Account

Biosurface Organic Chemistry: Interfacial Chemical Reactions for Applications to Nanobiotechnology and Biomedical Sciences

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In this review, the field of biosurface organic chemistry is defined and some examples are presented. The aim of biosurface organic chemistry, composed of surface organic chemistry, bioconjugation, and micro- and nanofabrication, is to control the interfaces between biological and non-biological systems at the molecular level. Biosurface organic chemistry has evolved into the stage, where the lateral and vertical control of chemical compositions is achievable with recent developments of nanoscience and nanotechnology. Some new findings in the field are discussed in consideration of their applicability to nanobiotechnology and biomedical sciences.

Key Words : Biosurface organic chemistry, Surface organic chemistry, Self-assembled monolayers, Surface-initiated polymerization, Micropatterns

Introduction

Biosurface organic chemistry is an emerging research field in organic chemistry, the ultimate goal of which is to control the interfaces between biological and non-biological systems at the molecular level for the fundamental understanding of biological interactions at interfaces and for the potential applications to (nano)biotechnology and biomedical sciences. The term, "biosurface", implies that the field focuses on surfaces (or interfaces in general) and the surfaces are functional in the interactions with biological entities, such as biomolecules (DNAs, proteins, and polysaccharides) and cells. The biological interactions would occur at non-biological (in other words, man-made) surfaces via either organic or inorganic functionalities, and in the field of biosurface organic chemistry, organic functional groups are designed and introduced onto man-made surfaces to control the biological interactions.

Biosurface organic chemistry is composed of three mutually interacting research fields: surface organic chemistry, bioconjugation, and micro/nanofabrication (Figure 1). One of the aims of surface organic chemistry is to control the physicochemical properties of man-made surfaces by the functionalization of surfaces, yielding "tailor-made" surfaces. The tailor-made surfaces could be either "static", playing any designated roles, or "dynamic", playing switchable roles in response to demands. In addition to the molecular control of interfaces by introducing organic functional groups onto surfaces, another aim of surface organic chemistry is to investigate the similarities and differences between chemical reactions in solution (three-dimensional reactions) and interfacial chemical reactions (two-dimensional reactions)[1] because the rules that govern chemical reactions in solution would be different from the rules that govern chemical reactions at interfaces.[2]

Several factors, including solvent effect, steric effect and electronic effect, could affect the chemical reactivity of functional groups at surfaces: the characteristics in the local solvation of functional groups at interfaces could be different from those in the bulk solvation, and the local concentration of reagents near the interface also could be different. Sterically demanding reactions may be hindered at

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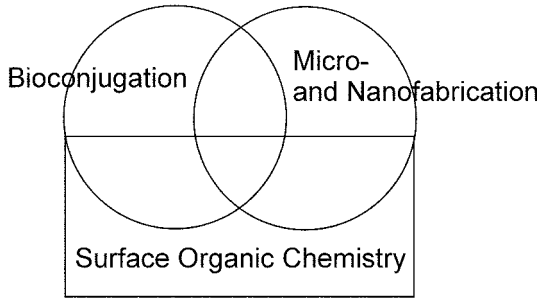

Figure 1. Biosurface organic chemistry, composed of surface organic chemistry, bioconjugation, and micro/nanofabrication.

surfaces. and it is true especially for well-packed monolayers. The pK_a values of certain molecules at interfaces were found to be different from those in solution,[2,3] and chemical and biological reactivities are also altered at interfaces. For example. Mrksich and Houseman studied the role of surface density (χ) of a ligand (*N*-acetylglucosamine. GlcNAc) in the enzymatic activity of bovine β-1.4-galactosyltransferase and reported that the maximum glycosylation occurred with the GlcNAc density of $\chi = 0.7$ (not 1.0).[4] The decreased reactivity of the glycosylation at higher densities than 0.7 is probably due to the steric crowding of GlcNAc at the surface. which would inhibit the enzymatic glycosylation. Similarly. Ryswyk and co-workers investigated the reaction rates of hydroxide-mediated ester hydrolysis in monolayers of 11-mercaptoundecyl isonicotinate ("the ester"). They varied the relative surface density of the ester with decanethiol in the form of mixed monolayers. The hydrolysis of the single-component monolayer of the ester ($\chi = 1.0$) was extremely slow: after the 72-h hydrolysis, no more than 5% of the ester was hydrolyzed at the surface and pseudo-first order rate was calculated to be less than 10^{-6} min^{-1}. In contrast, at $\chi = 0.25$ the initial rate of the hydrolysis greatly increased to be 0.2×10^{-3} min^{-1}.[5]

The acceleration of reaction rates in monolayers could be achieved by either enforced juxtaposition of the reactive functional groups or favorable orientation of the reactive groups. Oliver and Kumar investigated the kinetics of the acyl transfer reaction between thioester and amine (*i.e.*, the amide bond formation) in a monolayer at the air-water interface.[6] The monolayer provided an effective molarity for the reaction of ~500 M as compared with the bimolecular reaction in chloroform solution. The rate acceleration was due to the proximity effect in the monolayer. and the effective molarity in the monolayer was greater than that obtained in related intramolecular reactions in solution (~20 M).

