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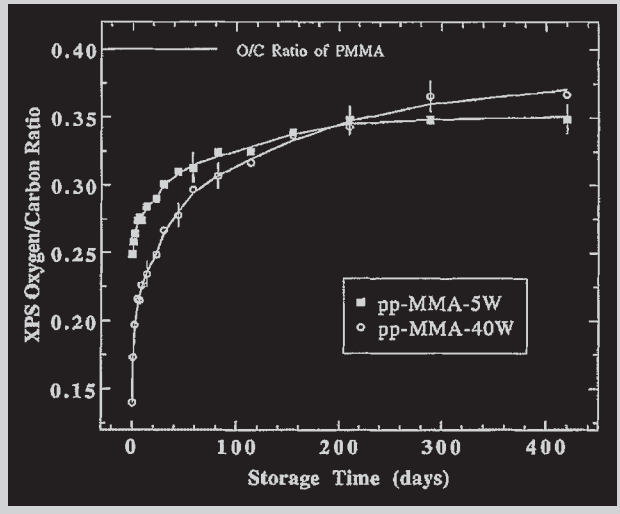
Summary: This review surveys methods for the fabrication, by plasma surface treatments or plasma polymerization, of polymeric surfaces and thin plasma polymer coatings that contain reactive chemical groups useful for the subsequent covalent immobilization, by solution chemical reactions or vapor phase grafting, of molecules or polymers that can exert bio-specific interfacial responses. Surfaces containing amine, carboxy, hydroxy, and aldehyde groups are the subject of this review. Aminated surfaces have been fabricated using various plasma vapors or mixtures and have found wide use for bio-interface applications. However, in many cases the amine surfaces have a rather limited shelf life, with post-plasma oxidation reactions and surface adaptation leading to the disappearance of amine groups from the surface. Aging is a widespread phenomenon that often has not been recognized, particularly in some of the earlier studies on the use of plasma-fabricated surfaces for bio-interfacial applications, and can markedly alter the surface chemistry. Plasma-fabricated surfaces that contain carboxy groups have also been well documented. Fewer reports exist on hydroxy and aldehyde surfaces prepared by plasma methods. Hydroxy surfaces can be prepared by water plasma treatment or the plasma polymerization of alkyl alcohol vapors. Water plasma treatment on many polymer substrates suffers from aging, with surface adaptation leading to the movement of surface modification effects into the polymer. Both hydroxy and aldehyde surfaces have been used for the covalent immobilization of biologically active molecules. Aging effects are less well documented than for amine surfaces. This review also surveys studies using such surfaces for cell colonization assays. Generally, these surface chemistries show good ability to support cell colonization, though the effectiveness seems to depend on the process vapor and the plasma conditions. Carboxylate co-polymer surfaces have shown excellent ability to support the colonization of some human cell lines of clinical interest. Immobilization of proteins onto plasma-carboxylated surfaces is also well established.



XPS O/C ratios (0^0 emission) as a function of storage time, of plasma-polymerized methyl methacrylate deposited at power levels of 5 and 40 W.

Plasma Methods for the Generation of Chemically Reactive Surfaces for Biomolecule Immobilization and Cell Colonization - A Review

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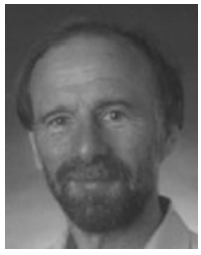
Introduction

Plasma-based approaches have gained considerable popularity for the creation of surfaces designed to be in contact with biological environments ('bio-interfaces'). Many studies have been reported using various approaches and process gases. Initially a number of studies explored the potential of plasma-produced surfaces or coatings to control bio-interfacial interactions. More recently, the majority of studies do not focus on the applicability of plasma



Hans J. Griesser is Professor of Surface Science and Deputy Director at the Ian Wark Research Institute of the University of South Australia. He is a Physical Chemist by training and has applied this background to interdisciplinary research in various fields, particularly surface science and bio-interface science, the analysis and modification of polymer surfaces, thin film deposition, biomaterials, and adhesion. He studied science at the Swiss Federal Institute of Technology (ETH), Zürich, obtaining a Diploma in Chemistry and a Ph.D. (Dr. sc. nat.) for research in molecular spectroscopy. Moving to Australia, he pursued postdoctoral studies in low temperature exciton spectroscopy at the Australian National University, followed by 4 years with the Research Laboratory of Kodak Australasia where he combined spectroscopy and surface science skills to develop novel polymer surface treatments and vacuum-coated thin films for non-photographic applications. Joining CSIRO in 1987, he continued research on surface modification and thin film coating processes, and surface analysis methods. This work led to the establishment of a research group that performed fundamental and applied research and development, using surface science and plasma expertise for various purposes, particularly for the development of novel biomaterial surfaces and coatings, membranes, and for achieving adhesive bonding of polymers. He joined the Ian Wark Research Institute in early 2002, where he has continued fundamental and applied research on interface science and coatings, particularly with polymers and bio-interfaces, and research on advanced methods of characterization. His research has led to outcomes of industrial and commercial significance, most notably in a large multi-disciplinary project that successfully developed a breakthrough contact lens that can be worn continuously for 30 days and nights. His current research interests comprise fundamental and applied studies on the design, fabrication, and characterization of novel surfaces and coatings for various purposes, particularly for the understanding and control of interfacial interactions between polymeric materials/devices and biological media and tissues.

Leanne Britcher is a Research Fellow at the Ian Wark Research Institute, University of South Australia. She received a B.Sc., B.Sc. (Hons), and a Ph.D. in Chemistry from the University of South Australia. In 1996 she spent some time at the Åbo Akademi in Turku, Finland to research metallocene polymerization catalysts. Since joining the Ian Wark Research Institute in 1997, she has been actively involved in the leadership of a number of applied research projects, including the development of copolymer coatings, modification and characterisation of E-glass fibers for use in composites, antibacterial coatings, and several projects on titania pigment modification with either inorganic and organic coatings. Her core research interest is the modification of surfaces such as polymers, glass, silica, and inorganic pigments with both polymer coatings and small molecules. The need to develop tailored coatings has also led to capability in synthesis and surface spectroscopy. This capability has been used for not only developing tailored coatings but also to ensure that the surface chemistry has been well understood. Recently, her interest has been in surface modification for biomaterial applications. She is also interested in understanding the interaction of biomaterials with proteins.

Sunil Kumar is a Senior Research Fellow with the Ian Wark Research Institute of the University of South Australia. Since obtaining his Ph.D. in thin-film electronic materials, he has been working in the fields of advanced thin-film materials and plasma surface engineering. For the last five years, the main focus of his research has been on improving the fixation of orthopaedic implants through surface coatings and modification and plasma processing. Currently, he is leading (as Chief Investigator) five major research projects in these areas mainly funded by the Australian Research Council and the National Health and Medical Research Council. He is a Committee Member of the Australasian Society for Biomaterials and a current member of the American Vacuum Society. Through invited visits, he has carried out collaborative work and continues to do so with researchers from the USA, Canada, Mexico and Singapore. In addition, he has several ongoing research links with Australian researchers. Dr Kumar routinely carries out peer-reviewing duties for a number of funding agencies and research journals. On knowledge application, Dr Kumar's research has resulted in patents and benefits to various industries.

K. S. Siow is doing his Ph.D. in the Ian Wark Research Institute, University of South Australia (UniSA). K.S. Siow graduated from Nanyang Technological University with a B.A.Sc. (Materials Engineering) with Honours in 1997 and an M.A.Sc. in 1999. Prior to joining UniSA, he worked with Infineon Technologies (formerly Siemens Microelectronics) and the National University of Singapore (NUS) as a materials engineer in the development of novel microelectronic packages. The latter was a collaborative project under the Nano-Wafer Level Packaging Programme between NUS, the Institute of Micro-Electronics (Singapore), and the Georgia Institute of Technology (USA). His current research interests include the use of surface analytical techniques to understand surface phenomena, notably in biointerface events and the surface modification of materials for various applications. He has published seven papers, and presented 18 conference papers.

polymers per se, but, instead, use plasma-treated surfaces or plasma polymers as interfacial bonding layers for the subsequent immobilization of molecules designed to elicit specific biological responses.

While a number of short, selective reviews have been published in recent years, there is no detailed overview of this research area, and this contribution is designed to address this gap. Given the size of the literature, a comprehensive review of all approaches to generate bio-interfaces by plasma methods would be prohibitively long. Therefore, we have decided to focus in this article on plasmas that generate surfaces with chemically reactive groups that can subsequently be used for the covalent immobilization of biologically active molecules. Here we will review work that is focused on surfaces with carboxy, hydroxy, amine, and aldehyde groups, which are the main chemically reactive groups amenable for the covalent immobilization of biologically active molecules.

Based on the outcomes of interactions with materials, plasmas can be classified into the broad categories of plasma polymerization, plasma treatment, and plasma etching. Plasma polymerization involves the fragmentation and subsequent deposition of organic monomers. Some etching can also happen simultaneously as a result of the bombardment of both the substrate and the growing coating by ions as well as radiation from vacuum ultra violet (VUV). Plasma treatment utilizes gases such as Ar, N_2, O_2, NH_3, and CF_4 to insert or substitute chemical functionalities onto a substrate or to create radicals for crosslinking or subsequent surface grafting. Depending on various factors such as the process vapor, substrates, and process conditions, deposition, substitution, or etching can dominate in modifications on the materials surfaces. These different processes have been described by Yasuda as competitive ablation polymerization (CAP).[1,2] A schematic diagram is shown in Figure 1.

Plasmas are often used to insert chemically reactive functionality onto otherwise non-reactive substrates. These functionalities can be chemical groups, which are the main focus of this review, or radicals. Generally, there are two methods of grafting onto radicals, that is, direct irradiation and post-irradiation. In post-irradiation grafting, the plasma creates radicals such as peroxide on the substrate, and then exposes the substrate to a grafting monomer. The direct-irradiation method irradiates the substrate, thus creating the radicals in the presence of grafting molecules. This direct-irradiation method is also known as plasma immobilization.[3] While the post-irradiation grafting method is derived from γ-ray activated grafting onto polymers,[4] direct-irradiation is based on the 'crosslinking by activated species of inert gas' (CASING) technique.[5]

The application of plasma technologies encompasses numerous fields, from promoting adhesion in the inking industry to improving the 'biocompatibility' of biomaterials. Its versatility stems from its many advantages, which include that different sizes, shapes, geometries, and type of materials can be treated, surface topography and bulk properties are usually not affected, specific functionality is possible (often by grafting), it can form dense coatings and good barrier layers against leaching, it may provide good adhesion to substrates, it has manufacturability aspects such as good reproducibility, minimal pollution, and waste, it can impart sterility to biomedical products as a result of the plasma exposure, and it is amenable to surface patterning.

