Chapter 4
Modification of Polymer Surfaces for Biofunctionalization

Guillaume Delaittre

4.1 Introduction

Molecular recognition is central to biological processes where it is usually the first step toward a cascade of events. Despite many years of intense research, still little is understood on how most biorecognition events actually operate. For instance, many questions remain unanswered on how proteins recognize one another, for the specific criteria of antibody-antigen binding, on the extent of (un)specificity in binding events, or on small molecule-based biorecognition, among others. Some systems are better understood than others. For instance, the biotin/avidin complex is, without a doubt, the most widely known biorecognition system since its discovery in the 1940s [1] as it features the strongest binding constant known to date ($K_D = 10^{-14}$–10^{-15} M) [2]. It was consequently ubiquitously employed in many areas spreading out of the pure biological field. Nevertheless, even for such a popular system, it took many years to understand its mechanism [2] and to even manipulate it [3, 4]. Therefore, biorecognition is not only a wide field of research on its own but is also vastly employed as a tool—with the few systems that are understood to a minimal extent—in other fields such as biotechnology or materials science.

In any case, immobilization of one binding partner onto a solid substrate is an important technique. Indeed, on the one hand, this technique is used for identification of unknown binding partners in screening methods such as peptide microarrays [5]. On the other hand, known complementary binding partners are involved in biopurification [6], biosensors [7, 8], bioassays such as ELISA [9, 10], biomaterials for cell biology or tissue engineering through integrin-binding peptides [11], targeted

G. Delaittre (✉)
Institute of Toxicology and Genetics, Karlsruhe Institute of Technology, Hermann-von-Helmholtz-Platz 1, 76344 Eggenstein-Leopoldshafen, Germany
e-mail: guillaume.delaittre@kit.edu

Institute for Chemical Technology and Polymer Chemistry, Karlsruhe Institute of Technology, Engesserstrasse 18, 76131 Karlsruhe, Germany

J. Rodríguez-Hernández, A. L. Cortajarena (eds.), *Design of Polymeric Platforms for Selective Biorecognition,* DOI 10.1007/978-3-319-17061-9_4

delivery via biological ligands such as folic acid [12, 13], as well as in pure materials science to bring together entities that are covalently linked to either of them [14, 15] such as in the classic case of streptavidin-coated surfaces serving as docking sites for biotinylated molecules [16] or even to construct extremely complex nanoarchitectures, as in the case of DNA supramolecular assemblies [17]. Some techniques combine both approaches, i.e., exploiting a known biorecognition pair in combination with molecular biology tools to detect, extract, and study unknown biomolecules and often their complexes. For instance, tandem affinity purification consecutively utilizes IgG- and calmodulin-functionalized beads to purify multiprotein complexes expressed in native conditions [18].

Prior to immobilization of one of the biorecognition partners, the presence of reactive groups on the surface of the material is necessary. One can distinguish three major classes of materials: metals, ceramics, and polymers. The first two are hard and either ductile and conductive, or brittle and nonconductive, while polymers are light and can possess any of these features, depending on their chemical composition. Thus, polymers offer a significantly wider range of applications than metals and ceramics. Depending on the targeted application, one can choose among an overwhelming variety of reported and even commercial polymers. Premade (commercial) polymers are usually inert, for obvious reasons. This implies that surface modification will usually be necessary in order to covalently bind recognition motifs. This is certainly the easiest option when one has limited chemical facility access or chemistry skills. Alternatively, one can indeed opt for fabricating an advanced polymeric material through specific monomer design or simply mixing different monomers in the so-called "copolymerization" method. Thus, in these cases, it is not only possible to tune the mechanical properties of the bulk material but also to introduce functionality, including at the surface. This chapter, however, only focuses on the surface modification route—as it is the most commonly employed one—and especially on studies leading to surface biofunctionalization. For examples of prefunctionalization methods, the reader may refer to the existing literature [11, 19–21]. Importantly, conducting polymers, albeit major players in polymer-based biosensors, [22] are not described here as their surface functionalization is predominantly the result of a prefunctionalization approach either through the use of a functional (co)monomer [23–25] or via a doping method (i.e., blending of the conducting polymer with a functional nonconductive counterpart) [26], certainly due to the fact that direct backbone functionalization would lower their conductivity [27].

It must be noted that polymer coatings onto inorganic materials (e.g., polymer brushes, spin-coated or chemical-vapor deposited polymer films) will generally not be treated here as these methods do not exploit the polymer bulk properties cited above. Only cases with widely used polymers involved are mentioned as the chemical surface modification may be translated to bulk systems. Furthermore, we only present methods leading to covalent immobilization of biorecognition units, as opposed to noncovalent adsorption. Finally, this chapter does not aim at being comprehensive as there is an immense body of published work on the modification of polymers (for bio-related purposes) but rather intends to provide an overview of the basic methods to introduce surface functionality into polymeric substrates that are initially devoid of it.

4.2 General Aspects of the Modification of Polymeric Substrates

It is possible to distinguish two main categories of postpolymerization surface modification. In the first case, the modification is carried out by placing the polymer in contact with a solution containing chemicals, which modifies the chemical structure of the outermost layer of the material. Usually, the depth of modification depends on the exposure time, the harshness of the treatment, and the susceptibility of the polymer toward the employed chemicals. In the second case, physical methods are employed to generate reactive species at the surface of the materials, and often (but not always) in the gaseous environment of a close chamber where the polymer sample is placed. In this case, it is usually the method that acts as a determining factor for the extent of modification, rather than the specific polymer. In the following sections, we thus describe the most common routes employed to introduce the initial (primary) reactive handles into polymeric materials and sort them according to two main categories, namely, wet chemical methods or physical chemical methods. We then explain the subsequent steps that can be taken to immobilize recognition units.

4.3 Introduction of Primary Reactive Groups by Wet Chemical Methods

A major aspect regarding the employment of wet chemical methods (pure or in combination with physical activation) is typically the resistance of polymers to solvents. Indeed, the user will often desire to maintain the surface roughness of the initial material, or at least the bulk integrity (i.e., prevent the complete dissolution), and therefore will be limited in terms of possible solvents and reagents (which usually need to be dissolved) by the nature of the polymer. Generally, polar polymers such as poly(meth)acrylates, poly(meth)acrylamides, or polyesters are not soluble in solvents with a very low polarity index (e.g., pentane, hexane) and apolar polymers such as polystyrene are not soluble in polar solvents. But the picture is quite more complicated than this simple statement as substituents within a same class of polymers can have a dramatic effect on solubility and, sometimes, solvents may not molecularly dissolve the polymer but would at least induce swelling. The chemical resistance of some important polymers can actually be correlated to their low propensity to interact with solvents. For instance, elevated temperatures are required to dissolve polyethylene (PE) in “strong” solvents such as aromatic hydrocarbons or chlorinated solvents. Poly(tetrafluoroethylene) (PTFE) is generally considered as insoluble as only high-molecular-weight (per)fluoroalkanes can dissolve it (close to its melting temperature). These two polymers are among the most chemically inert ones. Therefore, wet chemical methods will be employed when it is possible to dissolve the reagents necessary to carry out the surface modification in a solvent which does not provoke extensive swelling on the bulk material. This greatly reduces the leeway.

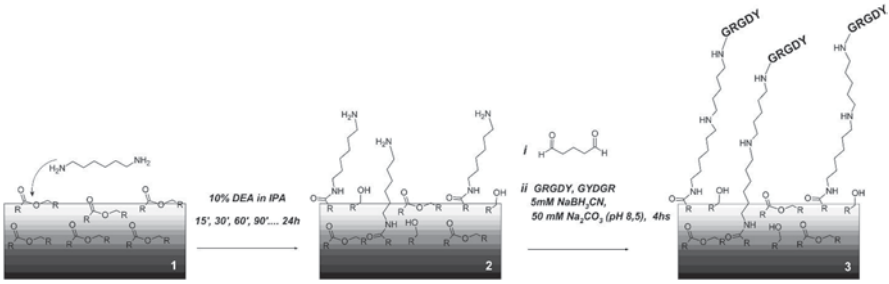

Fig. 4.1 Aminolysis/amination of poly(ε-caprolactone) surface by hexanediamine and subsequent glutaraldehyde-mediated peptide functionalization [29]

As a general rule, it actually is safe to state that the combination of most common organic solvents with most common polymers is not judicious. The safest organic solvents are probably the low-molecular-weight alcohols, particularly methanol and ethanol, but swelling cannot be excluded. Therefore, water is the solvent of choice for wet chemical treatment. Wet chemical treatments are generally highly dependent on the chemical structure of the polymer to modify, particularly on the presence of chemical groups that can be altered using chemicals. This implies that for each type of polymeric surface, a limited range of chemistries are available.

Without a doubt, hydrolysis and aminolysis have been the most popular solution-based methods to introduce reactive groups at the surface of polymeric materials. Obviously, only polymers that are able to undergo such degradations are concerned. This typically includes polyesters such as poly(ε-caprolactone) (PCL), poly(lactic acid) (PLA), and poly(lactic acid-*co*-glycolic acid) (PLGA). PCL is commonly treated with isopropanol solutions of diamines such as ethylenediamine (EDA) or hexanediamine (HDA), which induces a transamidification reaction and leave free amines on the surface (Fig. 4.1) [28−31]. The extent of surface functionalization, that is, the density of surface-displayed amines, depends on the temperature and duration of immersion. Nevertheless, satisfying results can be obtained at room temperature, even in water [32]. In one case, SEC characterization was utilized to prove the overall integrity of the polymer after aminolysis, thereby, demonstrating that the bulk material was intact [32]. For a specific study, it was shown that the density of surface-bound amines increased dramatically in the first 30 min, followed by a slight decrease and stabilization. At the same time, surface roughness evaluated by AFM increased for treatments longer than 30 min. The authors also witnessed rapid weight loss and drop in elastic modulus between 45–90 min with a further stabilization. These phenomena probably occur due to the formation and dissolution of oligomers. All in all, although not mentioned in this study, it is likely that the depletion of amines leads to a termination of the aminolysis process, which means that without standardized experiments (sample surface area and thickness), it is rather complicated to extract absolute values and only trends should be considered.

Aminolysis/transamidification has also been performed on PLA and PLGA. Probably due to the higher concentration of ester bonds in these (co)polymers as compared to PCL, which possesses a longer alkyl spacer, short exposure times are enough to reach the maximum effect [33]. A treatment as brief as 2 min at room temperature with a 6% w/v solution of HDA in isopropanol (i.e., 1 M amine) was efficient to introduce amines in an amount sufficient for further bioconjugation [34]. In the case of more sensitive PLGA materials such as microporous scaffolds, the slightest surface alteration can lead to a drastic change of the porosity. As a consequence, a treatment of only 10 s at room temperature with a 0.075 g mL^{-1} diamino-poly(ethylene glycol) solution in isopropanol (1500 g mol^{-1}, i.e., 0.1 M amine) revealed adequate [35]. As another member of the polyester family, poly(ethylene terephthalate) (PET) was also reported to undergo controlled aminolysis. Particularly, PET fibers were treated with a set of four amines exhibiting various chain lengths and alkyl or ethoxy spacers, nondiluted or in methanolic or aqueous solutions, at different temperatures and times [36]. The weight loss and the morphology of the fibers were monitored by gravimetry and by the use of scanning electron and atomic force microscopes, respectively, while the amount of grafted amines was measured by colorimetry. Overall, a temperature reaction of 50 °C with a 1 M methanolic amine solution was claimed to be the best compromise. Aminolysis/transamidification was also performed onto poly(methyl methacrylate) (PMMA) using diamines. In this case, the ester groups are lateral groups, which should not lead to an extensive degradation of the polymer [37, 38]. In this context, a method where these diamino linkers were first converted to their monoanion by treatment with *n*-butyl lithium was also reported by Soper and McCarley [39]. For more details on polymethacrylate surface modification for biosensors, the review of Djordjevic in the context of optical biosensors is recommended [40].