Bioconjugation is required for various applications in nanobiotechnology and biomedical engineering, where biologically-active molecules are attached/immobilized onto surfaces in the controlled manner and biospecific interactions are realized at the surfaces. Microarrays and biosensors mainly depend upon the interactions between the attached/immobilized biologically-active compounds ("ligands") and external biological entities. Target-directed drug delivery systems also require specific interactions between ligands and designated targets in the body, and tissue engineering does interactions between polymeric scaffolds and cells. Microfabrication and nanofabrication techniques give biosurface organic chemists another powerful tool in the control of the interfaces between biological and non-biological systems: spatial control became achievable by introducing organic functionalities onto localized areas of interest at the micro- and nanometer scale. The spatial control could be either two- or three-dimensional depending upon the applications. and would make it possible to further understand biological interactions at interfaces and to tightly control the interactions. It is clear that the basis of bioconjugation and micro/nanofabrication is surface organic chemistry and the fundamental understanding of interfacial chemical reactions would synergically be combined with bioconjugation and micro/nanofabrication.

In this review, we will present some of research results in the field of biosurface organic chemistry. Because of the wide use of self-assembled monolayers (SAMs) in surface sciences and technologies, a large portion of the review contains research results based on SAMs.[7]

Self-Assembled Monolayers (SAMs) and Dynamic Surfaces

Self-assembled monolayers (SAMs). particularly SAMs of alkanethiolates on Au(111) surfaces and of siloxanes on SiO_2 surfaces. have been used in a wide range of research fields (Figure 2a).[1,2,8] SAMs are ordered molecular assemblies formed by the adsorption of active surfactants onto a solid surface. The 2-dimensional order of structures in SAMs is produced by a spontaneous chemical synthesis at the interface. as the system approaches equilibrium. SAMs are excellent model systems for the fundamental understanding

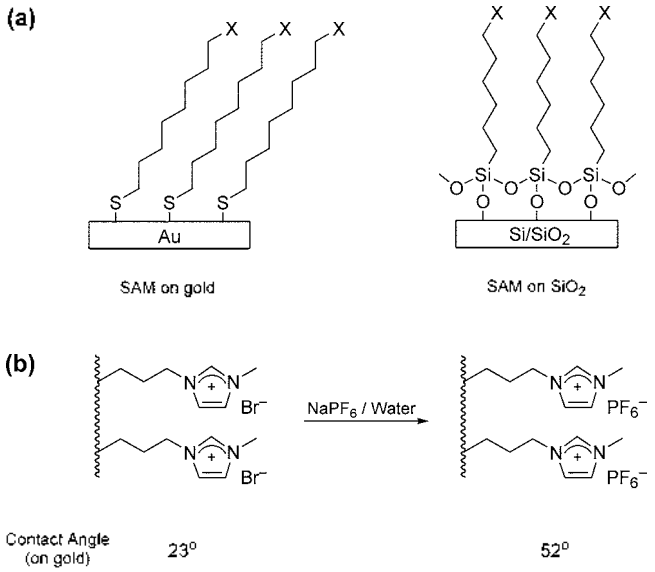

Figure 2. (a) Schematic representation of SAMs on gold and SiO_2. (b) Anion-directed control over water wettability based on imidazolium ion-terminated SAMs.

of self-organization, structure-property relationships, and interfacial phenomena such as wetting, adhesion, lubrication, and corrosion. SAMs also provide the needed design flexibility, both at the individual molecular and at the material levels, and offer the scaffold in the investigation of specific interactions at interfaces.

The structure of SAMs of alkanethiolates on gold was intensively studied by scanning tunneling microscopy (STM).[9] STM provides a visual picture of the SAMs at the atomic and molecular level: alkanethiols form a well-ordered, closely packed structure with a fundamental periodicity of simple hexagonal ($\sqrt{3} \times \sqrt{3}$)R30° with respect to Au(111). It is believed that the c(4 × 2) superlattice structure forms when the monolayers reach the equilibrium state. The alkyl chains are tilted from the surface normal by ~30°, according to IR and X-ray experiments. The closely packed structure of alkanethiols is the foundation of many interfacial phenomena and applications to various areas, such as passivation, lubrication, sensors, anti-stiction in MEMS, electron-transfer barrier, and resists for lithography.

The applications mentioned above are basically dependent upon the formation of "static" surfaces. In contrast, stimuli-responsive (or dynamic) surfaces are surfaces that switch their physicochemical properties (and consequently their functions) in response to external stimuli,[10] and one of their applications is the control of biological interactions such as bioadhesion.[11] The simplest control of physicochemical properties would be the control over water wettability of the surfaces, the applications of which include superhydrophobic surfaces,[12] control over the orientations of liquid crystals,[13] drug delivery and biomimetic materials,[10d,e] and microfabrication,[14] as well as biotechnological applications. Diverse strategies have been developed for controlling wettability of solid surfaces based on SAMs and polymeric films in response to environmental changes (*i.e.*, solvents,[15] pH,[16] temperature,[10a,17] and surface pressure[18]) and external stimuli (*i.e.*, light,[19] charge,[20] and oxidation-reduction[21]), and the methods are mainly based on the reorganization of the internal or surface structures of SAM-forming molecules on surfaces. For example, Langer and co-workers reported the design of surfaces that exhibited dynamic changes in water wettability in response to an electrical potential, where the change in water wettability was caused by conformational transition of methylene groups from all-trans to partially gauche oriented conformation.[20] The key strategy was the formation of low-density SAMs on gold to make room for the conformational transition. The low-density SAMs were generated by the formation of SAMs of 16-mercaptohexadecanoic acid 2-chlorotrityl ester and the cleavage of the acid-labile 2-chlorotrityl group. The 2-chlorotrityl group was chosen on the basis of the theoretical calculations, indicating that an area-per-molecule of 0.65 nm^2 was optimum for steric relaxation and substantial chain overlap.