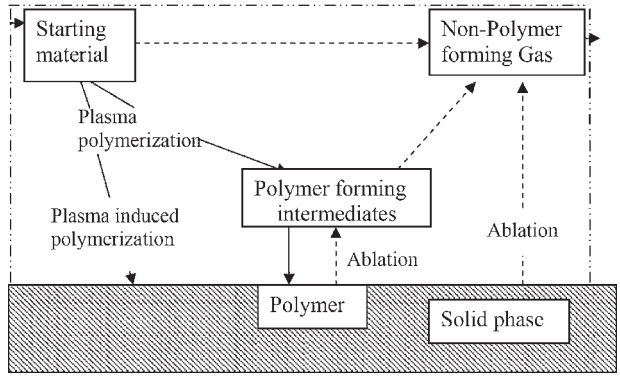


Figure 1. Schematic diagram of competitive ablation polymerization, reproduced from ref. [1] and [2].

One of the major limitations of plasma technologies is the diversity of functional groups produced by the multitudes of chemical reactions that occur in the plasma. The plasma causes various homolytic bond fissions and ionization events, as well as secondary collisions. Subsequent molecular fragmentations, reactions, and ionization processes result in a spread of functionalities on the plasma-treated or polymerized surfaces. Optimization of the desired functionality on a plasma surface can be performed by reducing the discharge power or increasing the off time in pulsed polymerization, although the type of monomer affects the effectiveness of the latter technique.[6] Plasma polymers also have irregular, non-repeat polymer structures with much branching and crosslinks. Techniques such as remote (or downstream) plasma polymerization and pulsed-plasma polymerization are utilized to counter these chemical divergences. Remote plasma polymerization makes use of physical location to prevent the direct bombardment by ions. Grafting of well-defined chemical species onto a plasma-activated surface is another approach to achieve more controlled surface chemistries. These techniques will be discussed within the context of chemical groups in this review.

The design of plasma methods for biomaterial applications must be based on an understanding of the requirements of the bio-interface. Definitions of 'biocompatibility'[7–9] are, unfortunately, vague in terms of what chemical composition a biocompatible surface should possess. Endothelial seeding[9] and pre-adsorbing fibronectin for endothelialization[10] are approaches for blood compatibility, but design rules do not

exist for plasma surfaces that are to promote these effects. Understanding interactions of plasma surfaces with proteins and cells is important. Control of surface chemistry not only enhances cell attachment but also cell spreading, which is important as it affects cell division and the synthesis of DNA and proteins.[11]

Several reviews exist on plasma polymerization and the treatment of biomaterials[12–16] but here we will review a select set of plasmas, those that create surface chemistries suitable for covalent interfacial reactions for the permanent immobilization of biologically active molecules. We will review plasma methods, both plasma treatments and plasma polymer depositions, which result in surfaces that contain amine, carboxy, hydroxy, and aldehyde groups. Other reactive surface chemistries that enable biomolecule immobilization are feasible, such as sulfhydryl and epoxy, but very few such plasma studies exist and no information on aging effects of such surfaces forthcoming; these will not be discussed here. The methods and process vapors that create surfaces with these chemical groups are first discussed, followed by discussion of aging effects of these surface chemistries. Finally, studies are reviewed that have investigated these same surface chemistries for the support of surface colonization by anchorage-dependent mammalian cells. Adequate characterization and understanding of plasma-produced surfaces is crucial to the successful implementation of plasma polymers for biomedical device applications.

Quantification and Identification of Chemical Groups

Various tools have been used to characterize biomaterial surfaces with varying degrees of success and detail. The most popular has been X-ray photoelectron spectroscopy (XPS). This tool is well suited to the purpose because a central theme of biomaterials research is the identification of chemical groups on biomaterial surfaces and their quantification (surface density). The C atomic percentage and fitting of the C 1s peak with different bond types such as –C–O, C=O, and –COOR can be used to estimate the density of chemical groups. However, some groups cannot be distinguished by XPS and derivatization methods have been used. The density and oxidation state of functionalities defines the success of subsequent steps such as the immobilization of biomolecules. XPS is also useful for the measurement of the thickness of thin plasma coatings, via overlayer equations.[17] Other techniques such as ellipsometry and atomic force microscopy (AFM) can be used to measure the thickness but will not be discussed here. Attenuated total reflectance (ATR) FT-IR spectroscopy can be used to identify the chemical groups if the thickness of a plasma polymer is in the micrometer range.[18,19]

A key challenge is the comparison of results from different laboratories. Differences in the design of plasma equipment affect the flow of process vapors and the shape of the plasma discharge zone, which affects the density and nature of species in the plasma.

Derivatization of surface chemical groups can suffer from factors such as failure of the reaction to proceed to completion,[17,20] but it has been successfully implemented using a number of reagents such as pentafluorobenzaldehyde[21,22] and fluorescein isothiocyanate for amine groups,[23] hydrazine for carbonyl,[21] trifluoroethanol (TFE) for carboxy (COOH),[21,24] and trifluoroacetic anhydride (TFAA) for hydroxy (OH) groups.[21,24] Both TFAA vapor phase[22] and liquid phase derivatization[21] have been used successfully to identify OH groups, though some believe that gas phase derivatization produces more reliable results. However, the use of TFAA to identify OH groups is controversial because it has been reported that it also reacts with epoxide,[25] amino, and amido groups.[26] FT-IR spectroscopy can be used to differentiate the presence of epoxide from other groups. In addition, derivatization is often carried out with poly(vinyl alcohol) to serve as a benchmark for appropriate reaction times.

A gravimetric technique has also been used to measure the density of groups but this normally overestimates the density because it measures the total weight gain caused by other groups as well as the intended chemical groups.[19] Another study has used solvents to extract low-molecular-weight material from plasma polymers followed by titration of amine and carboxy groups.[27] This approach is unlikely to give a reliable measure of functional groups because only a fraction of the chemical groups of interest can be eluted from the plasma polymer. As a minimum, it would have been advisable to use a complementary technique to analyze the remaining plasma polymers for the presence of unextracted functional groups.

Other methods to determine the density of chemical groups involve the use of dyes such as rhodamine[28] or Toluidine Blue O[29,30] that can complex with carboxy groups and the amount of bound dye can be quantitated by colorimetry. For amine groups, measurement of the optical density of acidic dyes (e.g., Acid Orange 7)[29,30] or 2 furoyl quinoline-2-carboxyaldehyde[31] has been used to determine density. Cationic dyes such as methylene blue have also been used to quantify the density of sulfonate groups but suffer from non-specific binding to COOH groups.[32] Radiolabeling, e.g., with ^{14}C formaldehyde[33] or ^{35}S heparin,[34] has also been used to probe for amine groups or alkylated amine groups. The ninhydrin method is the most widely used method to measure the surface density of amide chemical groups or the presence of bound protein.[35] While some of the radiolabeling results[33] are comparable to derivatization,[23] the dye-adsorption and derivatization approaches often suffer from non-specific binding.[36] Multiple control experiments and 'crossover derivatization XPS' should be carried out to increase reliability of the measured densities of chemical groups.[36] The crossover derivatization XPS approach

utilizes two similar probes that should give complete or zero covalent immobilization to a specific reactive group on the polymer surface. The probe that is incapable of covalent immobilization enables assessment of the extent of physisorption.[36]

Limitations and Pitfalls of Studies of Interactions Between Cells or Proteins and Plasma Surfaces

The choice of cell line can affect the result of cell attachment studies. Bovine corneal epithelial cells can attach and grow even on unmodified polystyrene (PS).[37] These cells can colonize various substrates in the absence of glycoproteins, vitronectin, and fibronectin.[38] These characteristics make these cells suitable for testing surfaces for critical applications. Keratinocyte cells are notorious for their variation of adhesion behavior between donors,[39] their number adhering on positive control collagen I can vary between 6 to 20 times relative to hydrocarbon plasma polymers.

The study of cell attachment can be complicated by the surface restructuring of polymers. Mobile polymer surfaces might adapt to an adsorbing protein layer by emergence of a buried polar group into the interface regions till an equilibrated interfacial energy is reached.[40] It is also important to take into account aging effects, as the ability of a plasma-prepared surface to support cell colonization might vary significantly as the surface chemical composition changes upon storage after plasma fabrication.

While plasma treatments are in industrial use for tissue culture plastics, products can be variable and thereby confound conclusions when comparing experimental surfaces with these 'controls'.[41–43] Argon plasma-treated PS has a 'saturated' O/C ratio of 0.30 which will reduce to a 'stable' O/C ratio of 0.20 after washing with methanol.[42] 'Saturation' is the highest level of attainable oxygen during treatment while 'stable' refers to the level of retained oxygen after low-molecular-weight polymer fragments have been removed by washing or evaporated upon exposure to air. Dissolution of fragmented and modified surfaces can also interfere with contact angle measurements and cell attachment.[41] Clean glass may be a more suitable control surface because of its inert nature.

Grafting Functionalities

Plasma produced reactive surfaces with amine, carboxy, hydroxy, and aldehyde groups have been used by many scientists because of their compatibility with well-established chemical reactions for grafting of bioactive moieties such as enzymes, antibodies, proteins, and glycosaminoglycans. Such interfacial immobilization should satisfy a number of criteria:

- The linkage should be a covalent bond to avoid displacement by biological media and be stable enough for the duration of the intended application.
- The binding site of the bioactive molecules should be situated away from the biologically active area.
- The active sites of bioactive molecules should face the biological medium.
- The bioactive molecules should not denature or change configuration upon binding.
- The anchoring reaction should only react with specific groups on the bioactive molecules.

Sometimes, spacer molecules such as succinic anhydride have been used to avoid denaturation of bioactive molecules[44] or to avoid steric hindrance of the bioactive molecules.[45] Other spacer molecules comprise poly(ethylene glycol) (PEG)[46] and polyamine.[47] A disadvantage of using spacers may be the reduction of stability of bioactive molecules in some cases.[9]

Amine Groups

Amine-containing surfaces have been prepared mainly by ammonia plasma treatment or by the plasma polymerization of alkylamine monomers. The ammonia plasma treatment approach suffers, on some polymer substrates, from the short-lived nature of the treatment as reptation leads to the disappearance of some of the treatment effects: treated chains reptate into the polymer bulk and the surface reverts partly toward the original untreated state.[48–50] The popularity of amine plasma polymers stems from the ease of fabrication; good coatings can be obtained over a relatively broad range of plasma parameters. Amine plasma polymers are useful surfaces as such for cell colonization,[51] but the large majority of reports utilizes them as chemically reactive platforms for the covalent immobilization of biologically active molecules.