Hydrolysis has been in lesser use than aminolysis, probably because it yields species (i.e., hydroxyl and carboxyl) that are less reactive and usually require further activation or the presence of catalysis for further coupling. Although acid hydrolysis is possible, base-catalyzed hydrolysis is preferred as it is proceeds faster. In addition, the former may induce bulk hydrolysis, at least in the case of PLA, [41] rather than surface-confined modification. Only strong acid treatments may be efficient but they would also lead to extended morphological changes at the surface. PCL [42, 43] as well as PLA [44] and PLGA [33] were treated with sodium hydroxide solutions. Again, temperature and concentration are the determining parameters regarding the reaction rate [42] and the increase of surface roughness [44]. Hydrolysis with an ethanolic sodium hydroxide solution on a PMMA substrate was directly compared to aminolysis in terms of subsequent surface functionalization and it clearly showed that the latter was more efficient [37]. In some cases, hydrolysis of the PMMA surface was performed as a preliminary step before amidification, particularly when using polymeric amines such as poly(ethyleneimine) and poly(allylamine) [38]. Saturated sodium hydroxide aqueous solutions were also efficiently employed to produce carboxylic acids at the surface of commercial PMMA investigated for DNA microarrays [45].

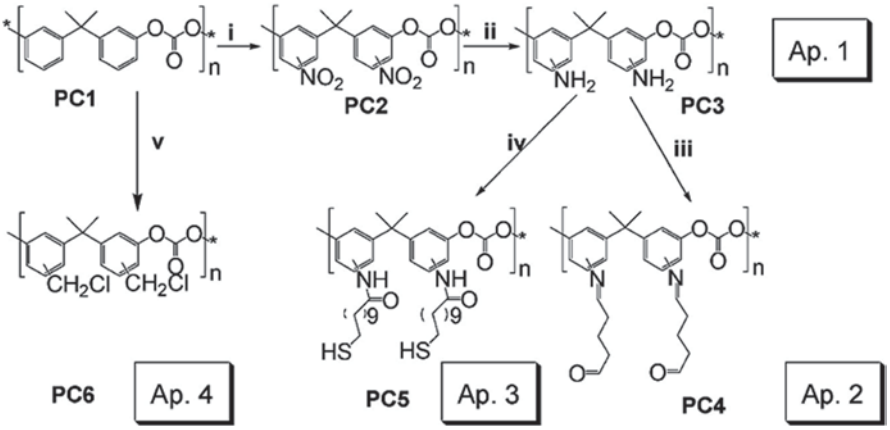

Fig. 4.2 Various methods to introduce reactive groups at the surface of polycarbonate substrates through initial aromatic substitutions [53]

A few other methods have been reported to enrich the surface of PET materials in hydroxyl and carboxyl groups, which otherwise are only present as end groups. Marchand-Brynaert reported an oxidation procedure to convert hydroxyl termini into carboxyl groups in PET microporous membranes by exposure to a potassium permanganate in sulfuric acid at 60 °C [46, 47], following assessment of diverse methodologies to maintain the porous structure [48]. In addition, Hubbell showed that the aromatic ring present in the PET repeating unit could be exploited to introduce further hydroxyl groups by treatment with an aqueous solution of formaldehyde and acetic acid at 20–37 °C for 4–8 h [49, 50].

Polycarbonate (PC) is an attractive polymer as it is transparent, has a high temperature and impact resistance, and can be thermoformed. This is advantageous for studies requiring well-defined topography and geometry, such as emulating blood vessels for instance [51]. PC is also the constituting material of compact discs (CDs) and has thus been considered in this very form to be an ideal platform for screening [52]. Therefore, Maquieira studied two routes to impart surface reactivities to CDs with the aim of immobilizing oligonucleotides for DNA detection (Fig. 4.2) [53]. The first method was based on a Friedel–Crafts alkylation with chlorodimethylether catalyzed by zinc(II) chloride, in cyclohexane at 60 °C for 2 h. These conditions were found to be the best compromise between a reasonable extent of functionalization and the conservation of the physical properties. Longer times and higher temperatures lead to increase in roughness and even cracking. It was noted that this method proved to be efficient for further attachment of aminated DNA strands and is particularly attractive as after only one treatment step the CDs are ready for biofunctionalization. However, the requirement for organic solvents and highly toxic chlorodimethylether lowers the attractiveness of this route. Indeed, the second alternative consisted of a nitration/reduction sequence to attach amine groups to the aromatic rings of PC, all performed in aqueous solutions (of nitric acid and sodium

borohydride, respectively). Nevertheless, we also found in our laboratory that this method was sometimes not conclusive for more fragile specimens of PC, such as porous thin membranes, as cracking also occurred [54].

Polyethersulfone (PES) is another interesting polymer as it is a highly chemical resistant, semitransparent thermoplastic, and sometimes replaces PC in more demanding applications. Its backbone is also made of multiple aromatic rings, this time connected together via sulfone and ether groups. Therefore, Marra employed a Friedel–Crafts alkylation procedure similar to Maquieira's to halogenate the polymer surface using chlorodimethylether with tin(IV) chloride as the Lewis acid catalyst [55]. This exact same procedure was developed earlier by Higuchi in the frame of poly(*N*-vinylpyrrolidone) grafting onto PES [56].

PTFE and other perfluorinated polymers are usually considered nonreactive and are widely used for biomedical applications. Strong reducing agents are, nevertheless, able to alter PTFE. Often, alkali metals are employed but yield blackened products. Importantly, McCarthy reported a procedure in which a benzoin dianion formed by reaction of benzoin with potassium *tert*-butoxide in deoxygenated dimethyl sulfoxide or *N*-methylpyrrolidone was able to reduce the surface of PTFE in a more controlled manner, producing films with a metallic visual aspect [57, 58]. This process was later used by Hubbell in order to produce insaturations at the surface of PTFE, which were then modified to immobilize cell-adhesive peptides [50]. Another protocol for introducing surface insaturations, initially reported by McCarthy [59] and later employed by Shoichet [60] for poly(tetrafluoroethylene-*co*-hexafluoropropylene) (FEP), was exploited by Gabriel on the PTFE homopolymer [61]. In this case the (strong) reducing agent is sodium naphthalide, which must be prepared fresh by stirring sodium and naphthalene together in dry tetrahydrofuran as the resulting product degrades in presence of water.

Finally, a very important polymer that has been used to a lesser extent in the frame of biorecognition is polydimethysiloxane (PDMS). For instance, we can mention the report of Sheardown who grafted a polymethylsiloxane layer to introduce Si–H groups that can later be involved in palladium-catalyzed hydrosilylation [62]. For further possible procedures, the reader may refer to reviews dealing with the surface modification of PDMS for microfluidic [63] and biomedical [64] applications. A typical alternative is oxidation to create silanol groups amenable to further silanization, as in the case of silicon wafers.

4.4 Introduction of Primary Reactive Groups by Physical Chemical Methods

The introduction of functional groups at the surface of a material using physical methods is significantly less substrate-dependent than that with wet chemical procedures and usually relies on the employed reagents (elemental gases or small molecules) rather than the chemical structure of the polymeric surface. An important difference between wet chemical and "dry" physical chemical methods is that in the

latter case, the surface topography is generally less altered, at least for compared treatment times leading to similar degrees of functionality. Nevertheless, these physical methods often present the disadvantage of simultaneously generating a broad range of species rather than a precisely defined single species and of requiring specialized equipment.

Plasma-induced modifications are the most widely employed techniques. Other methods include ozone treatment, as well as UV- or electron beam-based methods. Importantly, physical treatments generally allow spatially resolved modification due to the radiative nature of these modifications. Typically, a simple masking procedure with a blocking grid is sufficient [65–67].

4.4.1 Plasma-Based Modifications

Plasmas basically originate from the ionization of gases. They can be created via different methods, the most popular—at least in the area of surface modification for biorelated applications—being glow discharge, a nonthermal process. In that case, the plasma is generated by applying a radio-frequency electrical field into a gas-filled container. Plasma procedures are easily transferable from one polymer to another, only requiring optimization in some cases [68]. Depending on the gas or liquid vapors introduced in the plasma chamber as well as on other potentially coupled energy sources, plasma treatments can be categorized as follows:

- Direct plasma treatment (DP) utilizing nondepositing gases, commonly argon, nitrogen, or oxygen, or small molecule vapors [68]
- Plasma (grafting) polymerization, also named plasma-enhanced chemical vapor deposition (PECVD), employing vapors of diverse molecules, typically enes [69]
- Plasma-immersion ion implantation (PIII), which is based on a direct plasma treatment setup coupled to a high voltage pulse generator (Fig. 4.3) [70]

These three treatments result in rather different functionalities on the surface:

- DP will introduce elementary functional groups by oxidation directly on the treated polymer surface such as hydroxyls, aldehydes/ketones, carboxyls, or amines, depending on the nature of the gas [68]. DP treatment is usually not extremely stable over time: it was for instance commonly observed that it resulted in a sharp decrease of water contact angle (increase of hydrophilicity) in comparison to the native materials but that the hydrophobicity slowly increased when samples were exposed to air or water [72, 73].
- PECVD yields a thin polymeric layer grafted on the modified substrate and the functionality directly arises from the employed monomers. Obviously, milder plasma-generating conditions will preserve functionality to a higher extent [69]. PECVD usually leads to stable functionalization over time.
- PIII is reported to produce unpaired electrons inside the material, these electrons slowly migrating to the surface enabling radical-based grafting. [74]. The activated substrate procedure maintains a bonding ability of several months, if for instance freeze-dried.

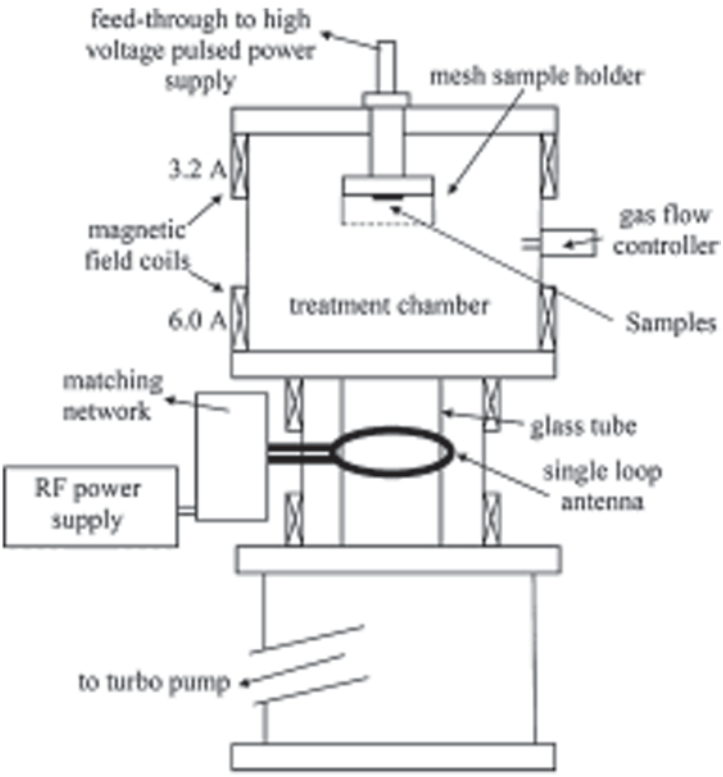

Fig. 4.3 Schematic diagram of a PIII treatment system [71]

Below, we explain in detail the sort of reactive groups that are produced in each method, depending on the employed gas/vapor phase. How these primary groups are further exploited in order to bring biorecognition units onto the surface is described later in Sect. 4.5.

4.4.1.1 Direct Plasma Treatment (DP)

In DP, flows of inert gases such as argon and nitrogen have been employed at low pressure. These two gases present great advantages in terms of safety of storage and handling. After such plasma exposure, the polymeric samples are usually exposed to air or even to pure oxygen in order to generate oxygen-based reactive species such as carboxylic acid or peroxides. Callens [75], Ho [71], Maeji [76], and Yin [77] have done so on PCL, PE, polyvinylidene difluoride (PVDF) [78], and PP, respectively. Nitrogen additionally leads to the introduction of amines [71, 79].