Azobenzene derivatives have widely been studied for photoswitching systems due to their *cis-trans* isomerization. The cis-trans isomerization is heavily biased toward the trans isomer under ambient conditions. Irradiation with UV light (λ = ~365 nm) causes isomerization to the cis isomer, provided that the azo moiety is not sterically hindered at surfaces. Reversal to the trans state can be achieved either by irradiation with blue light (λ = ~436 nm) or by thermal relaxation. Various alkanethiol-derivatized azobenzenes were synthesized and the possibility of photoswitching was investigated.[22] The photoswitching was not observed in single-component SAMs because the SAMs were closely packed structures and the photoswitching (*i.e.*, conformational transition) was sterically hindered.

The increase of the free volume was achieved by the incorporation of the azo moiety to a macrocyclic amphiphile, *O*-carboxymethylated calix[4]resorcinarene (CRA-CM), where the sufficient free volume was ensured even if the cyclic skeleton of CRA-CM formed a densely packed monolayer.[19b] The photoresponsive SAM was prepared by immersing an aminosilylated silica plate in a dilute solution of CRA-CM, yielding a robust monolayer with dense packing. The photoirradiation of the monolayer with UV resulted in the formation of ~90% cis isomer, leading to an increase in surface free energy (*i.e.*, water wattability), and the photoirradiation of the cis isomer-rich surface with blue light caused the cis isomer to reverse into the trans isomer. Based on the finding, Ichimura and co-workers demonstrated the directional motion of liquid droplets on the flat silica surface by asymmetrical photoirradiation.[19b] The asymmetrical photoirradiation caused a gradient in surface free energy, and the direction and velocity of the motion were controlled by the direction and steepness of the gradient in light intensity.

Another approach to the control of water wettability was to utilize the anion effect on water miscibility of 1,3-dialkylimidazolium salts (known as one of ionic liquids). The hydrophobicity of dialkylimidazolium salts is modulated by changing the length of alkyl groups and/or counteranions, and consequently water miscibility can be varied in solution. The SAMs terminating in imidazolium salts were formed on gold and SiO_2 and the anion effect on water wettability of the SAM-coated surfaces was measured in terms of water contact angle (Figure 2b).[23] On the basis of the observed contact angles of the SAMs, the effects of counteranions on hydrophobicity of the SAMs were quantified in the following order: $NTf_2^- > PF_6^- > CF_3SO_3^- > ClO_4^- > NO_3^- > BF_4^- > Br^-$. Additionally, the *N*-alkyl chain length of 1-(12-mercaptododecyl)-3-alkylimidazolium salts was varied from n = 1 (methyl) to n = 6 (*n*-hexyl) and the effect of the *N*-alkyl chain length on water wettability was investigated. Lee and Lee found that water wettability was greatly affected by counteranions in the cases of the SAMs bearing short alkyl chains (n = 1 to 4) but the anion effect diminished in the cases of SAMs with *n*-pentyl and *n*-hexyl group.[23c] The little change in the contact angle implies that the anions may be embedded in the relatively long alkyl chains and be in close contact with imidazolium cations.

Organic Reactions on SAMs and Bioconjugation

SAMs are one of the topics intensively studied in the last

decade because of fundamental interest in interfacial reactions and many technological applications such as microarrays, (bio)sensors, catalysis, and biocompatible coating.[24] Especially, the SAMs of alkanethiolates on gold are structurally well-defined, and therefore they are an excellent model system for studies in biosurface sciences. Two methods are currently used for the generation of surfaces or surface films presenting biomolecules or ligands based on SAMs on gold:[25] (1) direct method: any desired molecules (usually alkanethiols carrying biomolecules or ligands for the formation of SAMs on gold) are designed and synthesized separately in solution, and the synthesized molecules are assembled on gold surfaces, and (2) common intermediate method: SAMs are first formed on gold surfaces, where the SAM-forming thiols contain (potentially) reactive chemical groups at their tail ends. Carboxylic acid groups are usually utilized[26] for an amide bond formation via acid chloride,[27] interchain anhydride,[28] pentafluorophenyl ester,[29] or *N*-hydroxysuccinimide (NHS)-activated carboxylic acid.[30] Compared with the direct method, the common intermediate method does not require cumbersome separate synthesis of molecules in solution and could easily be applied to the generation of micro- and nanoarrays with the existing techniques for generating micro- and nanopatterns such as spotting,[31] ink-jet printing,[32] micro-and nanocontact printing,[33] dip-pen nanolithography (DPN),[34] and other scanning probe microscope (SPM)-based methods.[35] Another disadvantage of the direct method is a limited compatibility of functional groups (especially for SAMs of siloxanes on glass or silicon oxide surfaces). As an example of the common intermediate method, nitrilotriacetic acid (NTA) was coupled with NHS-activated carboxylic acid-presenting SAMs on gold for the orientation-controlled immobilization of histidine-tagged proteins (His-tagged proteins) (Figure 3).[36] three different SAMs terminating in carboxylic acids were formed with $HS(CH_2)_{15}COOH$ (C15-COOH), $HS(CH_2)_{11}(OCH_2CH_2)_3$-$OCH_2COOH$ (EG3-COOH), and $HS(CH_2)_{11}(OCH_2CH_2)_5$-$OCH_2COOH$ (EG5-COOH), and the lateral packing densities of the SAMs were calculated to be 4.32 (for C15-COOH), 3.49 (for EG3-COOH), and 2.65 (for EG5-COOH) molecules/nm^2, respectively. The yields of the coupling reaction at the surfaces were estimated to be 25-30% by the X-ray photoelectron spectroscopy (XPS) analysis, generating the surfaces presenting approximately one NTA molecule/nm^2. The surface plasmon resonance (SPR) experiments on the immobilization of a His-tagged protein revealed that the ethylene glycol linker was of importance in minimizing non-biospecific adsorption of proteins.