Carbodiimide chemistry is by far the most popular approach. Reaction between the surface amine groups and carboxy groups on the molecules to be immobilized leads to the formation of interfacial amide bonds. An alternative reaction scheme is to react the surface amine groups with aldehyde groups on biomolecules by reductive amination. Aldehyde groups do not normally exist on biological molecules but can be produced on sugars by periodate oxidation.[52] Examples of bioactive molecules that have been successfully immobilized onto plasma-prepared amine groups are DNA,[53,54] protein A,[30] hyaluronic acid,[45] heparin,[55] immunoglobulin G,[33,56] enzymes such as glucose oxidase[57] and glucose isomerase,[58] lysozyme,[44] and polysaccharides such as dextran[52] and carboxylmethyl-dextrans.[47,59]

What has often been overlooked, however, is that carbodiimide activates carboxy groups that can then react with amine groups on the same protein or another protein rather than with an amine group on the polymer surface, thereby

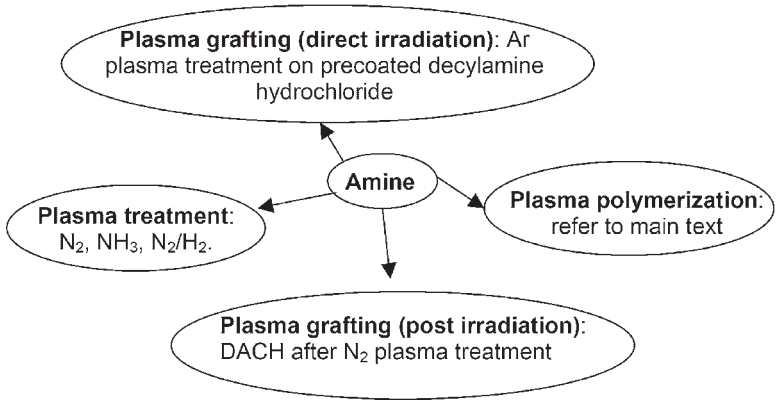


Figure 2. Approaches used to create amine groups on biomaterial surfaces.

causing crosslinks and oligomeric protein agglomerates. Such agglomerates become less soluble and can then adsorb on surfaces. Thus, not all molecules found on the surface may be interfacially covalently linked, they could be adsorbed agglomerates, and biological function may be compromised. Carbodiimide linking of proteins to amine surfaces should be viewed with caution and re-examined. It is much more appropriate from a chemist's point of view to turn the scheme around, that is, to use a carboxylated surface to react with amine groups on the protein. This can be done by using carboxy plasma surfaces, as discussed below, or by the use of an interlayer that contains carboxy groups, such as carboxymethylated dextran.[60]

In order to produce protein-resistant surfaces, PEGs with different terminating groups have also been successfully bound to aminated surfaces after appropriate treatment.[61–63] It has been shown by Kingshott et al.[63,64] that the density of amine functional groups on the surface is important when shorter PEG chains are used. With longer PEG chains, however, even the functional group density of a heptylamine plasma polymer is sufficient for dense immobilization of such polymers. This point is important because a number of studies have used pulsed plasma polymerization in order to boost the surface density of amine groups, however, since most biologically active molecules to be immobilized have a diameter that substantially exceeds the spacing between surface amine groups, such maximization of surface coverage may be unnecessary for many applications. For example, the relatively small protein lysozyme has a diameter of 3–4 nm, hence, even a surface density of $\approx$0.5 groups $\cdot$ nm^{-2}, as applicable to heptylamine plasma polymer,[23] is well in excess of the reactive group density needed for effective monolayer immobilization.

Four main classes of plasma processes have been used to produce NH_2-functional surfaces. Figure 2 shows representative examples. Experimentally the simplest is plasma treatment using NH_3[34,49,65] or ammonia with H_2[66] or Ar,[45,67] a combination of N_2 and H_2[31,34,65] or C_2H_4,[68] or N_2 alone.[65,69] The admixture of Ar to an NH_3 plasma increased the N/C and O/C ratios on a polysulfone membrane because of an increase of free radicals generated by the Ar plasma in the polymer.[67] Whereas most plasma-based amination has been carried out on polymeric substrates, diamond like carbon is also a suitable platform for inserting NH_2 groups [55].

The success of surface amination is readily detected using XPS. The elemental survey spectrum shows a N peak. It is, however, essential that a high-resolution scan of the N 1s signal also be acquired and properly charge compensated to establish the peak binding energy (BE). Some reports and conference presentations have shown an N 1s signal at 400.0 eV as 'proof' that amination had occurred. However, this BE value is characteristic of ***amide*** groups. The authors have failed to recognize the effect of post-plasma oxidation that converts amines into amides.[70] Amines have a BE of 399.2 (+/− 0.2) eV. Another error is the assignment of a signal at 400.0 (+/− 0.3 eV) to imines. XPS BE values correlate with the electron density on atoms, and thus one would expect imines to have a BE not higher than amines. In fact, aromatic C appears below aliphatic C in the C 1s signal, and in the absence of definitive data we would expect imines to be slightly below amines in the BE scale because of the slightly higher electron density that results from conjugation. Incorrect assignments of XPS signals have marred many studies, not cited here to protect the guilty.

An XPS N 1s signal with a binding energy of $\approx$399.2 eV is good evidence for amine groups on surfaces but it is advisable to complement this with derivatization to positively identify the presence of amine groups.

A higher NH_2 density is promoted by short treatment times (in seconds), high discharge power, and a high flow rate of H_2 monomer.[66] A short treatment time has also been suggested by Meyer–Plath and others to create a high NH_2 density.[65] They attributed this behavior to the limited supply of NH_3, which decomposes to form a hydrogen rich plasma. During this short treatment duration, this plasma preferentially inserts NH_2 groups on the substrate.[65] On

the other hand, long treatment times of more than 5 min, a low discharge power, and a low flow rate of H_2 favors high N contents. Therefore, a two-step process that consists of a pure NH_3 plasma followed by a pure H_2 plasma will convert the abundance of nitrogen in the first step into NH_2 in the second step. It has been postulated that the H radicals, from the homogeneous dissociation of H_2 molecules, reduce the various oxidation states of nitrogen to NH_2.[66] Besides the reduction of nitrogen groups, Favia et al. have proposed that the competing steps of positive ion bombardment reduce the density of grafted NH_2 by hydrogen abstraction and/or total removal of NH_2 from the substrate.[66] Therefore, remote plasma polymerization enhances the preferential deposition of NH_2 groups as a result of the absence of ion bombardment. Similarly, a high density of NH_2 created at short treatment time is also caused by the absence of ion bombardment.[66]

Meyer–Plath et al. have studied the influence of duty cycle and effective treatment duration (product of duty cycle and total treatment duration) in terms of amine selectivity (NH_2/N in %), amine efficiency (NH_2/C in %), and in-situ N efficiency (N/C in %).[65] They have also conducted continuous wave (CW) plasma treatment of similar vapors as comparison. An increase of duty cycle reduces the gas renewal rate of NH_3, which decomposes to N_2 and H_2 during plasma treatment. Therefore, NH_3 treatment becomes more similar to N_2–H_2 plasma treatment with increased duty cycle. The decomposition of NH_3 to N_2 and H_2 also explains why N_2–H_2 plasmas do not vary with increases of duty cycle.

Although it was possible to immobilize NH_2 on PS with N_2 plasma treatment, its efficiency and selectivity is very low.[65] The π system in PS leads to greater formation of radicals that can react with plasma-induced species.[69] Oxgyen plasma treatment of PS and polyethylene show differences in the rate and distribution of chemical groups in the plasma-treated surfaces.[69]

An alternative efficient way to create amine surfaces is the plasma polymerization of amine-based monomers. Examples of these monomers are allylamine,[22,58,71–74] diaminocyclohexane (DACH),[75,76] 1,3-diaminopropane,[77] heptylamine,[63,73] ethylenediamine,[78] butylamine,[58] propargylamine,[72] and propylamine.[72] The plasma polymerization of acetonitrile[79–81] and acrylonitrile[80,82] has also been reported but it is not well established as to what extent these plasmas create amine groups on their plasma polymers. Nitrogen monomers with higher oxidation states such as dimethylformamide and dimethylacetamide have also been successfully plasma polymerized,[23] but as expected the resultant plasma polymer contains more amide groups than amine groups, as evidenced by the XPS N 1s signal. Although most of the plasma polymerizations were conducted on two-dimensional polymeric materials, allylamine plasma polymer has also been successfully deposited on three-dimensional poly(D,L-lactic acid) scaffolds[83] and titanium alloy.[44]

The choice of monomer as well as plasma conditions can affect the densities of amine surface groups on plasma polymers. For heptylamine, for example, a higher discharge power leads to a reduction of amine groups and a more hydrocarbon-like plasma polymer (unpublished data). A recent report shows dehydrogenation and an increased percentage of unsaturated C–N bonds with increased discharge power levels for alkylamine plasma polymer.[84] The density of amine groups is observed to be DACH > allylamine > heptylamine.[31,63] The presence of two amine groups per monomer of DACH encourages the formation of a high density of amines on the plasma polymer.[31] In the case of allylamine, its double bond encourages higher deposition rates by a combination of plasma polymerization and conventional radical addition polymerization. The reaction between double bonds and radicals is well known in the literature on polymer growth mechanisms. Amide-containing monomers produce lower densities of NH_2 on the plasma polymer because of its inefficient reaction pathway, which involves elimination of CO and subsequent attachment of H.[23] An interesting approach is the copolymerization of allylamine with octa-1,7-diene,[43] which has been carried out in order to control the surface densities of amine groups.

The density of amines created by plasma polymerization of allylamine or DACH were reported to be higher than for NH_3 or N_2/H_2 plasma-treated substrates, although the highest density has been produced by the grafting of DACH onto N_2 plasma-pretreated poly(vinylidenefluoride) (PVDF) membrane.[31] However, quantification was by surface fluorescence spectroscopy and they do not find any correlation between the percentage of N measured by XPS and the content of primary amino groups detected with a fluorescent probe. The results may have been affected by non-specific adsorption of the dye onto the surface, which would result in over-estimation of NH_2 groups. The problem of non-specific adsorption of probe dyes and derivatization agents has been pointed out[36] but is often not addressed. Inefficiency of amine grafting on O_2 plasma-treated surfaces had also been demonstrated by a comparatively lower density of amines on such surfaces than on an allylamine plasma polymer.[83] Another possibility is that the depth distribution is not uniform across the top 10 nm probed by XPS, in which case XPS N signals and surface derivatization might deviate.

Other effects of molecular structure on retention of NH_2 groups and deposition rates are the degree of saturation of the monomer and the presence of low energy bonds such as Si–C and C=C bonds. Unsaturation of the monomer also affects the types of functional groups on the plasma polymer.[72] Deposition rates increase with the degree of unsaturation and amine-based monomers are more 'robust' than alcohol-based monomers in their increase of deposition rates with increase of discharge power before exces-

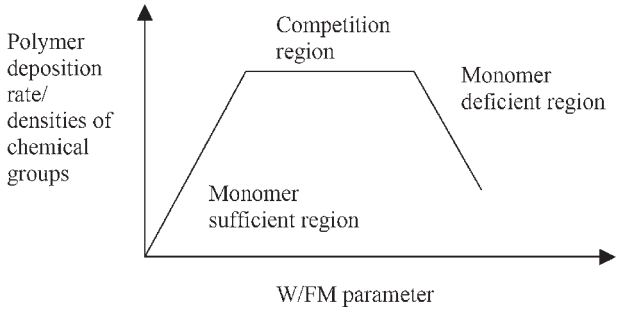


Figure 3. Regions of plasma polymer deposition, adapted from ref. [2]

sive amine loss occurs. The influence of unsaturation of monomers on the retention of amine groups is inconclusive because of complications in derivatization XPS. A plasma polymer from γ-aminopropylethoxydimethylsilane was found to retain significant NH_2 structure.[85] Methyl(trimethylsilydimethylamine) and vinyl(bis(dimethylamino)methylvinylsilane) show less fragmentation and higher deposition rates than monomers without these side groups, e.g., bis(dimethylamino)methylsilane.[86] The authors concluded that Si–H structures were unfavorable while vinyl and methyl groups promote plasma polymer formation.