Oxygen, which is clearly more hazardous than argon or nitrogen, has been extensively utilized as the plasma source in DP. Quite obviously, it generates a mixture of species ranging from oxygen radicals and anions to hydroxyls, carbon-

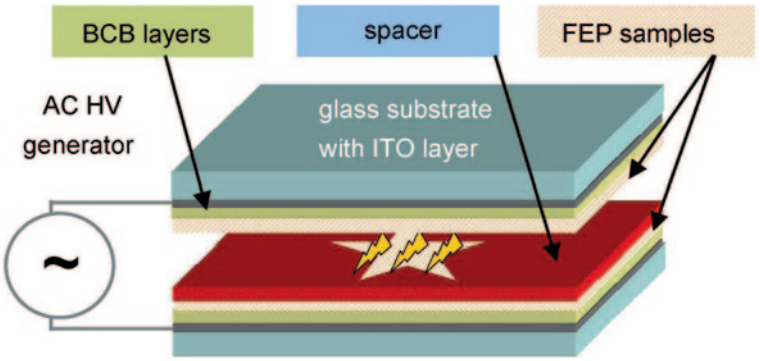

Fig. 4.4 Schematic setup for surface modification of two polymer samples at a time, which are sandwiched between two indium tin oxide (ITO) coated glass slides covered with the dielectric benzocyclobutene (BCB) and separated by a patterned spacer-mask [89]

yls, carboxyls, and peroxides that can be exploited in a range of chemistries with the final aim of immobilizing biomolecule ligands. Oxygen plasma was applied to numerous polymeric substrates. For instance, it has proven particularly useful to introduce functionality at the surface of vinyl polymers: polyolefins [80, 81] and their copolymers such as cyclic olefin (co)polymers (COP or COC) [72, 73, 82, 83], and PS-*b*-poly(ethylene-*co*-butylene)-*b*-PS (SEBS) [84], as well as their perfluorinated counterparts, for example, PTFE [73]. Interestingly, carbon dioxide seems to yield similar species, as reported by Vassile for PVDF functionalizations [79]. Polymers made by step-growth polymerization were also treated with oxygen plasma: polyesters such as PET [73], PLA [85, 86], and its copolymers [87]; polycarbonates [73]; poly(ether carbonate urethane) [88]. PDMS usually undergoes plasma oxidation similarly to silicon wafers, thereby producing silanol (Si-OH) groups [73].

A unique method, not involving low molecular gas pressure but air at atmospheric pressure was reported by Graz [89]. The plasma is generated by dielectric barrier discharges (DBD), a process first reported by Siemens in the nineteenth century [90]. The setup consists of a sandwich procedure where two polymeric films—in the present case FEP—can be treated at once if placed between two metal electrodes generating a high voltage alternating current with at least one insulating film in the discharge gap (Fig. 4.4). This process leads to the formation of weakly ionized plasma. In such a setup, patterning is also possible by placing a mask between the two polymeric substrates. Not only the presence of oxygen species such as aldehydes, but also of nitrogen species, was evidenced on the surface of the DBD-treated FEP films.

As mentioned above, DP can also be utilized in the presence of chemical vapors. For instance, Aebischer employed a mixture of helium and methanol vapors to create hydroxyl groups at the surface of FEP substrates [65, 91]. More commonly, polymeric materials were exposed to ammonia plasma for the specific grafting of amino

groups on PP [78, 79], PVDF [92], polyesters such as poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) [93], or PLA [94], and poly(ether ester urethane)s [95].

Friedrich had recourse to bromoform ($HCBr_3$) as the plasma source in order to introduce bromide groups at the surface of PE and PP, species later involved in nucleophilic substitutions [80].

Finally, DP can also result in the formation of grafted polymer but after additional thermal or radiative activation of DP-generated species in presence of radically polymerizable monomers. This process is called postplasma-grafting and will not be distinguished from DP as the same primary species (e.g., peroxides) are also present in DP processes. Corresponding examples of secondary functionalization will be detailed in Sect. 4.5.

4.4.1.2 Plasma Polymerization or Plasma-Enhanced Chemical Vapor Deposition (PECVD)

DP suffers from the requirement for renewed optimization for each polymer to be treated [68], although far less than in the case of wet procedures. However, plasma polymerization exhibits a higher transferability. PECVD can be considered a covalent coating method that relies to a large excess onto the nature of the radically polymerizable monomers present in the vapor phase (Fig. 4.5). It is applicable not only to polymers but also to metals and ceramics. The relative mildness of the process is important with regards to functional substituents of the vinyl monomers. A customarily applied method is shortening the plasma duty cycle by pulsing periods interspersed with "off" times during which the polymerization continues to proceed. In some cases, continuous wave conditions can still be applied during a short initiation period.

By far, carboxylic acid-containing monomers, and particularly acrylic acid (AA), have been graft-polymerized the most, onto a variety of polymeric materials: PE and PP [80], PS [97], PLA [98−100], polyurethanes [98]. Urban reported a PECVD-based method where he used maleic anhydride (MAnh) in place of AA [101]. MAnh is a special monomer owing to the fact that it is not able to homopolymerize. Therefore in that study, a monolayer of maleic acid—that can be hydrolyzed to yield two carboxylic acid functions—was certainly formed onto PE and PP substrates.

Usually, carboxylic acids require activation for subsequent conjugation reactions. A recent alternative has been explored via the plasma polymerization of pentafluorophenyl methacrylate, first on silicon substrates [102, 103] and then on PS [96].

Allyl monomers were not forgotten in the area of PECVD where they are rather popular to introduce alcohol and amine groups. Allyl alcohol (AllOH) as well as allylamine ($AllNH_2$) were graft-polymerized by Friedrich onto PP and PE [80]. Eberhart covalently attached poly($AllNH_2$) onto PCL and PLA [104], so did Sheardown with P(AllOH) onto PDMS [105]. Klee utilized a protected, hydrolyzable version of AllOH, namely vinyl acetate (VAc) [106].

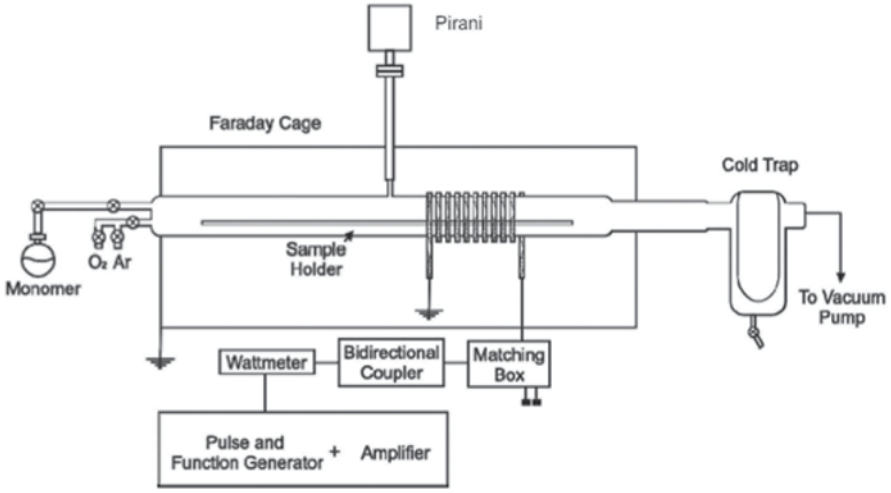

Fig. 4.5 Schematic diagram of a plasma reactor employed for plasma-enhanced chemical vapor deposition (PECVD) and its electrical components [96]

4.4.1.3 Plasma-Immersion Ion Implementation (PIII)

PIII has been extensively employed in the frame of bioconjugation by the team of Bilek [107, 108]. Although PIII certainly yields similar species as classic DP, it is usually employed in order to exploit the radicals that are formed along with these oxygen and nitrogen species. It is usually referred to as a linker-free method for biomolecules, particularly enzymes, which can be immobilized without intermediate procedures [109]. The treated substrates generally become hydrophilic (as in DP, which confirms the previous statement on the similarity of produced species) but seem to retain this character for extended period of time, which results in prolonged protein activity albeit this absence of linker that could regulate protein-surface interaction and prevent denaturation. In the frame of PIII, inert gas plasmas have generally been used: nitrogen onto PE [71, 74, 110, 111], PTFE [74, 112, 113], PMMA [74], PS [74, 114], and PC [115] as well as argon onto PE [71] and PS [116].

In a different approach, Choi employed PIII together with a masked irradiation to pattern implanted areas that would yield peroxide surface patterns after exposure to air for 24 h [117].

4.4.2 Ozone Treatment

Ozone treatment is related to plasma techniques in the fact that it also relies on a reactive gas phase. Early studies on the action of ozone onto PE films evidenced the formation of oxidation products similar to those produced by oxygen plasma, i.e., hydroxyls, carbonyls, and carboxyls [118, 119]. For instance, Diaz-Quijada investigated the ozone treatment for the fabrication of DNA microarrays on COP

and PMMA [45]. The activation of ozone by UV was also investigated and demonstrated a higher degree of modification, unfortunately yielding an increase of autofluorescence of the substrates, which can be detrimental for applications involving spectroscopic methods.

4.4.3 Photoirradiation

Light-based techniques can play a major role in the area of surface modification, as they allow facile temporal and spatial control [54, 120−122]. Notably, they usually do not require harsh chemicals as in the case of wet chemical modifications, and avoid the requirement for vacuum techniques as in plasma-based techniques or for hazardous gases such as ozone. An exception to that is however the UV activation of a mercury- and ammonia-containing chamber to introduce amines at the surface of FEP fibers [123].

The incorporation of photoreactive species in the polymer itself is out of the scope of this chapter, therefore the following described examples concern the use of soluble photoactive moieties yielding a direct grafting onto the surface. The first class of such studies is devoted to UV-induced polymerization, which is conceptually very similar to PECVD: radicals are created in the surroundings of and onto the surface in presence of radically polymerizable monomers. This process has been carried out using either gaseous or liquid monomer formulations.

For instance, Kessler reported the direct grafting of acrylated integrin ligand peptides—precisely, cyclic RGD—on PMMA in the presence of camphorquinone acting as a radical source, in solution, and under UV light [124, 125]. The same team also reported the grafting polymerization of photoisomerizable cyclic RGD peptide-based acrylamides at the surface of PMMA without the use of a photoinitiator. In that case, the PMMA films were first irradiated for 2 h at 254 nm, in order to create surface-bound radicals [126].

Albertsson reported a similar strategy using benzophenone for gas-phase grafting polymerization with acrylamide, vinylpyrrolidone, and MAnh [127]. The vapor pressure of the monomers had an influence on the grafting yield: the higher, the better. As we suggested in the case of PEVCD reported by Urban, MAnh yielded a low grafting density, for which impossible homopolymerization was here explicitly designated as the cause. While this study did not involve subsequent biofunctionalization, the method was adapted by other teams for this purpose. For instance, Xu graft-polymerized acrylic acid on PP [128]. In that case, PP membranes were first soaked in a solution of benzophenone and dried before placing them in an aqueous solution of AA and irradiating them.

Larsen also utilized a benzophenone derivative (benzoyl benzylamine hydrochloride, BzBAm) to create protein-repellent coatings from a solution of PEG and BzBAm [129]. In this case, no polymerizable monomer is present, but benzophenone derivatives are known to readily abstract hydrogen from hydrocarbons and create radicals. This feature was thus exploited for a second step of functionalization to graft, directly on the passivating layer, an enzyme as well as an antibody,

which both maintained activity. Tan reported a few years before a rather similar method exploiting the hydrogen abstraction ability of benzophenone—with the coupling of 4-benzoylbenzoic acid and an RDG peptide followed by its direct UV-induced grafting of the latter onto poly(carbonate urethane)s [130].

Yang reported another simple UV-induced grafting method. His team found that irradiation of a phenol solution in acetone resulted in the attachment of the aromatic compound onto polymeric surfaces, via a mechanism involving the triplet state of acetone [131]. This method is compatible with several functional groups (sulfonic and carboxylic acids, amine, thiol). In the case which interests us, bromo-4-hydroxyacetophenone was chosen to introduce bromine groups at the surface of PP in a spatially resolved way using a metallic mask [132].