One of the newly-developed and intensively-used SAM-based reactions is Diels-Alder (D-A) reaction of cyclopentadiene and quinone (Q), developed by Mrksich.[37] The SAMs terminating in hydroquinone (HQ) were formed on gold and HQ was oxidized to Q electrochemically (Figure 4a). Advantages of the *in situ* oxidation of HQ to Q on the gold substrates over the formation of SAMs terminating in Q include (1) "oxidation-on-demand", where Q was generated when required, changing the D-A-inert surface to the D-A-reactive surface ("dynamic surface"), and (2) "localized activation", where the oxidation of HQ to Q could be controlled spatially in combination with the existing microfabrication and nanofabrication techniques since the oxidation is achieved by electrochemical method. Mrksich reported the physical organic studies of substituent,[37a,b] solvent,[37c] and steric effect[37d] on the D-A reactions at surfaces, and also demonstrated the chemically controlled attachment/detachment of various biologically-active molecules (such as biotin,[37e,38a] peptides,[37f-h,38b,c] proteins,[38d] and saccharides[37i,38e]) onto/from the surface. For example, Mrksich and co-workers reported the detachment and subsequent attachment of a biologically-active Arg-Gly-Asp (RGD) peptide by incorporating *O*-silyl group into HQ and facilitating the hydrolysis of the silyl ether (Figure 4b).[37h] In addition, the electrochemical oxidation of HQ to Q was sophisticatedly utilized to remove the protecting groups (Q

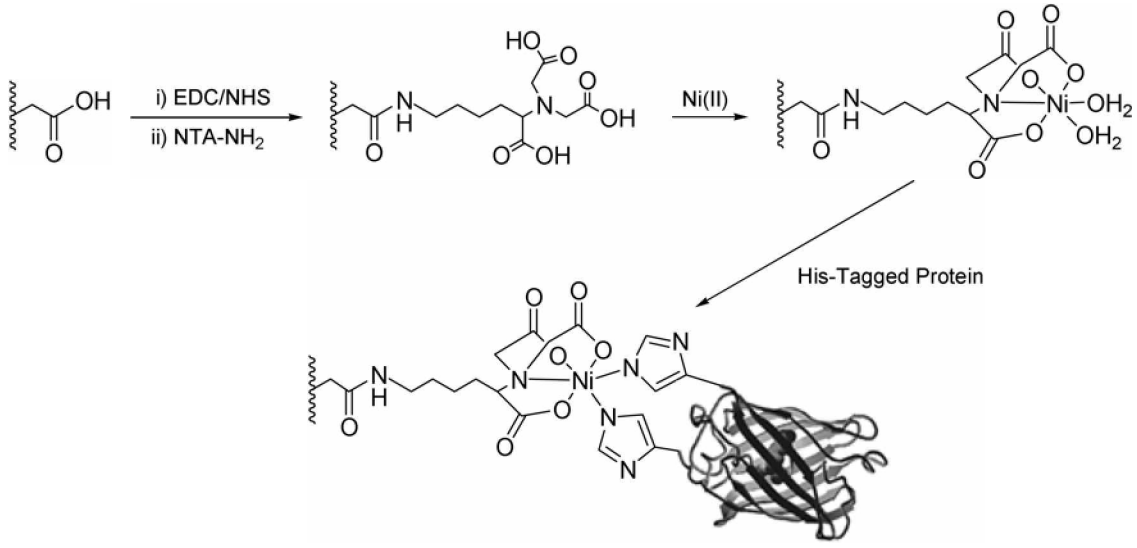

Figure 3. Immobilization of histidine-tagged proteins onto NTA-presenting surfaces.