The influence of process parameters on the chemical composition of plasma polymers can be interpreted in terms of 'discharge power deficient/monomer sufficient' and 'monomer deficient/power sufficient' regions (Figure 3).[1,2] An increase in discharge power will result in an increase of fragmentation of monomers, which is manifested as an increase of pressure. In the 'monomer deficient' region, the discharge power is more than sufficient to fragment the monomers, and further power increases will not result in increased deposition. Most work that aims to produce chemically reactive surfaces for further interfacial immobilization aims to retain a substantial density of the reactive groups, such as amines, which requires less extensive fragmentation of the monomer. Therefore, most studies use plasma conditions that fall into the 'power deficient' region, with excess monomer present in the reaction chamber. Experimentally, low discharge power favors the formation of NH_2 groups because there is less fragmentation of monomers in the plasma.[22,31,75] A way of normalizing the parameters of discharge power and amount of monomer is the *W/FM* ratio (W = discharge power, F = flow rate, and M = molecular weight of the monomer). *W/FM* represents the apparent power input for monomers of different molecular weight. Others have modified this composite parameter to include residence time in the plasma, which is a function of flow rate, pressure, and volume of the plasma reactor.[75] There is a range of *W/FM* values for which fragmentation of monomers is in the 'competition range'. In this range, there is no increase of deposition rates or plasma treatment efficiency with increasing discharge power or decreasing flow rate. In pulsed plasma polymerization of allylamine, longer off times promote higher densities of amines,[87,88] but the same approach was ineffective for acetonitrile plasma polymer, which seems to be insensitive to the duty cycle of pulsed plasma polymerization, although analysis was only by FT-IR spectroscopy.[80]

As to plasma grafting, the direct-irradiation technique involves adsorbing decylamine hydrochloride onto a polyethylene (PE) surface and treating with Ar plasma,[89] which 'stitches' the surfactant to the surface by newly formed interfacial covalent bonds. Derivatization has estimated the density of NH_2 groups to be 1×10^{-6} mol $\cdot$ m^{-2} for the optimum Ar plasma treatment time of 2 s. The other variation of plasma grafting involves treating a high-density PE surface with Ar plasma and grafting with acrylamide.[30,90] The amide is then derivatized by a Hoffman degradation procedure that yields densities of NH_2 as high as 1×10^{-7} mol $\cdot$ cm^{-2},[30] which depended on initial amide density, reaction time, and concentration of NaOH. Although interactions between the different process parameters have not been reported, a plateau of the maximum density is observed at a high concentration of NaOH (8 N). Ar pretreatment induces radical formation on the substrate surface, and on exposure to the atmosphere, radicals will react with oxygen and graft acrylamide directly or via peroxides.

Carboxylated Surfaces

Plasma-fabricated carboxylated surfaces have been found to be good supports for some anchorage-dependent cell lines.[91–93] The copolymerization of two monomers is shown to be a convenient and versatile method for adjusting the surface density of carboxy groups.[43,93–95] More widely used, however, are plasma-produced carboxy surface groups for their ability to provide a convenient platform for the interfacial immobilization of bioactive molecules that contain amine groups, such as proteins, by carbodiimide chemistry. Examples of bioactive molecules that have been successfully bound onto surface COOH groups are the oligopeptide Arg-Gly-Asp-Ser (RGDS),[96] collagen,[97–99] anticoagulants such as human thrombodulin,[19,100,101] heparin-albumin conjugate,[102–104] and the enzyme urokinase.[105] Some studies use spacers such as bis-amino-terminated PEG or 1,2-diaminoethane on carboxylated surfaces[46] to immobilize bioactive molecules such as heparin and sulfated hyaluronic acid.

As for amines, four main strategies have been used to create COOH groups on various substrates (Figure 4). One approach is plasma treatment with CO_2[106–108] or CO.[106] Depending on the substrate, different mechanisms have been proposed but in general, CO_2 plasma treatment not only produces COOH groups but other C and O groups such as hydroxys, aldehydes, ketones, and esters (as well as, we

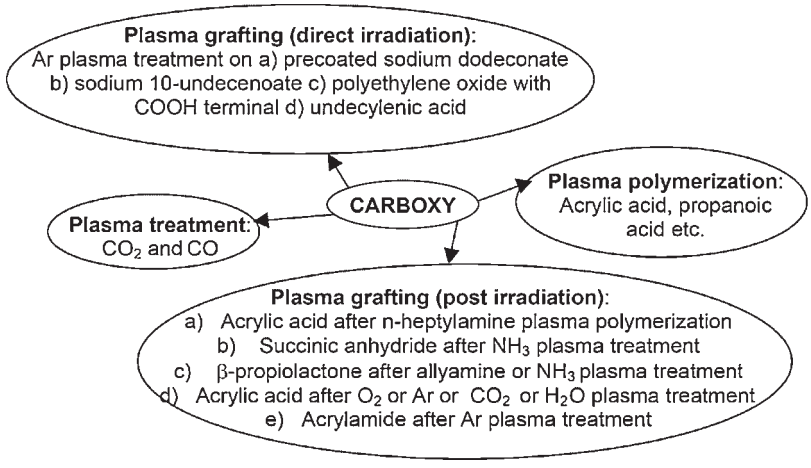


Figure 4. Plasma-based methods for producing carboxy groups on substrates.

assume, hydroperoxides from post-plasma reactions). In the CO_2 plasma treatment of polyimide, imide groups are cleaved to form COOH and amide groups.[106] In comparison to polyimide, the CO_2 plasma treatment of polysulfone is more complex; with diverse chain scissions and crosslinking.[109] CO_2 plasma treatment of isotactic poly(propylene) (PP) results in selective degradation of the syndiotactic phase on the surface of PP.[110] This preferential etching is shown by the re-emergence of infrared signals associated with the isotactic phase at 1 256 and 2 723 cm^{-1} with time. Further analysis has also detected oxidized PP oligomers on the surface. Degradation of aromatic rings occurs during CO_2 plasma treatment of PS.[27] While a variety of chemical functionalities appears to be produced in such plasma treatments, carboxy groups are evident after CO_2 plasma treatment as the surface is found to be acidic.[27,109,111]

As shown in Table 1, CO_2 plasma treatment causes an increase in the O/C ratios in most polymers though in the case of poly(methyl methacrylate) (PMMA), there is a reduction of oxygen because of the degradation of ester groups.[112] A rapid post-plasma increase in O/C has been attributed to ambient oxidation. An increase in discharge power results in higher O/C ratios for CO_2 plasma-treated LDPE because of increased fragmentation and concentration of reactive species in the plasma gas phase.[107] However, insertion of COOH groups only accounts for 4.8 to 7.6% of the C 1s peak for a range of gas plasmas including CO, O_2, and CO_2.[106] Terlingen and others have reported that 14% of the oxygen in CO_2 plasma-treated LDPE is assignable to COOH groups.[111] This again shows that many other reaction pathways occur. They propose zero-order incorporation kinetics and first-order etching for their plasma-treatment process. Although CO_2 plasma treatment as such has low efficiency, a plasma of 50 mol-% CO_2 and 50 mol-% acrylic acid produces almost twice as many COOH groups than that without CO_2.[113]

Carboxylated surfaces have been produced by plasma polymerization using monomers such as acrylic acid. However, the carboxy functionality is easily cleaved off, presumably as CO_2, and low power needs to be used, which reduces the deposition rate. The addition of CO_2 during plasma polymerization assists the fabrication of carboxy surfaces.[113] In more recent work, co-polymerization of acrylic acid with other monomers such as octa-1,7-diene[43,92,93,95] or hexane[94] has been successful. In addition to controlling the surface density of COOH on the plasma polymer, the addition of octa-1,7-diene also prevents the dissolution of the plasma polymers by water.[93] The ability to withstand dissolution is important to prevent false negative results from the underlying substrate during cell adhesion or protein adsorption studies.

Table 1. O/C ratios of different substrates subjected to O_2, CO_2, and CO plasmas.

No.	Monomer/gas	Substrate	Initial O/C	Final O/C	Ref.
1	O_2	Polyimide	0.201	0.419	[106]
2	CO	Polyimide	0.201	0.382	[106]
3	CO_2	Polyimide	0.201	0.469	[106]
4	CO_2	PET	0.37	0.43	[108]
5	CO_2	LDPE	Not available	0.389–0.695	[107]
6	CO_2	PS	0.03	0.298–0.364	[27]

Nevertheless, plasma polymerization of acrylic acid as such has also been reported.[46,71,74,114–116] Other carboxy monomers such as propanoic acid[114] and propargyl acid[117] have also been used as monomers for plasma polymerization. Carboxy groups can also be produced by plasma-induced reactions during the plasma polymerization of esters, for example methyl methacrylate,[118] though the density is lower.

Whereas CO_2 plasma treatment produces only a relatively low surface density of carboxy groups, plasma polymerization can create densities as high as 15 to 20.5% of the C 1s peak.[114,116] The influence of discharge power and/or unsaturation of monomer on the COOH density has been investigated by several researchers.[114,117] Although Yoshimura et al. have shown that plasma polymers made from propargyl acid have higher deposition rates than those from acrylic acid, the results are not comparable because of different *W/FM* ratios.[117] Others have reported that the deposition rate of acrylic acid plasma polymers was five times higher than that using propanoic acid.[114] The higher rates of acrylic acid plasma deposition can be traced to its polymerization pathway. At low discharge power, plasma polymerization of acrylic acid involves a significant extent of radical addition of C=C bonds,[119] which resembles cationic chain growth.[114] C=C bond opening requires 2.74 eV compared to cleavage of a C–C bond, which requires 3.61 eV.[119] Thus, part of the plasma polymerization of acrylic acid occurs via a classical polymerization mechanism and the polymer is expected to have a substantially more linear chain structure than typical plasma polymers from saturated monomers. Spectroscopic data of plasma polymers from propanoic acid show the usual diversity of structures, as expected from the absence of a clear reaction pathway for its plasma polymerization. There is a higher degree of branching in the propanoic acid plasma polymer compared with that formed by acrylic acids.