Isopropylthioxanthone (ITX) is a common photoinitiator, which was used by Yin to grow polymer brushes at the surface of PE and PS. Particularly, methacrylated microperoxidase and poly(L-lysine) could be copolymerized from the surface of PE films and PS well plates, respectively. The method relies on two steps: (i) the UV-induced grafting of ITX onto the polymer surface proceeding by hydrogen abstraction and coupling with the newly formed polymer-bound radical and (ii) the visible light-induced formation on an equilibrium between ITX-bound species and propagating radicals next along with the ITX intermediate radical. This mechanism somehow resembles that of photoinduced reversible-deactivation radical polymerization such as nitroxide-mediated photopolymerization [133].

Finally, photogeneration of highly reactive species, nitrenes and carbenes, able to insert into C–H bonds was utilized as a simple means to introduce not only chemical groups but also, more commonly, directly the biorecognition ligand. The most employed class of precursors is that of *p*-azidophenyl derivatives, which form nitrenes upon UV irradiation. Many *p*-azidophenyl-functionalized peptide systems have been reported for grafting onto PS [134], PET [134] poly(vinyl alcohol) [135], poly(ester carbonate)s [135], and polyurethanes [136]. A biotin derivative was also described for the functionalization of the epoxy-based photoresist SU-8 (Fig. 4.6) [137]. Lee also had recourse to this method to immobilize a brominated compound for further surface-initiated atom-transfer polymerization from COC. Chevolot opted for a related method based on the generation of carbenes from diazirine-functionalized mono- and disaccharides for PS surface functionalization [138].

4.4.4 Electron-Beam Irradiation

Electron-beam irradiation (eBeam) was seldom used on polymeric materials in the context of biorecognition-based systems. It has nevertheless been employed extensively for the patterning/crosslinking of PEG derivatives directly involved in bioconjugation/biorecognition [139]. eBeam is performed to create radicals at the surface of a polymeric material. Albertsson has for instance shown that it is possible to treat topographically patterned PCL substrates with eBeam and maintain the morphology of the sample [140]. The irradiated samples could be stored without loss of reactivity by immediate immersion into liquid nitrogen. A simple subsequent exposure to a deoxygenated solution of acrylic acid allowed surface grafting polymerization. Quite

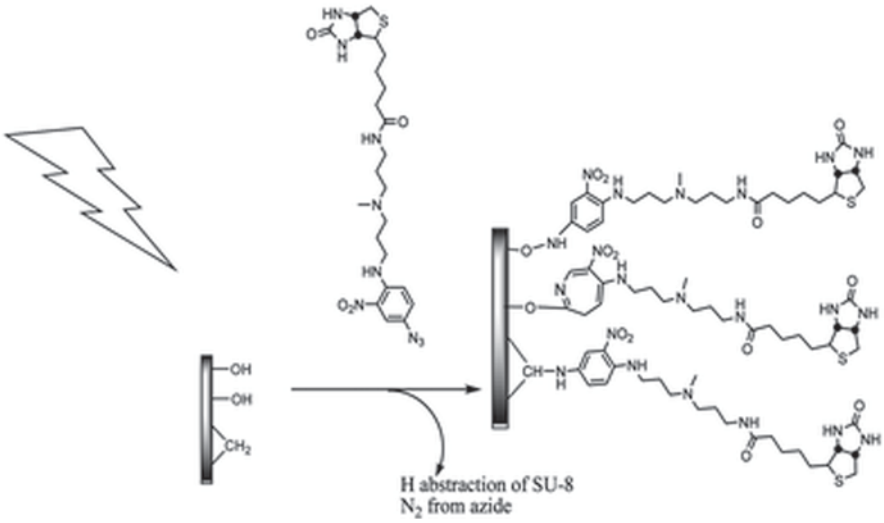

Fig. 4.6 UV-induced grafting of a *p*-azidophenyl-functionalized biotin [137]

differently, Okano proceeded to the direct eBeam of solutions of *N*-isopropylacrylamide and a carboxyl-functionalized analogue to yield patterned crosslinked layers through masked irradiation onto tissue culture polystyrene dishes [67].

4.5 Subsequent Surface Functionalization Methods and Attachment of Biorecognition Modules

Depending on the chemical groups introduced through methods described in Sect. 4.3 and 4.4, several synthetic routes open. In the following, the description of these routes is ordered according to the type of functional group present on the surface after the initial treatment, irrespective of the method employed to achieve this state.

4.5.1 Oxygen Species

Through the use of oxygen DP, diverse oxygen species are produced at the surface of a polymer. We detail their derivatization one by one below but it is perhaps interesting to first mention that Friedrich reported the global conversion of these groups into alcohols through the application of strong reductants such as B_2H_6/H_2O_2, $LiAlH_4$, or vitride/NaOH [80]. Sheardown did the same with sodium borohydride [105].

4.5.1.1 Peroxides

Surface-bound peroxides are generally exploited for a subsequent grafting-polymerization procedure. This can be triggered either by UV exposure [75, 77, 87] or by thermal treatment [76, 117] in presence of the monomer solution. UV sources usually employed are in the UVA range and irradiation is performed for a few tens of minutes. Thermal treatment occurs in the 65–75 °C range for a few hours. Again, as in PECVD, AA is the most widely utilized monomer [87, 117]. After polymerization, peptide coupling reagents such as 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC) and *N*-hydroxysuccinimide (NHS) are employed to immobilize amino containing bioligands [87]. Copolymerization of styrene and MAnh to yield alternating copolymers that can be hydrolyzed to yield carboxylic acids was reported [76]. Amine-containing monomers such as 2-aminoethyl methacrylate [75] and 2-methacryloyloxyethyl phosphorylcholine [77] were also used. Sometimes inhibitors such as Mohr's salt are added to the monomer mixture to prevent solution-phase homopolymerization and promote exclusive grafting [141].

4.5.1.2 Alcohols

There are many ways surface-bound alcohols can be derivatized to ultimately lead to biomolecular immobilization. In many reports, silanization is employed. 3-(aminopropyl)triethoxysilane is clearly the most popular reagent, yielding aminated surfaces that can then react further with aldehydes (such as glutaraldehyde [80] or reduced dextran [82]) or activated esters [85]. Through silanization atom-transfer radical polymerization (ATRP) initiators have also been introduced at the surface of PP, to yield brushes of poly(ethylene glycol) methacrylate (PEGMA) and glycidyl methacrylate (GMA) copolymers, whose epoxide rings were then exploited for amine-based protein attachment [81]. An interesting route was reported by Chiari with the use of a copolymer bearing silane side chains (as well as either NHS ester or epoxide groups) that could be coupled to oxidized surfaces for DNA microarraying (Fig. 4.7) [73].

Hydroxyl groups can also be reacted with diisocyanates to produce isocyanate surfaces that can be used as such for coupling with amine-containing biomolecules or again converted into amines by hydrolysis [80]. Esterification using acyl chloride was also performed to introduce a chloromethylbenzyl group which could potentially serve as an ATRP initiator but was actually converted to a dithiocarbamate (also called photoiniferter) instead, in order to perform UV-initiated "living" polymerization of PEGMA and AA, followed by IgG immobilization mediated by EDC/NHS [83]. Carbodiimidazole and tresyl chloride activations supplied handles for peptide immobilization through their N-terminus, forming urethane linkages, [65] and secondary amine and amide linkages, [105] respectively. Finally, Klee reported a protocol involving benzoquinone as a bridging molecule between the hydroxyl groups of hydrolyzed PVAc and the N-terminus of peptides [106].

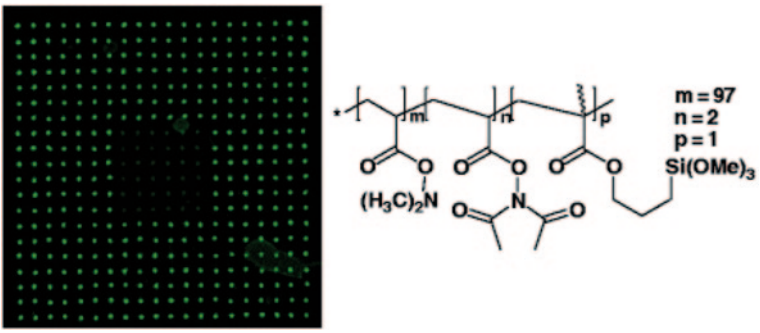

Fig. 4.7 (*Left*) Oligonucleotide hybridization experiment on a cyclic olefin (co)polymers (COC) surface treated by O_2 plasma, coated with an *N*-hydroxysuccinimide (NHS) ester-containing silane copolymer, functionalized with a 5′-NH_2-functionalized DNA strand, and incubated with a Cy3-labeled complementary oligonucleotide. (*Right*) Chemical structure of the coating copolymer. Adapted from [73]

Bromination of surface hydroxyl groups on plasma-oxidized SEBS could be achieved by HBr/H2SO4 treatment in order to perform surface-initiated ATRP [84].

4.5.1.3 Carbonyls

Carbonyl groups such as aldehydes and ketones have been less considered than other oxygen species although they can lead to interesting materials, as it is possible to reverse the immobilization of amine-containing (bio)molecules via the labile Schiff base adduct. Actually, most reports involving carbonyl-amine coupling describe the permanent fixation of this linkage by reduction using sodium cyanoborohydride (Fig. 4.8) [72, 89].

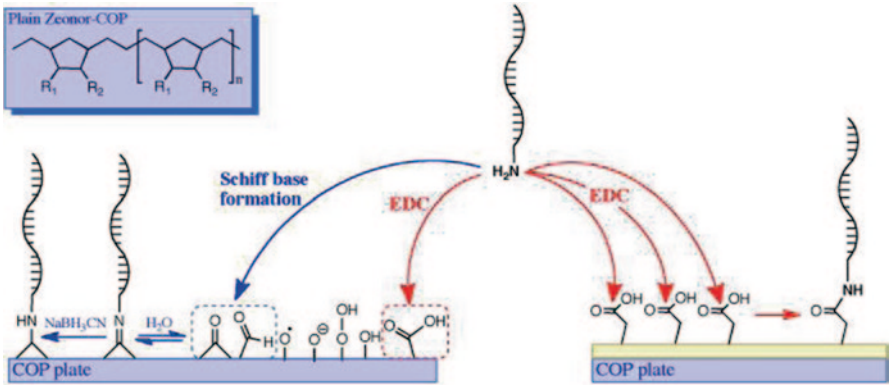

Fig. 4.8 Chemical structure of a cyclic olefin (co)polymers (COP; inset) and functional groups generated on the surface of oxidized COP (*left*) and graft-polymerized acrylic acid (AA; *right*), as well as possible chemistries to immobilize amine-terminated DNA [72]

4.5.1.4 Carboxylic Acids and Activated Esters

The derivatization of carboxylic acids offers much less variations. Basically, one method has been generally applied and relies on peptide coupling chemistry through the ubiquitous EDC/NHS couple (Fig. 4.8) [67, 72, 77, 88, 128, 140]. Sometimes the sulfonated variant of NHS is used to increase water solubility [86].

In most cases, the so-formed NHS-activated ester serves as a direct reactive site for biomolecule immobilization: peptides, [67, 86, 88] proteins, [67, 77] or aminated DNA strands [72].

In other cases, the NHS ester serves as an intermediate handle to incorporate another functional bioconjugation-amenable group via an amino derivative: 2-(2-pyridinyldithio)-ethanamine for thiol exchange immobilization of cysteine-containing peptides, [140] or propargyl amine for attachment of azidosugars involved in lectin recognition by *click* chemistry [128]. Urban also published a procedure to introduce alkyne groups but via acyl chloride formation and subsequent reaction with propargyl amine [101].

There are otherwise very few examples of activated ester directly introduced by a surface treatment. Borros showed that protein covalent immobilization could readily be achieved after the PECVD of pentafluorophenyl methacrylate without any additional treatment [96].

4.5.2 Amines

Besides the surprising examples of direct protein attachment onto amino groups without any catalyst or previous activation, [142, 143] amines have been reacted with bifunctional linkers to bridge surfaces and biomolecules. A very popular linker is glutaraldehyde which possesses two aldehyde groups, thereby allowing attachment of peptides and proteins through their N-termini [94]. Diisocyanates have also been used for the same purpose but present the disadvantage of being less stable, requiring a rapid bioconjugation event [92, 95].