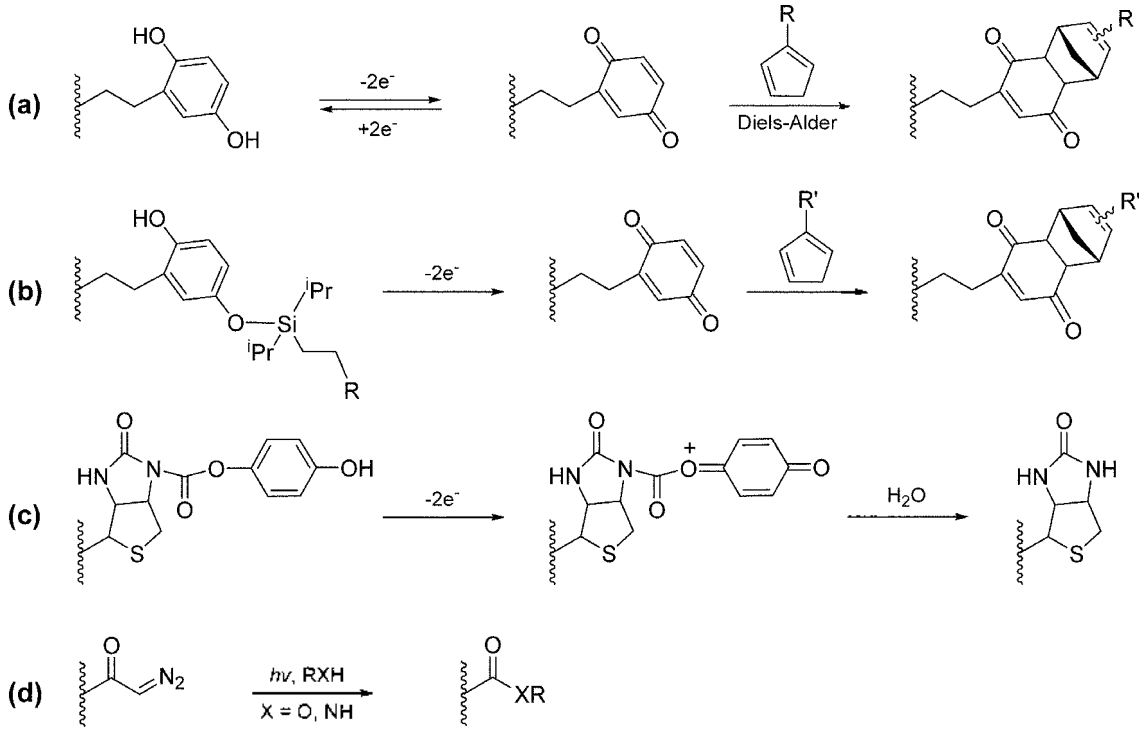

Figure 4. Some examples of organic reactions on SAMs. (a) Oxidation of hydroquinone to quinone and Diels-Alder reaction, (b and c) oxidation of hydroquinone to quinone and hydrolysis, and (d) Wolff rearrangement.

and CO_2) from biotin, which were attached onto gold, and to site-selectively control the bioactivity of surfaces (from bioinactive protected biotin to bioactive deprotected biotin) (Figure 4c).[38f] Another D-A reaction at surfaces was tested: vinyl-terminated SAMs were formed on SiO_2 and 2-(13-hydroxy-2-oxatridecanyl)furan was delivered to the surface by an atomic force microscope (AFM) tip, resulting in nanopatterns through the D-A reaction at the surface.[39] D-A reactions were also investigated at polymeric surfaces:[40] a furan ring-presenting surface was fabricated by pulsed plasma polymerization of furfuryl methacrylate and the D-A reaction with maleic anhydride was achieved at the polymeric surface.[40a]

Photochemical reactions were advantageous in the generation of micro- and nanopatterns because they can easily be combined with existing photolithographic techniques. One of the examples is Wolff rearrangement of surface-anchored α-diazo ketone groups upon the UV irradiation (Figure 4d).[41] The study on Wolff rearrangement in the SAM of 2-diazo-13-mercaptotridecan-2-one showed that only the sterically least demanding methanol formed the corresponding ester.[41a] and an adamantane-based tripodal surface anchor was developed to reduce the end group density of SAMs.[41b]

Control over the surface density of specific functional groups is also of importance in the bioconjugation and subsequent biological interactions, because the optimal density of ligands for the biological recognition is not the highest surface density but would depend upon the systems.[42a-d] A conically shaped dendrimer, dendron, was utilized to control the space between functional groups[42e] and the enhanced interactions between surface-immobilized biotin and external streptavidin were demonstrated.[42f]

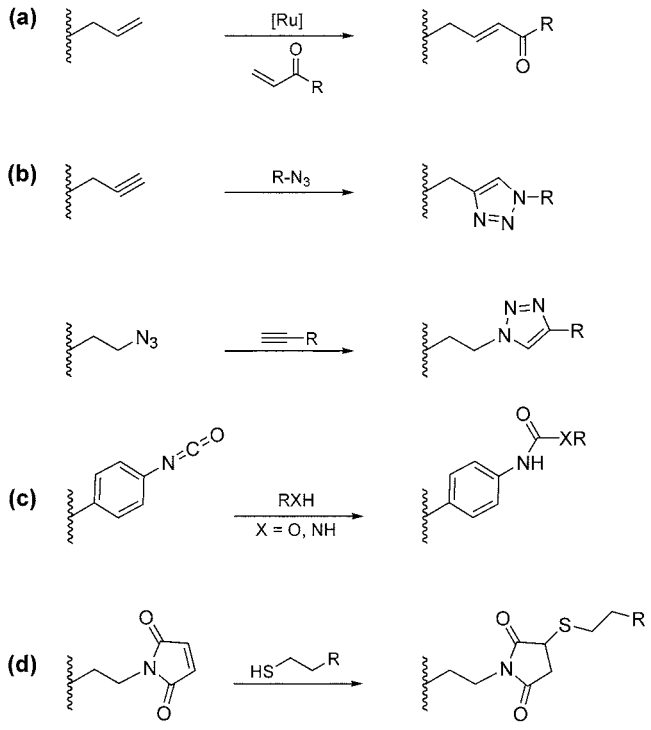

Figure 5. Some examples of organic reactions on SAMs. (a) Olefin cross-metathesis, (b) triazole formation, (c) addition to isocyanate, and (d) Michael addition.