The influence of discharge power on the structure of propanoic acid plasma polymers differ more from that of conventional poly(acrylic acid) with increasing power. In the case of acrylic acid, free radical reactions dominate the polymerization process at high discharge power.[114] Retention of COOH is only 9.7% of the C 1s peak because of fragmentation to CO and CO_2.[114] Using optical emission spectroscopy CO has been identified as a marker to optimize the density of COOH on the plasma polymer.[115] On the other hand, a cationic growth mechanism at low discharge power allows retention of carboxy groups as high as 20.5% of the C 1s peak.[114] This high retention of COOH at low discharge power has also been reported in other studies.[115,119,120] Higher retention of the original molecular structure of acrylic acid is promoted in the plasma polymer when the substrate temperature is reduced with liquid nitrogen.[121] At low discharge powers, most of the O–C=O functionalities are carboxylic acid groups rather than ester groups as indicated by tetrafluoroethylene (TFE) labeling and XPS analysis, whereas a mixture of acids and esters result at high discharge powers.[120] Increased fragmentation in the high power regime results in greater crosslinking. No linear structures longer than two acrylic acid units are observed by static secondary ion mass spectroscopy (SSIMS) analysis, whereas up to five acrylic acid units result at low discharge power.[120] The presence of the C=C double bond in acrylic acid leads to radical grafting and its deposition rate is larger than that with propanoic acid.[122]

In order to increase the specificity and density of carboxy groups, grafting techniques have been investigated in various studies. As shown in Figure 4, the grafting techniques include direct irradiation and post-plasma grafting by chemical reactions. Early attempts at direct irradiation used acrylic acid to immobilize COOH groups on substrates. These researchers used different organic solvents to swell the polymer substrate before exposing them to acryclic acid and treating with different plasmas such as N_2, Ar, ammonia, and acetylene.[123,124] Since surface analytical methods such as XPS were not widespread during that period, samples were analyzed by methods such as gravimetry or staining to show the success and/or extent of polymerization. These indirect methods have their inherent limitations. Later work by Terlingen et al. has shown that acrylic acid undergoes extensive decarboxylation during the first seconds of Ar plasma treatment.[125,126] The challenge in direct irradiation is to find surfactants that can withstand longer immobilization times by Ar plasma treatment. Some surfactants have met the criteria, the sodium salt of undecylenic acid,[102] sodium 10-undecenoate[127–130] with co-immobilization with 10-undecene sulfate,[131] sodium dodecanoate,[127,128] and alkyl poly(ethylene oxide) (PEO) with terminal COOH groups.[127,130,132] Optimum treatment times are relatively short, at 5–10 s, yet the percentage loss of functional groups is still 72 to 94%.[127] The main advantage of this technique is the high selectivity for COOH: 47% of the detected oxygen-containing groups are COOH groups.[127,130]

Several factors have been identified as crucial for successful plasma immobilization of surfactants on substrates. Factors include the wettability of substrates by surfactants,[129,132] degree of saturation of substrates,[128] degree of saturation of surfactants,[127,128] chain length of surfactants,[132] and presence of electromagnetic radiation.[130] The influence of chain length on immobilization efficiency has been studied with alkyl-PEOs, though the presence of COOH is not expected to change the conclusions. Failure of a surfactant to wet the substrate prevents immobilization. Pretreating the substrate with Ar plasma[132] or mixing the surfactants with organic solvents such as hexanol[129] or chloroform[132] could rectify wetting problems. Both methods provide comparable efficiency of immobilization but the former has a higher reproducibility. The efficiency of immobilization on polymeric substrates is related to the ease of formation of radicals

on the polymers.[128] Since PE has a greater amorphous region than PP, radicals are more likely to form on PE and hence immobilization takes place more efficiently on PE. However, radicals in close proximity to one another can recombine instead of immobilizing the surfactant. This self-extinguishing behavior is found on a poly(*cis*-butadiene) substrate. The presence of unsaturated bonds in the surfactant results in a higher efficiency of immobilization.[127,128] They suggest two mechanisms to explain this increase of efficiency. Unsaturated surfactants form allyl radicals more readily than saturated surfactants. The other mechanism involves radicals on the polymeric substrate that reacts with the double bond of the surfactant.

Many approaches to chemical grafting onto plasma-fabricated surfaces have been reported. One approach is to use a gas plasma treatment to create peroxides on polymers, which can then be used for radical grafting with COOH-terminated compounds, or to attach chemical groups such as amines onto which carboxylated compounds can be immobilized. Examples comprise treating the surface with amine plasmas NH_3,[107] NH_3-Ar,[45] heptylamine,[133] or allyamine,[44,107] followed by exposure to chemistries that contain COOH such as β-propiolactone,[107] succinic anhydride,[44,45] or acrylic acid.[133] Different reasons have been advanced for the use of two-step processes instead of a single step plasma polymerization but the most frequent is to create spacers between bioactive molecules and the solid surface.

Another variation of post plasma grafting involves treating polymeric substrates with different gases or water plasma treatment and then immobilizing acrylic acid. The choice of gas treatment is a balance between creating peroxide or hydroperoxide and avoiding excessive surface ablation of the substrate. It has been reported that it is difficult to modify poly(tetrafluoroethylene) (PTFE) with oxygen plasma because the substrate does not ''adsorb the 130.5 nm emission from oxygen plasma''.[134] Instead, water[105] or CO_2[19,101] plasma treatment has proven to be effective to bind acrylic acid on PTFE substrates. Other substrates such as PE and poly(ethylene terephthalate) (PET) have been successfully treated with air plasma[97,98] and Ar plasma[135] before grafting with acrylic acids. Likewise, acrylamide has been grafted onto polymeric substrates after gas plasma pretreatments with Ar, O_2, dry air, and hydrogen.[30,90] When acrylamides are subjected to alkaline hydrolysis, they are modified to carboxylic acid groups.[30]

Factors that affect the grafting density of acrylic acid comprise factors that influence the creation of peroxide or amine and factors that affect the grafting reaction. The generation of peroxide is affected by plasma treatment, time, and temperature before grafting. While one might assume higher power and longer times will result in a higher grafting density, exceptions exist.[90,135] The grafting yield is affected by reaction temperature, concentration of acrylic acid, reaction time, and presence of salts/activators.[19,135]

Hydroxy Groups

In contrast to the abundance of literature on plasma-generated amine and COOH surfaces, a limited number of reports is available on hydroxy groups. One reason is that alcohol monomers appear to fragment substantially with loss of –OH.

Hydroxy surfaces have been used for cell colonization.[51,136] Monomers such as ethanol and methanol can be plasma polymerized readily,[23] though the plasma power level must be kept low in order to avoid excessive loss of hydroxy groups, and the resultant coatings are attractive for their relatively hydrophilic nature.

As platforms for covalent immobilization of biologically active molecules by chemical reactions in aqueous solution, hydroxy surfaces are less attractive than amines and carboxyls on account of their lesser nucleophilic character and hence lesser chemical reactivity. Reactions designed to target surface hydroxy groups can suffer from interference by water molecules. Hydroxyl groups have been reported to bind a range of peptides including YIGSR (a lamin-derived peptide, Tyr-Lle-Gly-Ser-Arg) without any crosslinking or coupling agent,[137] although it appears more likely that binding is a result of physisorption rather than a covalent interfacial linkage. With further modification, OH can bind with other bioactive molecules such as glucose isomerase[138] and modified heparin.[139] It can also serve as a platform for silylation steps in biomaterials studies.[139,140] Others have used OH groups as a primer for immobilizing PEO.[61]

The two main routes for producing hydroxy groups on substrates are plasma treatment and plasma polymerization. In the case of plasma treatment, the strategies of Ar plasma followed by exposure to atmospheric oxygen[42] or oxygen plasma,[2,141,142] have been used with varying degrees of success. In the first scheme, exposure to atmospheric oxygen induces the creation of OH groups while the plasma chemistry itself incorporates OH in the second scheme. However, both techniques suffer from hydrogen abstraction, which results in crosslinking and/or etching rather than dominant insertion of OH groups on the polymer (ref. [42,143] and references therein). It has been shown that the density of OH groups per 100 carbon units is only 3.2 for O_2 plasma[2] compared to 31 OH per 100 carbons using the plasma polymerization of allyl alcohol.[144]. In certain circumstances, a low concentration of OH is desirable because the reactivity of OH increases in the proximity of C–F bonds from the FEP substrates.[137] At a high concentration of OH, its reactivity decreases because there are fewer C–F bonds for each OH group. This reduced reactivity is reflected in the lower efficiency of binding with peptide.

In the case of plasma polymerization, monomers used to create hydroxylated plasma polymers are: methanol[23,73,136] and methanol with hydrogen,[137] ethanol,[23] isopropyl alcohol (IPA),[145,146] allyl alcohol,[74,117,136,144,147–150] methylbutylnol,[117] propan-1-ol,[147] propargyl alcohol,[117,147] furfuryl alcohol,[117] and isobutyl alcohol.[23] Several factors have been identified that influence the OH density and deposition rates. These factors comprise the molecular structure of the monomer (unsaturation), presence of co-monomers (e.g., octadiene or carrier gas), ratio of discharge power to flow rate, duration of plasma polymerization, and duty cycle in the case of pulsed polymerization.

Unsaturation in the monomer affects the deposition rate, the percentage of OH, and degree of crosslinking of the plasma polymer. The presence of triple bonds in propargyl alcohol encourages its fragmentation upon entering the discharge chamber and thus increases the deposition rates.[147] While some C≡C bonds remain in the plasma polymer, other C≡C bonds are converted into C=C or C–C at a higher discharge power. On the other hand, monomers that contain olefinic double bonds need longer times to be activated and as a result, deposition rates are reduced. Saturated monomers required the longest time. Similar trends of deposition rates have also been observed when plasma polymerization is carried out with hydrocarbons of different degrees of saturation: acetylene, ethylene, and ethane.[151]

Despite the high O/C ratio of propargyl alcohol plasma polymer, its percentage of OH is relatively low, between 32 and 41% of the total O 1s peak, which indicates substantial molecular fragmentation and rearrangements of structural units. The percentage of OH for allyl alcohol plasma polymers is between 53 and 72%.[147] In the case of propanol plasma polymer, ether contributes 50% while OH makes up another 24% of the O 1s peak. The FWHM of the XPS C 1s C–C/C–H contribution of propargyl alcohol and propanol plasma polymers broaden, which suggests the presence of diverse structures and crosslinking.

When the 'discharge power (W) to flow rate (F)' ratio is increased, there is more cleaving of OH groups, which generates a highly crosslinked hydrocarbon-like surface and manifests itself in low O/C ratios.[117,149,150] In such high W/F conditions, SSIMS spectra of allyl alcohol plasma polymer show an increase of ions of low mass fragments at an m/z of 18 ($H_2O^{\bullet+}$), 26 ($C_2H_2^{\bullet+}$), 28 ($CO^{\bullet+}$ or $C_2H_4^{\bullet+}$), and 42 ($C_3H_6^{\bullet+}$), which suggests that the elimination of water or CO reduces the O/C ratios.[152] A reduction in discharge power reduces the concentration of C=C and C=O bonds in the allyl alcohol plasma polymer. At low discharge power, C=C bonds open and polymerize to retain the OH groups.[149,150] The C=O bonds are created presumably by subsequent oxidation of radicals generated during the plasma polymerization. A low W/F ratio also inhibits the formation of COOH in IPA plasma polymer[145] because of a reduced presence of H_2O and its related ions such as OH, which could oxidize the C=O groups to COOH.[146] The concentration of OH groups in the IPA plasma polymer could be increased by prolonging the plasma exposure time until it saturated at 17% of the C 1s photoelectron peak.[145]

In the pulsed plasma polymerization of allyl alcohol, analogous to pulsing of plasmas such as NH_2[88] and ethylene glycol,[153] an increased off-time increases the percentage of hydroxy groups because more growth occurs via the C=C double bonds through free radical addition.[148] It has also been suggested that the rapid disappearance of substrate bias during the off period minimizes bond breaking and ablation by high energy ions.