Increasingly popular are nowadays hetero bifunctional linkers, such as succinimidyl-4-(*N*-maleimidomethyl)cyclohexane-1-carboxylate (SMCC) [123] or NHS-PEG-maleimide, [93] which allow site-selective immobilization of polypeptides through rare cysteine residues by Michael addition on virtually any aminated substrate.

4.5.3 Halides

Albeit less common in polymer surface modifications, halides may gain a broader interest as they can be involved in a range of reactions. For instance, Friedrich derivatized the bromide group formed during bromoform plasma treatment of PE

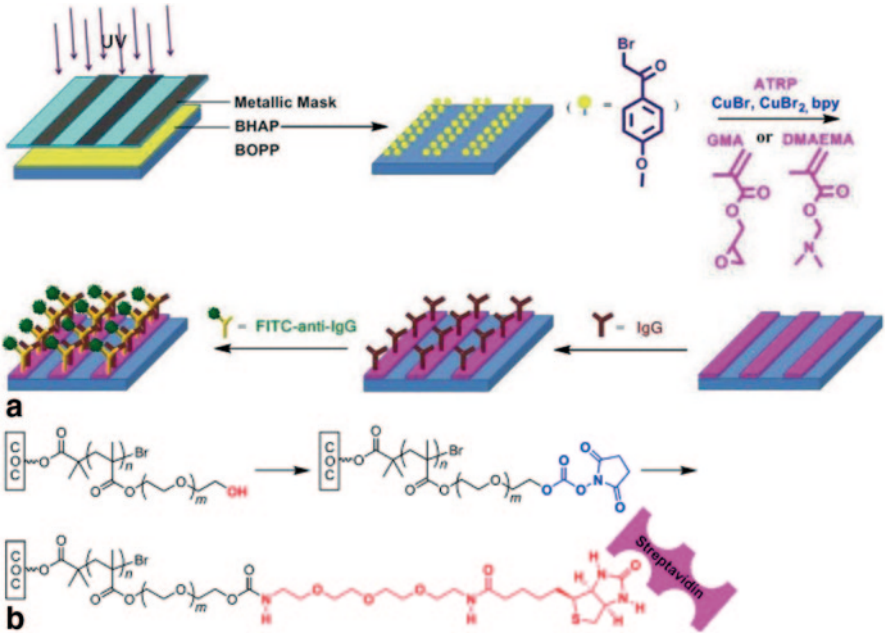

Fig. 4.9 **a** Schematic illustration of the surface-initiated *ATRP* of *GMA* (or *DMAEMA*) on *PP* functionalized by acetone-mediated UV grafting and subsequent protein immobilization [132]. **b** Formation of biotin-functionalized antifouling brushes onto *COC* after nitrene-mediated UV grafting [144]. *COC* cyclic olefin (co)polymers, *ATRP* atom-transfer radical polymerization, *GMA* glycidyl methacrylate

and PP into alcohols, by Williamson ether synthesis with a diol, and into amines by a nucleophilic substitution with diamines [80].

Halides are also commonly involved in radical-mediated processes such as ATRP. For example, Yang exploited the UV-grafted bromo-4-hydroxyacetophenone as an ATRP initiator to grow PGMA brushes and subsequently ring-open the epoxide side-chains to anchor IgG (Fig. 4.9a) [132]. Lee employed a similar strategy to grow brushes of poly(hydroxyPEGMA), followed by activation of the pendant hydroxyl groups by *N,N′*-disuccinimidyl carbonate, and coupling of amino-biotin (Fig. 4.9b) [144].

4.5.4 Insaturations

Insaturations resulting from strong reductant treatment on perfluorinated films usually are converted into alcohols. This can be done by oxidation using a hydroboration/oxidation sequence [50, 60, 61]. Alternatively, insaturations can give rise to carboxyl groups by direct oxidation in potassium chlorate/sulfuric acid [60].

4.6 Conclusion and Outlook

In this chapter, we have tried to cover as much as possible the range of chemical modifications, which have been carried out at the surface of commercial polymeric materials that are relevant for the field of biorecognition. Lately, physical methods seem to have taken the lead in that area for the introduction of primary reactive groups, owing to the easier access to advanced surface characterization techniques, such as X-ray photoelectron spectroscopy, FT-IR spectroscopy, or water-contact angle, that allow a better understanding of these processes. Of particular interest is that the surfaces are usually well-conserved in terms of topology, particularly with photoirradiation [145].

An aspect that has not been very much alluded to in the present literature review is that of passivation. Passivation is the key to biorecognition, in order to avoid non-specific adsorption [146, 147]. This is particularly true for biosensing applications where reduction of the background is essential to allow a decrease in detection limit down to the attomolar range. Over the last decade, there have been many advances in terms of designing such surfaces, particularly thanks to the advance in controlled polymerization methods allowing the grafting of dense hydrophilic polymer brushes [148−151]. Controlled radical polymerization processes [152−155] have thus a great future for the design of high-quality biochips, biosensors, and biomaterials as they are tolerant to a wide range of functionalities and can be combined with advanced photochemical strategies for patterning [120−122].

References

1. Gyorgy, P.; Rose, C. S.; Eakin, R. E.; Snell, E. E.; Williams, R. J. Egg-White Injury As the Result of Nonabsorption or Inactivation of Biotin. *Science* **1941**, *93*, 477–478.
2. DeChancie, J.; Houk, K. N. The Origins of Femtomolar Protein–Ligand Binding: Hydrogen-Bond Cooperativity and Desolvation Energetics in the Biotin–(Strept)Avidin Binding Site. *J. Am. Chem. Soc.* **2007**, *129*, 5419–5429.
3. Holmberg, A.; Blomstergren, A.; Nord, O.; Lukacs, M.; Lundeberg, J.; Uhlén, M. The biotin-streptavidin interaction can be reversibly broken using water at elevated temperatures. *Electrophoresis* **2005**, *26*, 501–510.
4. Chivers, C. E.; Crozat, E.; Chu, C.; Moy, V. T.; Sherratt, D. J; Howarth, M. A streptavidin variant with slower biotin dissociation and increased mechanostability. *Nat. Methods* **2010**, *7*, 391–393.
5. Breitling, F.; Nesterov, A.; Stadler, V.; Felgenhauer, T.; Bischoff, F. R. High-density peptide arrays. *Mol. BioSyst*. **2009**, *5*, 224–234.
6. Hage, D. S.; Anguizola, J. A.; Bi, C.; Li, R.; Matsuda, R.; Papastavros, E.; Pfaunmiller, E.; Vargas, J.; Zheng, X. Pharmaceutical and biomedical applications of affinity chromatography: Recent trends and developments. *J. Pharm. Biomed. Anal.* **2012**, *69*, 93–105.
7. Liedberg, B.; Nylander, C.; Lunström, I. Surface plasmon resonance for gas detection and biosensing. *Sensors and Actuators* **1983**, *4*, 299–304.
8. Do, T.; Ho, F.; Heidecker, B.; Witte, K.; Chang, L; Lerner, L. A rapid method for determining dynamic binding capacity of resins for the purification of proteins. *Protein Expression Purif.* **2008**, *60*, 147–150.
9. Engvall, E.; Perlmann, P. Enzyme-linked immunosorbent assay (ELISA) quantitative assay of immunoglobulin G. *Immunochemistry* **1971**, *8*, 871–874.

10. Van Weemen, B. K.; Schuurs, A. H. W. M. Immunoassay using antigen—enzyme conjugates. *FEBS Lett*. **1971**, *15*, 232–236.
11. Delaittre, G.; Greiner, A. M.; Pauloehrl, T.; Bastmeyer, M.; Barner-Kowollik, C. Chemical approaches to synthetic polymer surface biofunctionalization for targeted cell adhesion using small binding motifs. *Soft Matter* **2012**, *8*, 7323–7347.
12. Stella, B.; Arpicco, S.; Peracchia, M. T.; Desmaële, D.; Hoebeke, J.; Renoir, M.; D'Angelo, J.; Cattel, L.; Couvreur, P. Design of folic acid-conjugated nanoparticles for drug targeting. *J. Pharm. Sci.* **2000**, *89*, 1452–1464.
13. Low, P. S.; Henne, W. A.; Doorneweerd, D. D. Discovery and Development of Folic-Acid-Based Receptor Targeting for Imaging and Therapy of Cancer and Inflammatory Diseases. *Acc. Chem. Res.* **2007**, *41*, 120–129.
14. Li, M.; Wong, K. K. W; Mann, S. Organization of Inorganic Nanoparticles Using Biotin–Streptavidin Connectors. *Chem. Mater.* **1998**, *11*, 23–26.
15. Lee, J.; Govorov, A. O.; Kotov, N. A. Bioconjugated Superstructures of CdTe Nanowires and Nanoparticles: Multistep Cascade Förster Resonance Energy Transfer and Energy Channeling. *Nano Lett*. **2005**, *5*, 2063–2069.
16. Morais, S.; Marco-Moles, R.; Puchades, R.; Maquieira, A. DNA microarraying on compact disc surfaces. Application to the analysis of single nucleotide polymorphisms in Plum pox virus. *Chem. Commun.* **2006**, 2368–2370.
17. McLaughlin, C. K.; Hamblin, G. D.; Sleiman, H. F. Supramolecular DNA assembly. *Chem. Soc. Rev.* **2011**, *40*, 5647–5656.
18. Rigaut, G.; Shevchenko, A.; Rutz, B.; Wilm, M.; Mann, M; Seraphin, B. A generic protein purification method for protein complex characterization and proteome exploration. *Nat. Biotechnol.* **1999**, *17*, 1030–1032.
19. Goddard, J. M.; Hotchkiss, J. H. Polymer surface modification for the attachment of bioactive compounds. *Prog. Polym. Sci.* **2007**, *32*, 698–725.
20. Hersel, U.; Dahmen, C.; Kessler, H. RGD modified polymers: biomaterials for stimulated cell adhesion and beyond. *Biomaterials* **2003**, *24*, 4385–4415.
21. Reynhout, I. C.; Delaittre, G.; Kim, H.-C.; Nolte, R. J. M.; Cornelissen, J. J. L. M. Nanoscale organization of proteins via block copolymer lithography and non-covalent bioconjugation. *J. Mater. Chem. B* **2013**, *1*, 3026–3030.
22. Ates, M. A review study of (bio)sensor systems based on conducting polymers. *Mater. Sci. Eng., C* **2013**, *33*, 1853–1859.
23. Haddour, N.; Cosnier, S.; Gondran, C. Electrogeneration of a Poly(pyrrole)-NTA Chelator Film for a Reversible Oriented Immobilization of Histidine-Tagged Proteins. *J. Am. Chem. Soc.* **2005**, *127*, 5752–5753.
24. Xie, H.; Luo, S.-C.; Yu, H.-h. Electric-Field-Assisted Growth of Functionalized Poly(3,4-ethylenedioxythiophene) Nanowires for Label-Free Protein Detection. *Small* **2009**, *5*, 2611–2617.
25. Sosnowska, M.; Pieta, P.; Sharma, P. S.; Chitta, R.; Kc, C. B.; Bandi, V.; D'Souza, F; Kutner, W. Piezomicrogravimetric and Impedimetric Oligonucleotide Biosensors Using Conducting Polymers of Biotinylated Bis(2,2′-bithien-5-yl)methane as Recognition Units. *Anal. Chem.* **2013**, *85*, 7454–7461.
26. Song, H. K.; Toste, B.; Ahmann, K.; Hoffman-Kim, D.; Palmore, G. T. R. Micropatterns of positive guidance cues anchored to polypyrrole doped with polyglutamic acid: A new platform for characterizing neurite extension in complex environments. *Biomaterials* **2006**, *27*, 473–484.
27. Guimard, N. K.; Gomez, N.; Schmidt, C. E. Conducting polymers in biomedical engineering. *Prog. Polym. Sci.* **2007**, *32*, 876–921.
28. Santiago, L. Y.; Nowak, R. W.; Peter Rubin, J.; Marra, K. G. Peptide-surface modification of poly(caprolactone) with laminin-derived sequences for adipose-derived stem cell applications. *Biomaterials* **2006**, *27*, 2962–2969.
29. Causa, F.; Battista, E.; Della Moglie, R.; Guarnieri, D.; Iannone, M.; Netti, P. A. Surface Investigation on Biomimetic Materials to Control Cell Adhesion: The Case of RGD Conjugation on PCL. *Langmuir* **2010**, *26*, 9875–9884.