SAM-based reactions should be performed under mild reaction conditions (due to the chemical and thermal instability of SAMs) and be highly yielding (due to the difficulty in fully characterizing the SAM-based reactions) and in this respect some highly-yielding transition metal-catalyzed coupling reactions have also been investigated as a candidate for versatile SAM-based reactions. Ruthenium-catalyzed olefin cross-metathesis was performed on the vinyl-terminated SAM and α,β-unsaturated carbonyl groups were introduced onto the surface (Figure 5a).[43] Sharpless "click" chemistry, triazole formation between acetylene and azide, was also studied for introducing various organic functionalities directly onto the surface (Figure 5b).[44] Other examples include coupling reactions between surface-immobilized isocyanates and some functional groups, such as alcohols, amines and water (Figure 5c),[45] and Michael addition reactions of thiol compounds to maleimide-terminated SAMs (Figure 5d).[46] In particular, Michael addition was adapted to the DPN-based generation of nanopatterns:[46b] cowpea mosaic virus capsid particles were engineered to present cysteine on their outer surface and the engineered particles were immobilized onto maleimide-presenting surfaces by DPN.

Surface-Initiated Polymerization

Polymeric thin films have attracted a great deal of attention because of their applications to biocompatible medical implants, sensors, and microfabrication.[47] Among the methods for forming polymeric thin films, surface-initiated polymerization (SIP) has been a great success in grafting polymers onto solid substrates.[48] SIP, in which a polymerization initiator is directly bound onto a surface (mainly through the formation of SAMs) and a polymer chain is grown from the surface, has been investigated to improve the stability of grafted polymers and to increase grafting density of polymers on substrate surfaces (Figure 6a).

Various polymerization methods have been applied to SIP including radical,[49a-g] cationic,[49h] anionic,[49i-k] ring-opening metathesis,[49l-n] and ring-opening polymerization (ROP).[24d,f] The first example of SIP was surface-initiated, ring-opening polymerization (SI-ROP) of *N*-carboxyanhydrides of amino acids from amine-terminated monolayers, where a tripod linker containing primary amine was used to reduce steric crowding.[50] Russell and co-workers reported the formation of poly(2-(dimethylamino)ethyl methacrylate) (pDMAEMA) films by surface-initiated, atom transfer radical polymerization (SI-ATRP) (Figure 6b).[49g] The tertiary amine groups of the pDMAEMA film were quaternized using ethyl bromide, and the antibacterial activity of the surface was evaluated. Incubation of the substrate presenting the quaternized pDMAEMA film with either *Escherichia coli* or *Bacillus subtilis* demonstrated that the functionalized surface had substantial antimicrobial capacity, and the permanence of the antimicrobial activity was tested by repeated use of the substrate. The covalent attachment of pDMAEMA onto surfaces through SIP yielded permanent, nonleaching

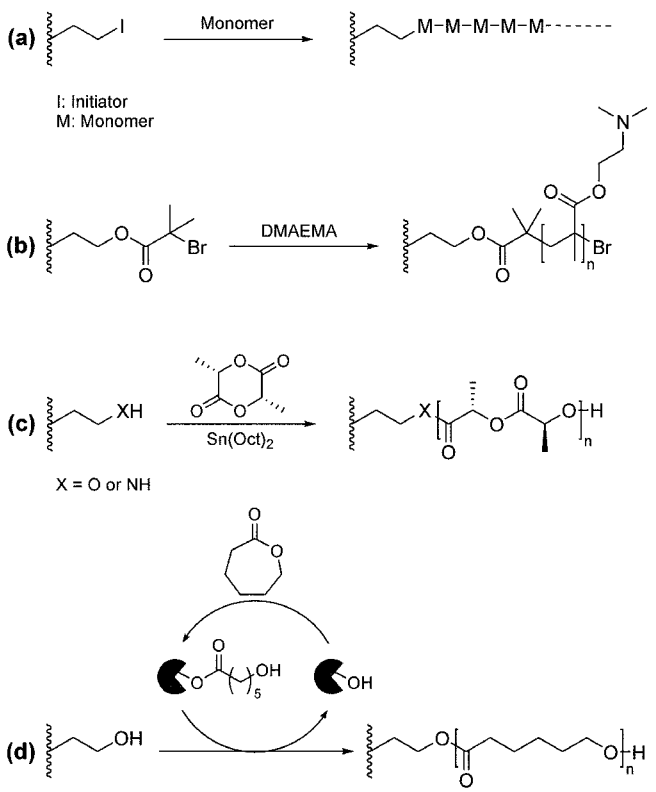

Figure 6. (a) General scheme of surface-initiated polymerization, (b) surface-initiated, atom transfer radical polymerization of 2-(dimethylamino)ethyl methacrylate, (c) surface-initiated, ring-opening polymerization of L-lactide, and (d) surface-initiated, enzymatic polymerization of ε-caprolactone.

antibacterial surfaces, where polymer composition, surface density, architecture and functionality could tightly be controlled compared with other grafting techniques.