To control the density of OH groups, co-deposition of allyl alcohol with octa-1,7-diene,[154] ethylene,[144] and buta-1,3-diene[144] has been successfully carried out. As shown in Figure 5 and 6, the variation of OH density with percentage of allyl alcohol varies between different co-monomers. At >50 mol-% allyl alcohol the co-monomer ethylene does not interfere with homopolymerization of allyl alcohol.[144] For butadiene at <60 mol-%, homopolymerization of butadiene dominates in the range 100 to 300 W, though such processes are more prevalent at a power input <100 W.[144]

As shown in Figure 6, a similar non-linear increase of OH groups is observed in the co-polymerization of octa-1,7-diene and allyl alcohol. The difference in the number of carbon atoms between allyl alcohol and octa-1,7-diene contributes to the non-linear increase in OH groups.[154] Time-of-flight secondary ion mass spectrometry (ToF-SIMS) analysis shows that the co-polymer of allyl-alcohol and octa-1,7,-diene is enriched with unsaturated moieties, and oligomerization reactions in the plasma phase results in dimeric allyl alcohol species present on the surfaces.[154]

In an alternative approach, Ar has been added to stabilize allyl alcohol during plasma polymerization.[138] The

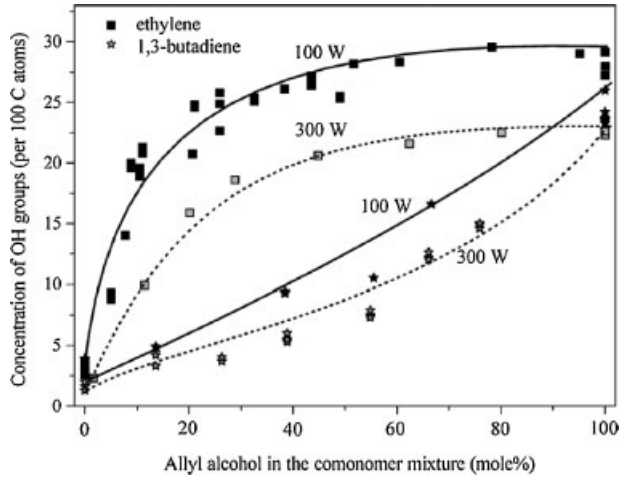


Figure 5. Densities of OH groups on ethylene/allyl alcohol and butadiene/allyl alcohol copolymers measured by XPS and derivatization with TFAA. Reproduced with permission from ref. [144]

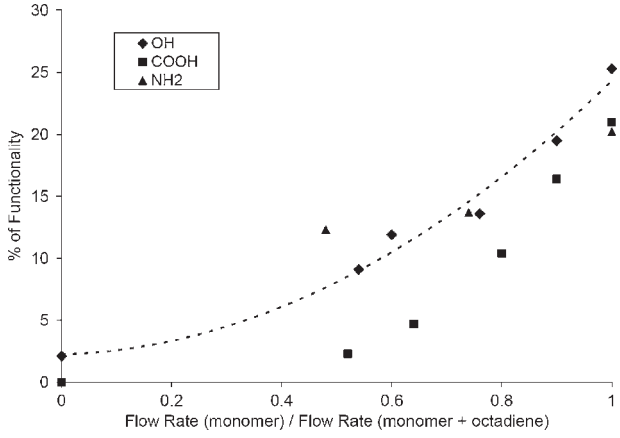


Figure 6. Densities of OH groups on octa-1,7-diene/allyl-alcohol plasma polymer derivatized with TFAA and measured by XPS. The line is drawn for illustration only, to connect the percentage of OH groups with respect to different ratios of flow rates. Data from ref. [43] and [154].

presence of Ar plasma is expected to increase the density of OH groups on account of an increase in the generation of radicals on the substrate surfaces, but experimental results show otherwise.[138] These unexpected results suggest dominance of ablation over deposition. Since the experiments utilize pulsed polymerization with a 25% duty cycle, an alternative interpretation is that the quenching of radicals amongst the polymer chains is dominant over binding with OH groups during the off time. Moreover, Ar plasma may cause increased C–O bond cleavage.

Another approach has reduced the carbonyl groups of *N*-vinyl-2-pyrrolidone plasma polymer to hydroxy groups.[18,155] Although the reduction does not proceed to completion, the percentage increase of OH groups is as high as 121%, though quantitatively the increment is only 2.3 $mmol \cdot g^{-1}$. Others have analogously used sodium borohydride to reduce carbonyl groups present on allyl alcohol plasma polymer to increase the content of OH groups.[139,140,155] Derivatization shows the density of OH to increase from 10.5 to 33.3% of the oxygen present in the plasma polymer.[140] Diborane/tetrahydrofuran (THF) has been used to reduce various oxygen functionalities of O_2 plasma-treated polymers to OH, and in the presence of H_2O_2, diborane can also convert C=C bonds into OH groups.[156] $LiAlH_4$ has also been used to reduce C–O–C groups present on an O_2 plasma-treated substrate to C–OH.[156]

Wickson and Brash have attempted to graft allyl alcohol onto Ar pretreated PE.[140] After derivatizing with trifluoroacetic acid (TFAA) to identify OH groups, the grafted surfaces show a 4.1 at.-% F compared to a 1.1 at.-% F for untreated PE.

Aldehyde Groups

In contrast to the popularity of amine and carboxy surfaces, very little has been reported on plasma-fabricated aldehyde surfaces, though reductive amination is an attractive interfacial immobilization chemistry. Several bioactive molecules or spacer groups have been successfully grafted onto aldehyde groups on plasma polymers: collagen,[157] cell-adhesive oligopeptides,[158] NH_2-terminated PEG,[159] albumin,[160] and composite PEG-albumin.[161] Polyamines followed by carboxylmethyl-dextran have also been successfully immobilized onto acetaldehyde plasma polymer layers.[47] Two different schemes have utilized acetaldehyde plasma polymer as a primer for binding hydrogel layers to bind Neutravidin.[162]

Aldehyde surfaces cannot be produced by plasma treatments as plasma polymerization is necessary. However, volatile aldehydes easily lose C=O. Various monomers have been investigated in plasma polymerization with varying degrees of success.[162–164] An acetaldehyde plasma polymer has been shown to contain aldehyde groups for the dense immobilization of molecules,[159–162] but low power had to be used, which reduces deposition rates. In contrast, a benzaldehyde plasma polymer did not show an infrared band characteristic of the C=O stretch band.[164] Instead, a strong ketonic band is detected. Leich et al. demonstrated that a higher peak power and a relatively large duty cycle favor plasma polymers with high densities of aldehyde moieties.[165] Aldehyde moieties are also promoted at low reactor pressure with the appropriate pulse cycle. They have postulated that the higher power density provides the energy for the cleavage of the aromatic ring to produce diradical species that propagate extensively during plasma polymerization. This extensive propagation retains the aldehyde moieties.

The influence of excitation frequency on aldehyde plasma polymerization has been investigated by Gong and Griesser on a range of aldehyde monomers.[163] A higher frequency results in a lower C=O content in capronaldehyde and ethylbutyraldehyde plasma polymers. Such dependence does not occur for nonylaldehyde plasma polymers. The extent of fragmentation is not considered the main reason for the differences in the structures of aldehyde plasma polymers because of similar pressure increases for the different monomers during plasma polymerization. Instead, the changes have been attributed to the chemistry of the plasma phases with variation of excitation frequencies.

Aging of Plasma-Treated Surfaces and Plasma Polymers

Aging studies of plasma polymers are important, as marked changes to the surface chemistry of the polymers may happen over the storage period, but such effects have often been overlooked or not recognized. In the absence of characterization of aging, erroneous conclusions can be drawn on relationships between surface chemical compositions and biological responses, for example, if cell attach-

ment and XPS analyses are performed after different times of aging. Likewise, to achieve understanding of the effects of surface chemistries on protein adsorption, the surface chemistry must be known at the time of the protein adsorption experiment. Finally, but most importantly, in light of the great interest in reactive plasma-prepared surfaces for the covalent immobilization of biomolecules, it is essential to ascertain that the intended reactive group is present on the surface, because many molecules will physisorb to plasma surfaces and such physisorption can be difficult to distinguish from covalent immobilization.[36] However, it is important to ensure that physisorption has not masked unsuccessful covalent linkage, because adsorbed molecules can be displaced in biological media by exchange processes, and thus the intended control over biointerfacial responses would be lost.

Plasma-treated polymer surfaces and plasma polymers have often been observed to undergo substantial changes in their surface chemical compositions and properties, such as contact angles (CAs), with time as they are stored. This ''aging'' is usually interpreted in terms of two fundamental processes: post-plasma oxidation, initiated by reaction between remaining radicals and in-diffusing atmospheric oxygen, and surface adaptation, which is a consequence of reptation motions that move some of the polymer chains from the surface into the bulk.

It is well known that plasma polymers from monomers that do not contain oxygen usually show signals in XPS and IR spectra from oxygen-containing groups.[23] While some early studies have speculated about residual oxygen or water vapor in the plasma reactor, extended studies of plasma polymers after various periods of storage have shown that the incorporation of oxygen occurs after the plasma polymers are removed from the plasma reactor.[70,73,77,78,118,166] The oxygen uptake has been interpreted in terms of addition of in-diffusing atmospheric oxygen with remaining trapped radicals in the plasma polymer.[166] Hydrolysis of chemical species created by plasma treatment such as imines is also possible.[84,167]

In addition, the surfaces of plasma-treated polymers and plasma polymers can change their interfacial chemistry and properties by surface adaptation, also known as surface restructuring or surface reorientation, in response to interfacial energy differences between the plasma polymer and its environment. This often leads to a substantial decrease in the surface density of functional groups. The extent of loss can be considerable for plasma-treated surfaces[48–50,168,169] but usually is small for plasma polymers.[73] Their relatively high crosslink density is thought to restrict their ability to engage in surface adaptation. In the vicinity of crosslinks, polar groups do not have sufficient mobility to respond to interfacial forces.[168] As shown by quantitative analysis of surface restructuring, there are immobile and mobile polar groups on plasma-treated surfaces, with a characteristics time constant (lifetime) for the reorientation process.[49,50] Others have called them reversible and irreversible components of surface modification.[170]

Surface adaptation is driven not only by interfacial enthalpy, but also by entropy, which strives to dilute the higher concentration of chemical groups on the surface in towards the bulk of the polymer to increase the translational entropy of the system.[50] This entropic force is also caused by the absence of geometrical constraints on the motion of polymer chains at the surface compared to the bulk polymer. As such, the surface has excess Helmholtz free energy.[2] However, mobile treated polymer chains may not bury deep into the bulk polymer because of the short effective range of entropy and interfacial enthalpy forces and the unfavorable energetic conditions in the depth. To address the unfavorable energy situation of polymer chains bearing polar groups inside a non-polar polymer, Griesser et al have hypothesized that these polar groups stabilize themselves by forming hydrogen-bonded dimers or micromicellar clusters in subsurface regions.[168] This surface adaptation appears to be irreversible for NH_3 plasma-treated polymers because subsequent soaking in water fails to bring the polar groups back to the surfaces.[48] Analogous behavior has been found on COOH-coated substrates created by CO_2 plasma treatment.[111]

Figure 7 summarizes factors involved in the aging behavior of plasma-treated surfaces and plasma polymer surfaces. Internal factors influence the mobility of the polymer chains while external factors refer to the influence of the environment on the aging behavior. The internal and external factors are strongly dependent on the polymer substrate and the plasma modification.[48,50]

It has at times been surmised that glassy polymers should not be able to undergo surface adaptation. However, plasmas often cause considerable extents of chain scissions in the surface layers of polymers such as polycarbonate and acrylics. As a result, the glass transition temperature of the *surface layers* may be lowered sufficiently to allow mobility.

