10) from our recent work[2a] provides nice support of the presented idea: use the cell metabolism as one of the stimuli. In particular, the novelty of this work is the design of the 3D designed surfaces with both space- and time-dependent functionality. The different surface functionality and reactivity can be controlled by surface patterning and formation of a sponge with active component in its composition. Ultrasonically formed silver/aluminum sponge-like surfaces as well as Ag-free surfaces were used for a self-assembled immobilization of pH-responsive triblock terpolymer micelles. The self-induced regulation which can be assumed as successful step in mimicking natural systems and antibacterial properties were tested by using *L. lactis 411* bacteria as a model system. These types of bacteria produce lactic acid in their life cycle and change the pH of the environment. The micelles change their conformation and bacteria are "pushed off" from the surface. The possibility to regulate cell adhesion was shown through patterned micelle adsorption. The antimicrobial activity clearly indicates antiseptic/disinfectant activity of the Ag/Al sponge surface.

## 4. Conclusions

In this article, the latest achievements in construction of "intelligent" systems that could mimic biofunctions are highlighted. Interfaces adapted to self-control and self-regulation require a complex hierarchical organization of stimuli responsive nano-organized blocks. In living systems, nature broadly exploits the principles of hierarchical organization for self-control and stimuli responsive switching and self-regulation. However, synthetic intelligence is still a challenge. Even more challenging is in vivo design.

We demonstrated how the blocks can be nanostructured and combined to provide system response to environmental changes, stimuli switchability. We focused on metal surfaces, that usually passive surface widely used for implantation. However, as shown in the here, nanostructuring of metal interfacial layer can provide novel characteristics that are attractive for intelligent surfaces. The potential of hybrids and composites for formation of active interfaces is also demonstrated. Polymer systems are suggested as nanoblocks for hierarchical organization in combination with metals.

The design of the 3D architecture surfaces with both space- and time-dependent functionality (cell attraction, pH- trigged self-cleaning, antiseptic/disinfection) is a modern issue in bionanoingineering and materials science. Spatial and temporal biomolecule performance at the surface provided by the surface nanoarchitecture offers advanced bio-applications of metal based materials, such as implantation, organ-on chip and lab-on-chip, biosensors, smart biomaterials, drug delivery systems.

We believe that the area will be explored further in nearest future. A next step in active surface construction can be the surfaces that are applicable for a generation of entire organs due to spatially and temporally resolved co-culture growth regulated by morphology and functions of the interfacial layer.

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