The surface-grafted pDMAEMA was also used as a synthetic counterpart to silaffins in diatoms. Silaffins are posttranslationally modified peptides where many of the lysines are modified to ε-*N*-dimethyllysine or oligo-*N*-methylpropyleneimine-linked lysine. The biosilicification in diatoms is achieved by specific interactions between silicic acid derivatives and silaffins: the self-assembled structure of the peptide part of the silaffins is thought to act as a template for the *in vivo* polycondensation of silicic acid derivatives catalyzed by the long-chain polyamines. The long-chain polyamines and other amines found in the silaffins are mostly methylated tertiary amines and therefore tertiary dimethylamino group-containing pDMAEMA was tested as a synthetic catalyst for the biomimetic formation of silica films on surfaces.[51]

The formation of non-biofouling surfaces, which prevent non-biospecific adsorption of proteins and adhesion of cells, is crucial in many biotechnological applications, including the passivation of implants and drug delivery systems, and the enhancement of sensitivity in microarrays and (nano)-biosensors. Although SAMs terminating in oligo(ethylene glycol) on gold have intensively been used to generate non-biofouling surfaces, there are some drawbacks in the use of

SAMs for practical applications, such as long-term instability. Polymer-based approach would be an alternative to SAMs because multivalent interactions of polymers with surfaces would increase the stability of grafted films and the molecular architecture could easily be controlled.[52] Chilkoti and co-workers used SI-ATRP to graft a poly(ethylene glycol)-containing polymer onto gold surfaces:[52c] poly(ethylene glycol) methyl methacrylate (PEGMA) was polymerized from a gold surface and the resulting surface was found to exhibit no detectable adsorption of proteins and to be cell-repellent for up to a month under typical cell culture conditions.

For possible applications in the biomedical sciences, such as passivation of drug delivery devices and implants and generation of microenvironments for tissue engineering, the SIP of biocompatible and/or biodegradable polymers was recently reported, such as SI-ROP of biodegradable polyesters[24d-f] and SI-ATRP of thermoresponsive poly(*N*-isopropylacrylamide) (PNIPAAm).[49f,53] Langer and Choi reported tin(II) octoate ($Sn(Oct)_2$)-catalyzed SI-ROP of L-lactide from hydroxyl- and amine-terminated SAMs (Figure 6c).[24d] The surface coated with biodegradable and biocompatible polymers, such as poly(L-lactic acid) (PLLA), could offer a model surface to investigate the biological interactions between biomedically relevant man-made surfaces and biomolecules and cells, as well as the biomedical applications mentioned above. Surface-grafted PLLA films could also be used for fundamental studies on interfacial phenomena:[54] the adsorption of PLLA and its enantiomer, poly(D-lactic acid) (PDLA), onto surface-grafted PLLA films was investigated. The entropic repulsion of free PLLA chains from the grafted PLLA layer caused the resistance of surface-grafted PLLA to the adsorption of free PLLA, while the entropic repulsion was suppressed in the case of free PDLA by stereocomplexation between the grafted PLLA and free PDLA chains.

PNIPAAm has intensively been studied for the controlled attachment/detachment of cells:[49f,53d] cells adhere, spread and proliferate at 37 °C, and at 25 °C the cultured cells are detached spontaneously from the surfaces without any enzymatic or mechanical means because of the phase transition of PNIPAAm. The lower critical solution temperature (LCST) of PNIPAAm is about 32 °C and the phase transition of PNIPAAm in water takes place over a narrow range of temperature (1-2 °C). Above the LCST, PNIPAAm is hydrophobic (cell-adherent) in water due to dehydration (loss of hydrogen bonding between the isopropylamide moiety and water molecules) and subsequent aggregation of the polymer chains, while PNIPAAm is hydrophilic (cell-repellent) in water due to the hydrogen bonding below the LCST.[55] Kang and co-workers reported SI-ATRP of NIPAAm and PEGMA from the hydrogen-terminated Si(100) (Si-H) surface.[53d] The surface coated with a copolymer of PEGMA and NIPAAm (1% PEGMA; film thickness: ~30 nm) yielded more rapid cell detachment during the temperature transition from 37 °C to 20 °C than the surface coated with only PNIPAAm.

Enzymatic, surface-initiated polymerization of aliphatic polyesters was reported for wider clinical use of aliphatic polyesters: the hydroxyl-terminated SAM acted as an initiation site for lipase B-catalyzed ROP of aliphatic polyesters, such as poly(ε-caprolactone) and poly(*p*-dioxanone) (Figure 6d).[24e] Another example of enzymatic SIP is the polymerization of poly(3-hydroxybutyrate) (PHB), where PHB synthase, fused with a His-tag at the *N*-terminus, was immobilized onto solid substrates through transition-metal complexes, Ni(II)-NTA, and the immobilized PHB synthase catalyzed the polymerization of 3-(*R*)-hydroxybutyryl-Coenzyme A (3HB-CoA) to PHB.[56] The difference between the two examples is that lipase B is free in the solution and acts as a monomer carrier (*via* the formation of enzyme-activated monomer (EAM) complex) while PHB synthase is immobilized onto surfaces and PHB is elongated from the surface-immobilized PHB synthase.