30. Zhang, H.; Hollister, S. Comparison of bone marrow stromal cell behaviors on poly(caprolactone) with or without surface modification: studies on cell adhesion, survival and proliferation. *J. Biomater. Sci., Polym. Ed.* **2009**, *20*, 1975–1993.
31. Gabriel, M.; Nazmi, K.; Dahm, M.; Zentner, A.; Vahl, C.-F; Strand, D. Covalent RGD Modification of the Inner Pore Surface of Polycaprolactone Scaffolds. *J. Biomater. Sci., Polym. Ed.* **2012**, *23*, 941–953.
32. Gabriel, M.; Van Nieuw Amerongen, G. P.; Van Hinsbergh, V. W. M.; Van Nieuw Amerongen, A. V.; Zentner, A. Direct grafting of RGD-motif-containing peptide on the surface of polycaprolactone films. *J. Biomater. Sci., Polym. Ed.* **2006**, *17*, 567–577.
33. Croll, T. I.; O'Connor, A. J.; Stevens, G. W.; Cooper-White, J. J. Controllable Surface Modification of Poly(lactic-co-glycolic acid) (PLGA) by Hydrolysis or Aminolysis I: Physical, Chemical, and Theoretical Aspects. *Biomacromolecules* **2004**, *5*, 463–473.
34. Ning, C.; Yingxue, G.; Kerm Sin, C.; Vincent, C.; Kin, L. Adhesion dynamics of porcine esophageal fibroblasts on extracellular matrix protein-functionalized poly(lactic acid). *Biomed. Mater.* **2008**, *3*, 015014.
35. Rohman, G.; Baker, S. C.; Southgate, J.; Cameron, N. R. Heparin functionalisation of porous PLGA scaffolds for controlled, biologically relevant delivery of growth factors for soft tissue engineering. *J. Mater. Chem.* **2009**, *19*, 9265–9273.
36. Bech, L.; Meylheuc, T.; Lepoittevin, B.; Roger, P. Chemical surface modification of poly(ethylene terephthalate) fibers by aminolysis and grafting of carbohydrates. *J. Polym. Sci., Part A: Polym. Chem.* **2007**, *45*, 2172–2183.
37. Patel, S.; Thakar, R. G.; Wong, J.; McLeod, S. D.; Li, S. Control of cell adhesion on poly(methyl methacrylate). *Biomaterials* **2006**, *27*, 2890–2897.
38. Bai, Y.; Koh, C. G.; Boreman, M.; Juang, Y.-J.; Tang, I. C.; Lee, L. J; Yang, S.-T. Surface Modification for Enhancing Antibody Binding on Polymer-Based Microfluidic Device for Enzyme-Linked Immunosorbent Assay. *Langmuir* **2006**, *22*, 9458–9467.
39. Henry, A. C.; Tutt, T. J.; Galloway, M.; Davidson, Y. Y.; McWhorter, C. S.; Soper, S. A.; McCarley, R. L. Surface Modification of Poly(methyl methacrylate) Used in the Fabrication of Microanalytical Devices. *Anal. Chem.* **2000**, *72*, 5331–5337.
40. Hosseini, S.; Ibrahim, F.; Djordjevic, I.; Koole, L. H. Recent advances in surface functionalization techniques on polymethacrylate materials for optical biosensor applications. *Analyst* **2014**, *139*, 2933–2943.
41. Tsuji, H.; Nakahara, K. Poly(L-lactide). IX. Hydrolysis in acid media. *J. Appl. Polym. Sci.* **2002**, *86*, 186–194.
42. Sun, H.; Önneby, S. Facile polyester surface functionalization via hydrolysis and cell-recognizing peptide attachment. *Polym. Int.* **2006**, *55*, 1336–1340.
43. Xu, F. J.; Wang, Z. H.; Yang, W. T. Surface functionalization of polycaprolactone films via surface-initiated atom transfer radical polymerization for covalently coupling cell-adhesive biomolecules. *Biomaterials* **2010**, *31*, 3139–3147.
44. Punet, X.; Mauchauffé, R.; Giannotti, M. I.; Rodríguez-Cabello, J. C.; Sanz, F.; Engel, E.; Mateos-Timoneda, M. A.; Planell, J. A. Enhanced Cell-Material Interactions through the Biofunctionalization of Polymeric Surfaces with Engineered Peptides. *Biomacromolecules* **2013**, *14*, 2690–2702.
45. Diaz-Quijada, G. A.; Peytavi, R.; Nantel, A.; Roy, E.; Bergeron, M. G.; Dumoulin, M. M.; Veres, T. Surface modification of thermoplastics-towards the plastic biochip for high throughput screening devices. *Lab Chip* **2007**, *7*, 856–862.
46. Boxus, T.; Touillaux, R.; Dive, G.; Marchand-Brynaert, J. Synthesis and evaluation of RGD peptidomimetics aimed at surface bioderivatization of polymer substrates. *Bioorg. Med. Chem.* **1998**, *6*, 1577–1595.
47. Marchand-Brynaert, J.; Detrait, E.; Noiset, O.; Boxus, T.; Schneider, Y.-J.; Remacle, C. Biological evaluation of RGD peptidomimetics, designed for the covalent derivatization of cell culture substrata, as potential promoters of cellular adhesion. *Biomaterials* **1999**, *20*, 1773–1782.
48. Boxus, T.; Deldime-Rubbens, M.; Mougenot, P.; Schneider, Y.-J.; Marchand-Brynaert, J. Chemical assays of end-groups displayed on the surface of poly(ethylene terephthalate) (PET) films and membranes by radiolabeling. *Polym. Adv. Technol.* **1996**, *7*, 589–598.

49. Massia, S. P.; Hubbell, J. A. Covalently attached GRGD on polymer surfaces promotes biospecific adhesion of mammalian cells. *Ann. N. Y. Acad. Sci.* **1990**, *589*, 261–70.
50. Massia, S. P.; Hubbell, J. A. Human endothelial cell interactions with surface-coupled adhesion peptides on a nonadhesive glass substrate and two polymeric biomaterials. *J. Biomed. Mater. Res.* **1991**, *25*, 223–42.
51. Hebeiss, I.; Truckenmuller, R.; Giselbrecht, S.; Schepers, U. Novel three-dimensional Boyden chamber system for studying transendothelial transport. *Lab Chip* **2012**, *12*, 829–834.
52. Kido, H.; Maquieira, A.; Hammock, B. D. Disc-based immunoassay microarrays. *Anal. Chim. Acta* **2000**, *411*, 1–11.
53. Bañuls, M.-J.; García-Piñón, F.; Puchades, R.; Maquieira, Á. Chemical Derivatization of Compact Disc Polycarbonate Surfaces for SNPs detection. *Bioconjug. Chem.* **2008**, *19*, 665–672.
54. Hirschbiel, A. F.; Geyer, S.; Yameen, B.; Welle, A.; Nikolov, P.; Giselbrecht, S.; Scholpp, S.; Delaittre, G.; Barner-Kowollik, C. Photolithographic Patterning of 3D-Formed Polycarbonate Films for Targeted Cell Guiding. *Adv. Mater.* **2015**, *27,* 2621–2626.
55. Lin, Y.-C.; Brayfield, C. A.; Gerlach, J. C.; Rubin, J. P.; Marra, K. G. Peptide modification of polyethersulfone surfaces to improve adipose-derived stem cell adhesion. *Acta Biomater.* **2009**, *5*, 1416–1424.
56. Higuchi, A.; Shirano, K.; Harashima, M.; Yoon, B. O.; Hara, M.; Hattori, M.; Imamura, K. Chemically modified polysulfone hollow fibers with vinylpyrrolidone having improved blood compatibility. *Biomaterials* **2002**, *23*, 2659–2666.
57. Costello, C. A.; McCarthy, T. J. Surface modification of poly(tetrafluoroethylene) with benzoin dianion. *Macromolecules* **1984**, *17*, 2940–2942.
58. Costello, C. A.; McCarthy, T. J. Surface-selective introduction of specific functionalities onto poly(tetrafluoroethylene). *Macromolecules* **1987**, *20*, 2819–2828.
59. Bening, R. C.; McCarthy, T. J. Surface modification of poly(tetrafluoroethylene-co-hexafluoropropylene): introduction of alcohol functionality. *Macromolecules* **1990**, *23*, 2648–2655.
60. Tong, Y. W.; Shoichet, M. S. Peptide surface modification of poly(tetrafluoroethylene-co-hexafluoropropylene) enhances its interaction with central nervous system neurons. *J. Biomed. Mater. Res.* **1998**, *42*, 85–95.
61. Gabriel, M.; Dahm, M.; Vahl, C. F. Wet-chemical approach for the cell-adhesive modification of polytetrafluoroethylene. *Biomed. Mater.* **2011**, *6*, 035007.
62. Mikhail, A. S.; Jones, K. S.; Sheardown, H. Dendrimer-grafted cell adhesion peptide–modified PDMS. *Biotechnol. Prog.* **2008**, *24*, 938–944.
63. Zhou, J.; Ellis, A. V.; Voelcker, N. H. Recent developments in PDMS surface modification for microfluidic devices. *Electrophoresis* **2010**, *31*, 2–16.
64. Abbasi, F.; Mirzadeh, H.; Katbab, A.-A. Modification of polysiloxane polymers for biomedical applications: a review. *Polym. Int.* **2001**, *50*, 1279–1287.
65. Ranieri, J. P.; Bellamkonda, R.; Bekos, E. J.; Gardella, J. A., Jr.; Mathieu, H. J.; Ruiz, L.; Aebischer, P. Spatial control of neuronal cell attachment and differentiation on covalently patterned laminin oligopeptide substrates. *Int. J. Dev. Neurosci.* **1994**, *12*, 725–35.
66. Sugawara, T.; Matsuda, T. Photochemical surface derivatization of a peptide containing Arg-Gly-Asp (RGD). *J. Biomed. Mater. Res.* **1995**, *29*, 1047–52.
67. Hatakeyama, H.; Kikuchi, A.; Yamato, M.; Okano, T. Patterned biofunctional designs of thermoresponsive surfaces for spatiotemporally controlled cell adhesion, growth, and thermally induced detachment. *Biomaterials* **2007**, *28*, 3632–3643.
68. Coad, B. R.; Jasieniak, M.; Griesser, S. S.; Griesser, H. J. Controlled covalent surface immobilisation of proteins and peptides using plasma methods. *Surf. Coat. Technol.* **2013**, *233*, 169–177.
69. Alf, M. E.; Asatekin, A.; Barr, M. C.; Baxamusa, S. H.; Chelawat, H.; Ozaydin-Ince, G.; Petruczok, C. D.; Sreenivasan, R.; Tenhaeff, W. E.; Trujillo, N. J.; Vaddiraju, S.; Xu, J.; Gleason, K. K. Chemical Vapor Deposition of Conformal, Functional, and Responsive Polymer Films. *Adv. Mater.* **2010**, *22*, 1993–2027.
70. Conrad, J. R.; Radtke, J. L.; Dodd, R. A.; Worzala, F. J.; Tran, N. C. Plasma source ion-implantation technique for surface modification of materials. *J. Appl. Phys.* **1987**, *62*, 4591–4596.