Some studies have aimed to prevent the occurrence of aging, but success usually has been limited. To overcome surface restructuring, pretreatment with Ar plasma has been used in several studies already mentioned above. It increases crosslinking at the polymer surface and thus

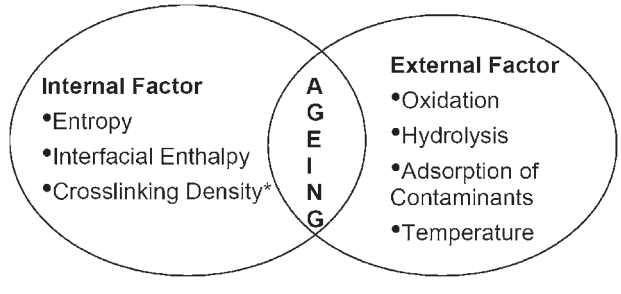


Figure 7. Factors that affect the aging behavior of plasma-treated polymers and plasma polymers.

reduces the extent of surface reorientation.[171] Friedrich et al. have used di-*tert*-butoxycarbonyl (BOC) to protect amine groups from oxidation.[144] The NH_2 groups can be retrieved when soaked in HCl and pyridine. However, they have not provided details on the efficiency, yield, and possible side reactions of the protection step. One report claims that a N_2–C_2H_4 plasma polymer produced by atmospheric pressure dielectric barrier discharge (DBD) did not register any significant changes in contact angles or XPS spectra after six months of air aging, though no detailed analysis of the XPS N 1s peak, which would reveal oxidation of amine groups, has been reported.[68] Most reports have tried to create stable amine surfaces. Similar work is absent for COOH and OH groups.

Without proper storage conditions, freshly plasma-treated surfaces can also attract atmospheric contamination, which increase its surface energy.[168] For air aging, storage in tissue culture PS dishes has proven to be effective in preventing the ingress of contaminants.[169] The importance of a clean surface prior to plasma treatment has been pointed out by Golub et al.[142]

In the following the aging behavior of the reactive surface chemistries reviewed above is discussed.

Aging Behavior of Plasma-Prepared Amine Surfaces

The aging behavior has been studied of surfaces produced by plasmas of ammonia,[48–50,169,172] ammonia with hydrogen,[66] allylamine,[71,73,116], heptylamine,[70,73], 1,3-diaminopropane,[73,77] ethylenediamine,[78] and diaminocyclohexane.[75] By measuring the concentration of pertinent elements as a function of depth (by angle dependent XPS) and time, one can assess the factors and their relative importance that contribute to aging. In the example of NH_3 plasma-treated FEP (a polymer of tetrafluoroethylene and hexafluoropropylene), O, N and F are depleted in the outermost region of the surface between 0.5 to 1 nm depth.[172] Analysis of the aging behavior of plasma polymers should adopt multiple techniques because each has a different depth of analysis. Among the most commonly used techniques are contact angle (CA) measurement and XPS. CAs are thought to be determined by the outermost nanometers of a surface, which is comparable to the interfacial forces involved in surface reorientation.[173] On the other hand, XPS has a probe depth of approximately 10 nm at normal emission and 2–3 nm at an emission angle of 75°, with an attenuation length of the photoelectrons of 3 nm,[169], a value typical of polymers.

Differences have been observed for different surfaces in aging studies, with similar trends in XPS results but different CA data. For example, angle-dependent XPS analysis of NH_3-treated FEP,[169] heptylamine,[73] and 1,3-diaminopropane[73] plasma polymers show higher O/C ratios at 75° than at 0° for the first few days and the situation is reversed after prolonged aging. This suggests the presence of two competing mechanisms: a continuing oxidation process, which dominates for the first few days and then slows down, and the rearrangement motions of polar groups, which become more detectable in the aging behavior after a few days. Post-plasma oxidation plays a significant role in creating a high percentage of oxygen.[169] The conclusion that most, perhaps all, oxygen is incorporated after the plasma process rather than by the presence of O_2 and H_2O during the plasma, is in agreement with a previous study in which nitrogen plasma treatment followed by in-situ XPS analysis (without breaking vacuum) produced no oxygen on the surface.[167] A more recent study with NH_3 and N_2/H_2 plasma-treated PS found that the in-situ O/C ratios are $2 \pm 1\%$ compared with $9 \pm 2\%$ for ex-situ XPS analysis.[65] A lower discharge power is thought to result in a lower post-plasma oxidation because of a lower density of radicals generated.[75]

However, CA measurements can reveal different trends. NH_3-treated FEP[169,171] and 1,3-diaminopropane plasma polymers show an increase of CAs while heptylamine plasma polymers show a decrease of CAs with aging.[73] Aging produces no detectable changes in the surface roughness.[50,73] Therefore, these differences have been attributed to differences in the relative importance of oxidation versus surface adaptation. If oxidation dominates, CAs continue to decrease. The higher mobility of the surface layers of NH_3-treated FEP and 1,3-diaminopropane plasma polymer results in a higher extent of surface restructuring compared with the more crosslinked heptylamine plasma polymer.

Plasma treatments can generate chain scissions in the substrate polymer, which then affects the surface restructuring dynamics. On the other hand, other plasma treatments, such as CASING,[5] achieve crosslinking of a treated surface. Generally, longer treatment times result, not unexpectedly, in more chain scissions and, therefore, higher mobility of the plasma-treated surface. For NH_3 plasma-treated FEP, for example, the fraction of mobile polar groups increases with increasing plasma treatment time up to 45s, which implies dominance of plasma-induced C–C chain cleavage over crosslinking reactions.[49] The migration to the surface of untreated chains to replace inwards-diffusing chains with polar groups is thought to be the rate determining step for surface restructuring, because the untreated chains have a higher molecular weight than the treated chains and, therefore, a lesser mobility.[49] In contrast, for heptylamine plasma polymer, the air/water CAs decrease on aging,[73,172] which suggests that the oxidation process is more efficient in incorporating the polar groups than the surface restructuring is able to move them into the bulk of the plasma polymer.

From the changes in CAs with time, a time constant for the surface adaptation process, which is thought o be driven mainly by reptation, can be derived. The time constant of surface adaptation is 10–50 d for heptylamine plasma polymer, whereas for NH_3-treated FEP surfaces it is 2–3 d.

Evidently, the plasma polymer has a much higher molecular weight and crosslink structure. Likewise, by measuring CAs versus time for NH_3-treated hexane plasma polymer surfaces a time constant of surface reorientation of 40–50 d has been obtained.[172] A similar trend has also been observed for NH_3 plasma-treated PE that has been pretreated with Ar plasma.[171] The Ar plasma pretreatment decreases the surface mobility as a result of crosslinking. In both cases (hexane/NH_3 and PE/Ar/NH_3) increases in CAs with time imply that surface reorientation is more efficient than the post-plasma oxidation.

The aging behavior of allylamine plasma polymer is complicated by its granular and uneven surface morphology, which complicates the CA measurements and XPS analysis,[73] however, observed increases in air/water CAs and O/C ratios over 2 months of aging suggest analogous phenomena. Whittle et al. have shown similar air aging results with allylamine plasma polymer,[116] with a rapid rise in O/C over the first 30 d followed by gradual oxidation for another 300 d. Similar plasma polymer systems have also been investigated by Ko and Cooper but without taking possible surface roughness into consideration.[71] They observed a reduction of air/ octane and air/water CAs regardless of the discharge power density used to deposit the allylamine plasma polymer. Simultaneously, the oxygen uptake increased and the maximum O/C ratio shifted to deeper depths during the first 7 d of aging. These short-term results indicate dominance of oxidation, which increases the hydrophilicity of the plasma polymer over this period.

However, aging can also yield volatile products. Interpretation of the XPS data upon aging of allylamine and 1,3-diaminopropane plasma polymers requires a third process (in addition to oxidation and surface adaptation), and the loss of some N suggests the generation of volatile reaction products. A similar loss of nitrogen has been observed by Whittle et al. over 330 d of air aging.[116] A recent study has reported that as much as $27 \pm 3\%$ of the N/C ratio is lost when NH_3 or N_2/H_2 plasma-treated substrates are exposed to air.[65] One possible mechanism for the reduction of N/C ratios is the hydrolysis of imine groups by reaction with atmospheric moisture since the presence of imines (C=N) in allylamine plasma polymers has revealed by near-edge X-ray absorption fine structure (NEXAFS) spectra.[74,84] A higher discharge power favors the formation of C=N and C≡N bonds in allylamine plasma polymers.[74] In contrast, Shard et al. have shown that only C≡N bonds are favored by a high discharge power while C=N bonds are typically invariant of discharge power.[84] Other researchers have reported that C≡N bonds are susceptible to attack from nucleophiles and radicals, which results in low concentrations in plasma polymers.[80] Previously, Gerenser had reported that N_2 plasma treatment creates imines that hydrolyze with atmospheric water vapor.[167]

In the case of 1,3-diaminopropane plasma polymer, Gengenbach and Griesser have proposed a two-step mechanism to explain the reduction of N/C with aging time, based on FT-IR spectra and angle-dependent XPS.[77] The initial sharp decrease of N/C is assigned to rapid volatilization of imines or NH_3 to the atmosphere while the subsequent slower decrease of N/C is assigned to the rearrangement motions of polymer chains.[77] An analogous two-step reduction of N/C is also observed with heptylamine plasma polymers.[73]

For the purposes of covalent immobilization, the key issue is that plasma-produced amine groups, whether from plasma treatment or plasma polymerization, will be partially or entirely lost from the surface with time. Amine groups can be lost from the surface not only by surface adaptation but also by oxidation reactions that convert amines to amides. This is attested by an increase in the XPS N 1s binding energy (BE)[73,78,172] from 399.2–399.3 eV, a position diagnostic of amine groups, to 399.8–400.0 eV, a position assignable to amides. It is, therefore, essential to characterize the time over which a sufficient density of amine groups is available for interfacial covalent reaction. To be safe, Dai et al. have suggested that even the less mobile heptylamine surfaces should be used within 2 d for interfacial reactions.[52] Favia et al. also report a reduction in reactive NH_2 with aging but without a change in the N/C ratio.[171] An alternative method is to reduce imines and nitriles in plasma polymers with $LiAlH_4$ to amines.[84]

Interestingly, XPS analysis of the N 1s signal shows that in all cases it is the C adjacent to the amine, not the N itself, that is oxidized, as no evidence for nitroso, nitro, and nitrate species is observed[77,169,172]. These groups would be discernible by contributions to the N 1s signal at BEs > 400.0 eV.