The lateral control of chemical compositions could be achieved by the combination of micro/nanofabrication and SIP:[53c,57] and the vertical control by block copolymerization.[49d,58] Mirkin and co-workers combined DPN and surface-initiated, ring-opening metathesis polymerization (SI-ROMP) to generate polymer brush arrays with nanoscale features. Of importance, they showed a possibility of generating combinatorial polymer brush arrays by controlling the tip-substrate contact time and using different monomers, in combination with block copolymerization.[57b]

Generation of Micro- and Nanopatterns

In the areas of nanobiotechnology and biomedical engineering, the pattern generation of biomolecules (DNAs, peptides, proteins, and saccharides) and cells has been investigated primarily for the development of microarray-based sensors. In particular, the generation of spatially well-defined, two-dimensional microstructures of cells has a great deal of potential in the development of high-throughput cellular analysis systems, ultrasensitive cell-based biosensors, and platforms for rare event detection,[59] as well as in the fundamental studies of cell biology on man-made surfaces.[60] The first example of micropatterns of cells was reported by Whitesides and co-workers[60a] and up to date the micropatterns of cells have been generated mainly by employing adhesion receptor ligands such as RGD peptides[61] and fibronectin[62] or nonspecific adhesion of cells with a number of organic functionalities.[37h,63] Biotechnologically important cell types, such as certain types of stem cells, lymphocytes and tumor cells, are, however, weakly adherent or non-adherent. With the aim of developing a versatile method for generating micropatterns of weakly adherent or nonadherent cells, Hammond and Cohen labeled live B cells with biotin by random biotinylation of membrane proteins of the B cells and achieved a near perfect, clean micropatterns of B cells over large area in combination with microcontact printing (Figure 7a).[64a] Bacterial spore-based micropatterns were also demonstrated as an alternative to conventional method for generating cell patterns, where spore surface display

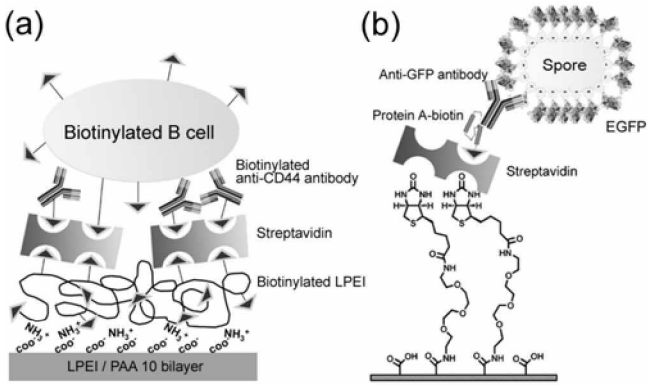

Figure 7. (a) Biotinylation of B cells and pattern generation, and (b) generation of spore patterns.

technique was utilized to display any arbitrary ligands on the surface of spores (Figure 7b).[64b] The combination of micropatterning techniques, such as microcontact printing, and surface organic chemistry for controlling the immobilization of biomolecules and cells would broadly be useful in fundamental studies and the applications mentioned above.

Biomedical devices are mostly manufactured from polymers and metals. For these materials, the main limitation is the lack of sufficient functional groups on the surfaces for surface engineering, exemplified by chemically inert aliphatic polyesters of poly(α-hydroxy acids), such as PLLA, poly(glycolic acid) (PGA) and copolymer of lactic and glycolic acids (PLGAs), which have widely been used for drug delivery systems and tissue engineering. In the fabrication of biomaterials, it is, therefore, an important technical challenge to develop methods for engineering biodegradable polymers in two and three dimensions.[65] The pattern generation of cells on the surface of biodegradable polymers[61d] would give an opportunity to investigate short- and long-term metabolism of cells attached onto biomedically relevant polymer surfaces.

Nanopatterns of organic functionalities would be of importance in studying fundamental cellular functions including motility, adhesion, proliferation, differentiation, and apoptosis (cell biology on man-made surfaces). In particular, it requires the generation of nanopatterns, where well-defined adhesive areas are separated by nonadhesive regions, to investigate cell-cell and cell-extracellular matrix (ECM) adhesion.[66] Various approaches to the generation of nanopatterns,[60d,67,68] such as DPN[68] and self-assembled of diblock copolymer micelles[60d] and to the spatial distribution of cell-adhesive ligands[25a,38c,69] have been developed to study cell adhesion, proliferation, and differentiation.

Conclusions

We reviewed some recent findings in the field of biosurface organic chemistry, by focusing on interfacial chemical reactions on self-assembled monolayers (SAMs). SAMs, molecularly ordered and nanometer-thick films on solid surfaces, have been a versatile platform for the fundamental understanding of biological phenomena, such as cell-cell interactions, and for the technologically important areas, such as (nano)biosensors, microarrays, and tissue engineering. Biosurface organic chemistry emerged as an independent research field mainly with the SAMs as a basis, where organic functional groups are controllably introduced onto surfaces, and has evolved into the stage, where lateral (such as surface density and nanopatterns) and vertical chemical compositions could be controlled at the molecular level. The *lateral and vertical control of chemical compositions* would be beneficial in the control over interfaces between biological and non-biological systems, which is of importance in (nano)biotechnology and biomedical engineering.

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