71. Ho, J. P. Y.; Nosworthy, N. J.; Bilek, M. M. M.; Gan, B. K.; McKenzie, D. R.; Chu, P. K.; dos Remedios, C. G. Plasma-Treated Polyethylene Surfaces for Improved Binding of Active Protein. *Plasma Processes Polym*. **2007**, *4*, 583–590.
72. Gubala, V.; Le, N. C. H.; Gandhiraman, R. P.; Coyle, C.; Daniels, S.; Williams, D. E. Functionalization of cyclo-olefin polymer substrates by plasma oxidation: Stable film containing carboxylic acid groups for capturing biorecognition elements. *Colloids Surf., B* **2010**, *81*, 544–548.
73. Zilio, C.; Sola, L.; Damin, F.; Faggioni, L.; Chiari, M. Universal hydrophilic coating of thermoplastic polymers currently used in microfluidics. *Biomed. Microdevices* **2014**, *16*, 107–114.
74. Bilek, M. M. M.; Bax, D. V.; Kondyurin, A.; Yin, Y.; Nosworthy, N. J.; Fisher, K.; Waterhouse, A.; Weiss, A. S.; dos Remedios, C. G.; McKenzie, D. R. Free radical functionalization of surfaces to prevent adverse responses to biomedical devices. *Proc. Natl. Acad. Nat. USA* **2011**, *108*, 14405–14410.
75. De Cooman, H.; Desmet, T.; Callens, F.; Dubruel, P. Role of radicals in UV-initiated post-plasma grafting of poly-ε-caprolactone: An electron paramagnetic resonance study. *J. Polym. Sci., Part A: Polym. Chem.* **2012**, *50*, 2142–2149.
76. Muir, B. W.; Barden, M. C.; Collett, S. P.; Gorse, A.-D.; Monteiro, R.; Yang, L.; McDougall, N. A.; Gould, S.; Maeji, N. J. High-throughput optimization of surfaces for antibody immobilization using metal complexes. *Anal. Biochem.* **2007**, *363*, 97–107.
77. Song, L.; Zhao, J.; Jin, J.; Ma, J.; Liu, J.; Luan, S.; Yin, J. Fabricating antigen recognition and anti-bioadhesion polymeric surface via a photografting polymerization strategy. *Mater. Sci. Eng., C* **2014**, *36*, 57–64.
78. Ameduri, B. From Vinylidene Fluoride (VDF) to the Applications of VDF-Containing Polymers and Copolymers: Recent Developments and Future Trends. *Chem. Rev.* **2009**, *109*, 6632–6686.
79. Pâslaru, E.; Baican, M. C.; Hitruc, E. G.; Nistor, M. T.; Poncin-Epaillard, F; Vasile, C. Immunoglobulin G immobilization on PVDF surface. *Colloids Surf., B* **2014**, *115*, 139–149.
80. Friedrich, J.; Kühn, G.; Mix, R.; Hoffmann, K.; Resch-Genger, U. Tailoring of Polymer Surfaces with Monotype Functional Groups of Variable Density Using Chemical and Plasma Chemical Processes. In *Characterization of Polymer Surfaces and Thin Films*; Grundke, K., Stamm, M., Adler, H.-J., Eds.; Springer Berlin Heidelberg, 2006; Vol. 132; pp 62–71.
81. Li, C.; Jin, J.; Liu, J.; Xu, X.; Yin, J. Improving hemocompatibility of polypropylene via surface-initiated atom transfer radical polymerization for covalently coupling BSA. *RSC Adv*. **2014**, *4*, 24842–24851.
82. Jonsson, C.; Aronsson, M.; Rundstrom, G.; Pettersson, C.; Mendel-Hartvig, I.; Bakker, J.; Martinsson, E.; Liedberg, B.; MacCraith, B.; Ohman, O.; Melin, J. Silane-dextran chemistry on lateral flow polymer chips for immunoassays. *Lab Chip* **2008**, *8*, 1191–1197.
83. Ma, J.; Luan, S.; Song, L.; Jin, J.; Yuan, S.; Yan, S.; Yang, H.; Shi, H; Yin, J. Fabricating a Cycloolefin Polymer Immunoassay Platform with a Dual-Function Polymer Brush via a Surface-Initiated Photoiniferter-Mediated Polymerization Strategy. *ACS Appl. Mater. Interfaces* **2014**, *6*, 1971–1978.
84. Hou, J.; Shi, Q.; Stagnaro, P.; Ye, W.; Jin, J.; Conzatti, L.; Yin, J. Aqueous-based immobilization of initiator and surface-initiated ATRP to construct hemocompatible surface of poly (styrene-b –(ethylene-co-butylene)-b-styrene) elastomer. *Colloids Surf., B* **2013**, *111*, 333–341.
85. Petersen, S.; Wulf, K.; Schunemann, S.; Teske, M.; Schmitz, K. P; Sternberg, K. Biofunctionalization of Polymer Implant Surfaces: From Drug Delivery to Stable Surface Functionality. *Biomed. Tech*. **2013**.
86. Paletta, J. R. J.; Bockelmann, S.; Walz, A.; Theisen, C.; Wendorff, J. H.; Greiner, A.; Fuchs-Winkelmann, S.; Schofer, M. D. RGD-functionalisation of PLLA nanofibers by surface coupling using plasma treatment: influence on stem cell differentiation. *J. Mater. Sci. Mater. Med.* **2010**, *21*, 1363–1369.
87. Wen, F.; Wong, H. K.; Tay, C. Y.; Yu, H.; Li, H.; Yu, T.; Tijore, A.; Boey, F. Y. C.; Venkatraman, S. S.; Tan, L. P. Induction of Myogenic Differentiation of Human Mesenchymal Stem Cells Cultured on Notch Agonist (Jagged-1) Modified Biodegradable Scaffold Surface. *ACS Appl. Mater. Interfaces* **2014**, *6*, 1652–1661.

88. Wang, F.; Li, Z.; Lannutti, J. L.; Wagner, W. R.; Guan, J. Synthesis, characterization and surface modification of low moduli poly(ether carbonate urethane)ureas for soft tissue engineering. *Acta Biomater*. **2009**, *5*, 2901–2912.
89. Graz, I.; Ebner, A.; Bauer, S.; Romanin, C.; Gruber, H. Micropatterned atmospheric pressure discharge surface modification of fluorinated polymer films for mammalian cell adhesion and protein binding. *Appl. Phys. A: Mater. Sci. Process*. **2008**, *92*, 547–555.
90. Kogelschatz, U.; Eliasson, B.; Egli, W. From ozone generators to flat television screens: history and future potential of dielectric-barrier discharges. *Pure Appl. Chem.* **1999**, *71*, 1819–1828.
91. Ranieri, J. P.; Bellamkonda, R.; Bekos, E. J.; Vargo, T. G.; Gardella, J. A.; Aebischer, P. Neuronal cell attachment to fluorinated ethylene propylene films with covalently immobilized laminin oligopeptides YIGSR and IKVAV. II. *J. Biomed. Mater. Res.* **1995**, *29*, 779–785.
92. Heuts, J.; Salber, J.; Goldyn, A. M.; Janser, R.; Möller, M.; Klee, D. Bio-functionalized star PEG-coated PVDF surfaces for cytocompatibility-improved implant components. *J. Biomed. Mater. Res., Part A* **2010**, *92A*, 1538–1551.
93. Wang, Y.-Y.; Lü, L.-X.; Shi, J.-C.; Wang, H.-F.; Xiao, Z.-D; Huang, N.-P. Introducing RGD Peptides on PHBV Films through PEG-Containing Cross-Linkers to Improve the Biocompatibility. *Biomacromolecules* **2011**, *12*, 551–559.
94. Hu, Y.; Winn Shelley, R.; Krajbich, I.; Hollinger Jeffrey, O. Porous polymer scaffolds surface-modified with arginine-glycine-aspartic acid enhance bone cell attachment and differentiation in vitro. *J. Biomed. Mater. Res., Part A* **2003**, *64*, 583–90.
95. Guan, J.; Sacks, M. S.; Beckman, E. J.; Wagner, W. R. Biodegradable poly(ether ester urethane)urea elastomers based on poly(ether ester) triblock copolymers and putrescine: synthesis, characterization and cytocompatibility. *Biomaterials* **2004**, *25*, 85–96.
96. Cifuentes, A.; Borrós, S. Comparison of Two Different Plasma Surface-Modification Techniques for the Covalent Immobilization of Protein Monolayers. *Langmuir* **2013**, *29*, 6645–6651.
97. Ito, Y.; Kajihara, M.; Imanishi, Y. Materials for enhancing cell adhesion by immobilization of cell-adhesive peptide. *J. Biomed. Mater. Res.* **1991**, *25*, 1325–1337.
98. Yanagi, M.; Kishida, A.; Shimotakahara, T.; Matsumoto, H.; Nishijima, H.; Akashi, M.; Aikou, T. Experimental study of bioactive polyurethane sponge as an artificial trachea. *Asaio J.* **1994**, *40*, M412–8.
99. Jung, H.; Ahn, K.-D.; Han, D.; Ahn, D.-J. Surface characteristics and fibroblast adhesion behavior of RGD-immobilized biodegradable PLLA films. *Macromol. Res.* **2005**, *13*, 446–452.
100. Jung, H. J.; Park, K.; Kim, J.-J.; Lee, J. H.; Han, K.-O.; Han, D. K. Effect of RGD-immobilized dual-pore poly(L-lactic acid) scaffolds on chondrocyte proliferation and extracellular matrix production. *Artificial Organs* **2008**, *32*, 981–989.
101. Pearson, H. A.; Urban, M. W. Simple click reactions on polymer surfaces leading to antimicrobial behavior. *J. Mater. Chem. B* **2014**, *2*, 2084–2087.
102. Francesch, L.; Garreta, E.; Balcells, M.; Edelman, E. R.; Borrós, S. Fabrication of Bioactive Surfaces by Plasma Polymerization Techniques Using a Novel Acrylate-Derived Monomer. *Plasma Processes Polym*. **2005**, *2*, 605–611.
103. Francesch, L.; Borros, S.; Knoll, W.; Förch, R. Surface Reactivity of Pulsed-Plasma Polymerized Pentafluorophenyl Methacrylate (PFM) toward Amines and Proteins in Solution. *Langmuir* **2007**, *23*, 3927–3931.
104. Carlisle, E. S.; Mariappan, M. R.; Nelson, K. D.; Thomes, B. E.; Timmons, R. B.; Constantinescu, A.; Eberhart, R. C.; Bankey, P. E. Enhancing hepatocyte adhesion by pulsed plasma deposition and polyethylene glycol coupling. *Tissue Engineering* **2000**, *6*, 45–52.
105. Aucoin, L.; Griffith, C. M.; Pleizier, G.; Deslandes, Y.; Sheardown, H. Interactions of corneal epithelial cells and surfaces modified with cell adhesion peptide combinations. *J. Biomater. Sci., Polym. Ed.* **2002**, *13*, 447–462.
106. Breuers, W.; Klee, D.; Höcker, H.; Mittermayer, C. Immobilization of a fibronectin fragment at the surface of a polyetherurethane film. *J. Mater. Sci.: Mater. Med.* **1991**, *2*, 106–109.
107. Hirsh, S. L.; Bilek, M. M. M.; Bax, D. V.; Kondyurin, A.; Kosobrodova, E.; Tsoutas, K.; Tran, C. T. H.; Waterhouse, A.; Yin, Y.; Nosworthy, N. J.; McKenzie, D. R.; dos Remedios, C. G.; Ng, M. K. C.; Weiss, A. S. Ion implanted, radical-rich surfaces for the rapid covalent immobilization of active biomolecules. *AIP Conf. Proc.* **2013**, *1525*, 364–369.

108. Bilek, M. M. M. Biofunctionalization of surfaces by energetic ion implantation: Review of progress on applications in implantable biomedical devices and antibody microarrays. *Appl. Surf. Sci.* **2014**, *310*, 3–10.
109. Bilek, M. M.; McKenzie, D. R. Plasma modified surfaces for covalent immobilization of functional biomolecules in the absence of chemical linkers: towards better biosensors and a new generation of medical implants. *Biophys. Rev.* **2010**, *2*, 55–65.
110. Nosworthy, N. J.; Ho, J. P. Y.; Kondyurin, A.; McKenzie, D. R.; Bilek, M. M. M. The attachment of catalase and poly-l-lysine to plasma immersion ion implantation-treated polyethylene. *Acta Biomater*. **2007**, *3*, 695–704.
111. Nosworthy, N. J.; Kondyurin, A.; Bilek, M. M. M.; McKenzie, D. R. Ion implantation treatment of beads for covalent binding of molecules: Application to bioethanol production using thermophilic beta-glucosidase. *Enzym. Microb. Technol.* **2014**, *54*, 20–24.
112. Kondyurin, A.; Nosworthy, N. J.; Bilek, M. M. M. Attachment of horseradish peroxidase to polytetrafluorethylene (teflon) after plasma immersion ion implantation. *Acta Biomater*. **2008**, *4*, 1218–1225.
113. Bax, D. V.; McKenzie, D. R.; Weiss, A. S.; Bilek, M. M. M. The linker-free covalent attachment of collagen to plasma immersion ion implantation treated polytetrafluoroethylene and subsequent cell-binding activity. *Biomaterials* **2010**, *31*, 2526–2534.
114. Hirsh, S. L.; Nosworthy, N. J.; Kondyurin, A.; dos Remedios, C. G.; McKenzie, D. R.; Bilek, M. M. M. Linker-free covalent thermophilic [small beta]-glucosidase functionalized polymeric surfaces. *J. Mater. Chem.* **2011**, *21*, 17832–17841.
115. Kosobrodova, E.; Mohamed, A.; Su, Y.; Kondyurin, A.; dos Remedios, C. G.; McKenzie, D. R.; Bilek, M. M. M. Cluster of differentiation antibody microarrays on plasma immersion ion implanted polycarbonate. *Mater. Sci. Eng., C* **2014**, *35*, 434–440.
116. MacDonald, C.; Morrow, R.; Weiss, A. S.; Bilek, M. M. M. Covalent attachment of functional protein to polymer surfaces: a novel one-step dry process. *J. R. Soc. Interface* **2008**, *5*, 663–669.
117. Jung, C.-H.; Hwang, I.-T.; Kuk, I.-S.; Choi, J.-H.; Oh, B.-K.; Lee, Y.-M. Poly(acrylic acid)-Grafted Fluoropolymer Films for Highly Sensitive Fluorescent Bioassays. *ACS Appl. Mater. Interfaces* **2013**, *5*, 2155–2160.
118. Beachell, H. C.; Nemphos, S. P. Oxidative degradation of polyethylene. *J. Polym. Sci.* **1956**, *21*, 113–124.
119. Cooper, G. D.; Prober, M. The action of oxygen corona and of ozone on polyethylene. *J. Polym. Sci.* **1960**, *44*, 397–409.
120. Dietrich, M.; Delaittre, G.; Blinco, J. P.; Inglis, A. J.; Bruns, M; Barner-Kowollik, C. Photoclickable Surfaces for Profluorescent Covalent Polymer Coatings. *Adv. Funct. Mater.* **2012**, *22*, 304–312.
121. Pauloehrl, T.; Delaittre, G.; Winkler, V.; Welle, A.; Bruns, M.; Börner, H. G.; Greiner, A. M.; Bastmeyer, M; Barner-Kowollik, C. Adding Spatial Control to Click Chemistry: Phototriggered Diels–Alder Surface (Bio)functionalization at Ambient Temperature. *Angew. Chem. Int. Ed.* **2012**, *51*, 1071–1074.
122. Richter, B.; Pauloehrl, T.; Kaschke, J.; Fichtner, D.; Fischer, J.; Greiner, A. M.; Wedlich, D.; Wegener, M.; Delaittre, G.; Barner-Kowollik, C.; Bastmeyer, M. Three-Dimensional Microscaffolds Exhibiting Spatially Resolved Surface Chemistry. *Adv. Mater.* **2013**, *25*, 6117–6122.
123. Shaw, D.; Shoichet, M. S. Toward Spinal Cord Injury Repair Strategies: Peptide Surface Modification of Expanded Poly(Tetrafluoroethylene) Fibers for Guided Neurite Outgrowth In Vitro. *J Craniofac Surg*. **2003**, *14*, 308–316.
124. Kantlehner, M.; Finsinger, D.; Meyer, J.; Schaffner, P.; Jonczyk, A.; Diefenbach, B.; Nies, B.; Kessler, H. Selective RGD-Mediated Adhesion of Osteoblasts at Surfaces of Implants. *Angew. Chem. Int. Ed.* **1999**, *38*, 560–562.
125. Kantlehner, M.; Schaffner, P.; Finsinger, D.; Meyer, J.; Jonczyk, A.; Diefenbach, B.; Nies, B.; Hölzemann, G.; Goodman, S. L; Kessler, H. Surface Coating with Cyclic RGD Peptides Stimulates Osteoblast Adhesion and Proliferation as well as Bone Formation. *ChemBioChem* **2000**, *1*, 107–114.

126. Auernheimer, J.; Dahmen, C.; Hersel, U.; Bausch, A; Kessler, H. Photoswitched Cell Adhesion on Surfaces with RGD Peptides. *J. Am. Chem. Soc.* **2005**, *127*, 16107–16110.
127. Edlund, U.; Källrot, M.; Albertsson, A.-C. Single-Step Covalent Functionalization of Polylactide Surfaces. *J. Am. Chem. Soc.* **2005**, *127*, 8865–8871.
128. Wang, C.; Ren, P.-F.; Huang, X.-J.; Wu, J.; Xu, Z.-K. Surface glycosylation of polymer membrane by thiol-yne click chemistry for affinity adsorption of lectin. *Chem. Commun.* **2011**, *47*, 3930–3932.
129. Larsen, E. K. U.; Mikkelsen, M. B. L.; Larsen, N. B. Facile Photoimmobilization of Proteins onto Low-Binding PEG-Coated Polymer Surfaces. *Biomacromolecules* **2014**, *15*, 894–899.
130. Li, J.; Ding, M.; Fu, Q.; Tan, H.; Xie, X; Zhong, Y. A novel strategy to graft RGD peptide on biomaterials surfaces for endothelization of small-diamater vascular grafts and tissue engineering blood vessel. *J. Mater. Sci.: Mater. Med.* **2008**, *19*, 2595–2603.
131. Xu, J.; Ma, Y.; Xie, J.; Xu, F. J.; Yang, W. Functionalization of polymeric surfaces by simple photoactivation of Cï£¿H bonds. *J. Polym. Sci., Part A: Polym. Chem.* **2011**, *49*, 2755–2760.
132. Li, C. Y.; Xu, F. J.; Yang, W. T. Simple Strategy to Functionalize Polymeric Substrates via Surface-Initiated ATRP for Biomedical Applications. *Langmuir* **2012**, *29*, 1541–1550.
133. Guillaneuf, Y.; Bertin, D.; Gigmes, D.; Versace, D.-L.; Lalevée, J.; Fouassier, J.-P. Toward Nitroxide-Mediated Photopolymerization. *Macromolecules* **2010**, *43*, 2204–2212.
134. Huebsch, J. B.; Fields, G. B.; Triebes, T. G.; Mooradian, D. L. Photoreactive analog of peptide FN-C/H-V from the carboxy-terminal heparin-binding domains of fibronectin supports endothelial cell adhesion and spreading on biomaterial surfaces. *J. Biomed. Mater. Res.* **1996**, *31*, 555–567.
135. Mizutani, M.; Arnold, S. C.; Matsuda, T. Liquid, Phenylazide-End-Capped Copolymers of ε-Caprolactone and Trimethylene Carbonate: Preparation, Photocuring Characteristics, and Surface Layering. *Biomacromolecules* **2002**, *3*, 668–675.
136. Lin, Y.-S.; Wang, S.-S.; Chung, T.-W.; Wang, Y.-H.; Chiou, S.-H.; Hsu, J.-J.; Chou, N.-K.; Hsieh, K.-H.; Chu, S.-H. Growth of endothelial cells on different concentrations of Gly-Arg-Gly-Asp photochemically grafted in polyethylene glycol modified polyurethane. *Artificial Organs* **2001**, *25*, 617–621.
137. Ortega, F. J.; Banuls, M.-J.; Sanza, F. J.; Laguna, M. F.; Holgado, M.; Casquel, R.; Barrios, C. A.; Lopez-Romero, D.; Maquieira, A.; Puchades, R. Development of a versatile biotinylated material based on SU-8. *J. Mater. Chem. B* **2013**, *1*, 2750–2756.
138. Chevolot, Y.; Martins, J.; Milosevic, N.; Léonard, D.; Zeng, S.; Malissard, M.; Berger, E. G.; Maier, P.; Mathieu, H. J.; Crout, D. H. G.; Sigrist, H. Immobilisation on polystyrene of diazirine derivatives of mono- and disaccharides: biological activities of modified surfaces. *Bioorg. Med. Chem.* **2001**, *9*, 2943–2953.
139. Kolodziej, C. M.; Maynard, H. D. Electron-Beam Lithography for Patterning Biomolecules at the Micron and Nanometer Scale. *Chem. Mater.* **2011**, *24*, 774–780.
140. Sun, H.; Wirsen, A.; Albertsson, A.-C. Electron beam-induced graft polymerization of acrylic acid and immobilization of arginine-glycine-aspartic acid-containing peptide onto nanopatterned polycaprolactone. *Biomacromolecules* **2004**, *5*, 2275–2280.
141. Bozzi, A.; Chapiro, A. Synthesis of perm-selective membranes by grafting acrylic acid into air-irradiated Teflon-FEP films. *Int. J. Radiat. Appl. Instrum. C Radiat. Phys. Chem.* **1988**, *32*, 193–196.
142. Sipehia, R.; Chawla, A. S.; Chang, T. M. S. Enhanced albumin binding to polypropylene beads via anhydrous ammonia gaseous plasma. *Biomaterials* **1986**, *7*, 471–473.
143. Sipehia, R.; Chawla, A. S.; Daka, J.; Chang, T. M. S. Immobilization of enzymes on polypropylene bead surfaces by anhydrous ammonia gaseous plasma technique. *J. Biomed. Mater. Res.* **1988**, *22*, 417–422.
144. Jeong, S. P.; Hong, D.; Kang, S. M.; Choi, I. S.; Lee, J. K. Polymeric Functionalization of Cyclic Olefin Copolymer Surfaces with Nonbiofouling Poly(oligo(Ethylene Glycol) Methacrylate). *Asian J. Org. Chem.* **2013**, *2*, 568–571.
145. Barish, J. A.; Goddard, J. M. Topographical and chemical characterization of polymer surfaces modified by physical and chemical processes. *J. Appl. Polym. Sci.* **2011**, *120*, 2863–2871.

146. Kochkodan, V.; Johnson, D. J.; Hilal, N. Polymeric membranes: Surface modification for minimizing (bio)colloidal fouling. *Adv. Colloid Interface Sci*. **2014**, *206*, 116–140.
147. Yang, R.; Asatekin, A.; Gleason, K. K. Design of conformal, substrate-independent surface modification for controlled protein adsorption by chemical vapor deposition (CVD). *Soft Matter* **2012**, *8*, 31–43.
148. Leckband, D.; Sheth, S.; Halperin, A. Grafted poly(ethylene oxide) brushes as nonfouling surface coatings. *J. Biomater. Sci., Polym. Ed.* **1999**, *10*, 1125–1147.
149. Hucknall, A.; Rangarajan, S.; Chilkoti, A. In Pursuit of Zero: Polymer Brushes that Resist the Adsorption of Proteins. *Adv. Mater.* **2009**, *21*, 2441–2446.
150. Tauhardt, L.; Kempe, K.; Gottschaldt, M.; Schubert, U. S. Poly(2-oxazoline) functionalized surfaces: from modification to application. *Chem. Soc. Rev.* **2013**, *42*, 7998–8011.
151. Schlenoff, J. B. Zwitteration: Coating Surfaces with Zwitterionic Functionality to Reduce Nonspecific Adsorption. *Langmuir* **2014**, *30*, 9625–9636.
152. Handbook of RAFT Polymerization; Barner-Kowollik , C., Ed.; Wiley-VCH Verlag GmbH & Co. KGaA, 2008.
153. Moad, G.; Rizzardo, E.; Thang, S. H. Toward Living Radical Polymerization. *Acc. Chem. Res.* **2008**, *41*, 1133–1142.
154. Braunecker, W. A.; Matyjaszewski, K. Controlled/living radical polymerization: Features, developments, and perspectives. *Prog. Polym. Sci.* **2007**, *32*, 93–146.
155. Nicolas, J.; Guillaneuf, Y.; Lefay, C.; Bertin, D.; Gigmes, D.; Charleux, B. Nitroxide-mediated polymerization. *Prog. Polym. Sci.* **2013**, *38*, 63–235.