In some cases, other reactions can also occur upon aging. The concentration of F atoms on NH_3-treated FEP has been found to *decrease* upon aging,[172] which is unexpected because surface reorientation should bring fluorocarbon chains to the surface. Three possible mechanisms have been proposed for the reduction of F/C: homolytic cleavage of C–F bonds, decomposition of peroxide radicals with volatilization of fluorine-rich molecules, and loss of HF as a result of the much higher reactivity of NH_2 groups when next to F, which results in HF and nitriles.[172] Another possible mechanism is the conversion of secondary amines ($–CF_x–NH–CF_y–$) by HF loss to imines ($–CF_{x-1}=N–CF_y–$).[172] Unfortunately, XPS cannot distinguish such reaction products; whereas some workers have assigned N 1s BE values of 400.0 eV to imines, such an assignment does not seem reasonable as BEs are governed by electron densities, and the electron density on the N of an imine is likely to be much closer to that of an amine N than an amide N.

The environment in which samples are stored also plays a role in the mechanisms and rates of aging reactions. When NH_3 plasma-treated FEP is aged in different environments of air, water, methanol, nitrogen, and oxygen, air/water CA measurements show an increase whose rate and magnitude vary with the environment.[172] It may seem

counter-intuitive that CAs would increase on storage in polar media, but the interfacial enthalpy of retaining polar surface groups at the treated surface appears to be more than compensated for by translational entropy that drives polar groups into the bulk polymer. Comparing the storage in air, nitrogen, and oxygen atmospheres suggests that oxidation with atmospheric oxygen competes with crosslinking that quenches any remaining radicals. In the nitrogen atmosphere, the crosslinking process reduces the translational mobility required for surface reorientation and, hence, the increase in CAs. In an oxygen environment, crosslinking is less effective as radicals react with inwards-diffusing O_2 and, hence, there is a more rapid increase in CAs.

Harsch et al. have used other tests, which have included three autoclaving cycles, 4 weeks of soaking in warm culture medium, and various cleaning procedures, to evaluate the stability of allylamine pulsed plasma polymer on polysiloxane.[88] Results show only minimal reduction of amine densities after autoclaving,[87] and a re-used allylamine plasma polymer supports cell growth as effectively as a freshly plasma polymerized coating.

With regard to the influence of the substrate on the aging behavior of NH_3-treated substrates, PTFE, FEP, and LDPE surfaces do not show fundamental differences in CA behavior but there are quantitative differences in the surface restructuring parameters.[50] The same mechanisms of surface reorientation and post-deposition oxidation operate, but to different extents, in the aging of NH_3 plasma-treated HDPE and PP surfaces.[172] Specific combinations of substrate and plasma treatment, however, can show markedly different aging behaviors. Water and ammonia plasma treatments modify PTFE and FEP to different depths, with the water plasma achieving deep modification whereas the ammonia plasma treatment is very shallow. As a result, the compositional gradient across the treatment depth differs markedly and leads to a minimal driving force for surface reorientation for the water plasma-treated substrates, which show stable air/water CAs.[48] A steep compositional gradient has also been reported for N_2/H_2 plasma-treated PFA [a composition of TFE and perfluoro(alkoxy vinyl ether)].[174] For water plasma-treated fluoropolymers the F/C ratio decreased to a lesser extent than during NH_3 plasma treatment, which suggests less crosslinking, yet the absence of a significant compositional gradient means that despite potential mobility there is no significant driving force for surface restructuring.[48]

The effect of crosslinking on the aging behavior is difficult to evaluate because there is no direct analytical technique to measure the densities of crosslinks in plasma-treated surfaces and coatings. Studies have relied on the reduction of XPS F/C ratios and apparent surface hardening observed with AFM or STM for indication of crosslink densities.[169] Based on a series of assumptions, which may not be applicable to other monomers, NMR and IR spectra have been used to derive an apparent crosslink density of ethylene, ethylene-acetylene, butadiene, and benzene plasma polymers.[175] Others have used the total secondary ion yield of SSIMS spectra to measure the degree of crosslinking in the plasma polymers.[176] While the total ion yield is found to be inversely related to the degree of crosslinking, it is directly related to the oxygen concentration. A lower ion yield could also be caused by a reduction of oxygen on the surfaces instead of a higher degree of crosslinking.[149] Another study has utilized the ratio of total yield of C_6-C_8 hydrocarbon secondary ion clusters to the total yield of C_2-C_8 clusters $[\Sigma(C_6-C_8)/\Sigma(C_2-C_8)]$, which increases with the degree of crosslinking.[177] This parameter, however, may not discriminate branching from crosslinking in the polymers. An alternative approach involves dissolving a plasma-treated polymer in *p*-xylene at 110 °C until the untreated substrate is completely dissolved,[178] and the degree of crosslinking is estimated from the undissolved gel weight.

Crosslinking densities may evolve with aging as a result of oxidation and radical reactions. A complication in the study of crosslinking in plasma polymers is that by increasing the discharge power with the aim to increase the density of crosslinking also results in an increase in trapped radicals that may affect the extent and reaction pathways of post-deposition oxidation. In addition, the increase of power density will increase the fragmentation of the monomers in the gas phase, which may change the composition of the plasma polymer. There is also the practical limitation of having a highly crosslinked plasma polymer because it may become too brittle to handle because of mechanical incompatibility with the substrate materials.[73]

Aging Behavior of Plasma-Prepared Carboxylated Surfaces

A similar aging behavior of CO_2 plasma-treated substrates has been reported by Ko et al.[107] and Terlingen et al.,[111] though the exact pathways appear to be slightly different. During 7 d of air aging, water and octane CAs increase while angle-dependent XPS (at 75°) shows a reduction of O/C ratios.[107] Ko et al. have attributed this to diffusion of functional groups into the bulk and/or loss of low-molecular-weight oxygen-containing fragments.[107] On the other hand, Terlingen and others have reported that their XPS oxygen level is almost invariant but their water CAs show an increase over 90 d of aging in air.[111] They have also found that aging temperature can influence the rate of increase of water CAs but subsequent immersion of treated samples into water for 24 h at a temperature of 50 °C fails to fully recover the original surface hydrophilicity. The recovered CA depends on the aging time instead of aging temperature. These results suggest that there may be two components of polar groups: reversible and irreversible,[111] which is analogous to the mobile and immobile

groups used in the surface restructuring model of Chatelier et al.[49,50]

The short-term aging of plasma polymer from acrylic acid appears to be similar to that of surfaces created by CO_2 plasma treatment. Over 9 d of air aging there is an increase of water and octane CAs while O/C ratios at various XPS emission angles show substantial reductions after 6 d of aging in air.[71] The possibility of hydrocarbon contamination is eliminated because the reduction of O/C is dissimilar between the different plasma polymers. Hydrocarbon contamination is expected to cause similar reductions of O/C ratios because of a similar storage environment. However, some researchers have reported reduction of the COOH groups in the plasma polymer upon rinsing or soaking in water and thus suggest that dissolution of low-molecular-weight oligomers increases the air/water CAs.[115,119] This hypothesis is supported by the reduction of plasma polymer thickness during adhesion force measurements in water.[119] Migration of hydrophilic groups into the bulk polymer could also cause a reduction in O/C. The putative migration mechanism has been further investigated by varying the humidity.[119] Surprisingly, the CA increases even in a humid environment of 92% relative humidity. A reason for this could be the formation of a hydrogel at high humidity, which induces the hydrophilic groups to orient towards the interior. The water in the hydrogel would insert into hydrogen bonds amongst the polymer chains and increase the mobility. On the other hand, Whittle et al. have shown almost invariant O/C ratios and percentage of COOH groups over 420 d of aging (Figure 8).[116] Thus, the mechanisms of aging of acrylic acid plasma polymers may depend on various factors including the composition, which varies with plasma conditions and apparatus.

The deposition parameters can also influence the aging behavior of plasma polymers, as shown by Gengenbach and Griesser (Figure 9), though they use methyl methacrylate as the monomer.[118] A higher discharge power results in higher oxidation and more variety of chemical groups than with a lower discharge power. However, they also report that the rapid increase of O/C ratios within the first few days is followed by tapering off of these ratios over the next

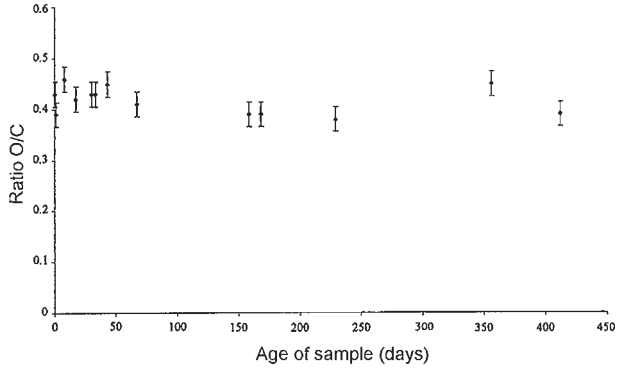


Figure 8. O/C ratio of acrylic acid plasma polymer as a function of sample age. Reprinted with permission from ref. [116]

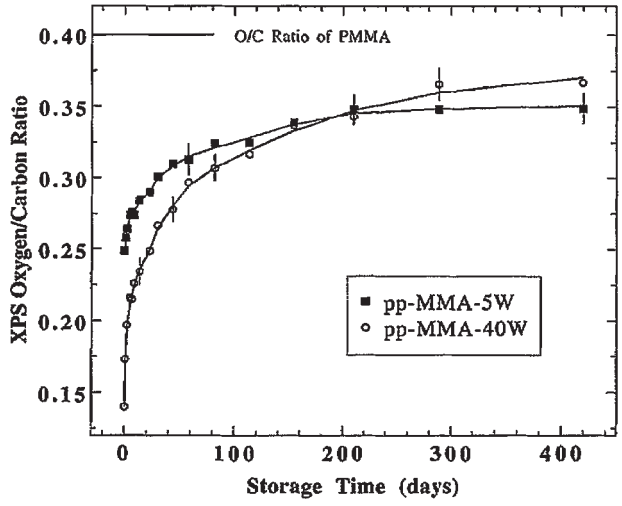


Figure 9. XPS O/C ratios (0^0 emission) as a function of storage time, of plasma-polymerized methyl methacrylate deposited at power levels of 5 and 40 W. Also included is the theoretical value of O/C for PMMA (0.4). Error bars represent one standard deviation. Reproduced with permission from ref. [118]

300 d. Another consequence of higher discharge power is the higher degree of crosslinking in plasma polymers, which reduces surface reorientation regardless of the storage environment.[119,127,132]

A high density of crosslinking has been postulated as the cause for stability of COOH surfactants immobilized by Ar plasma treatment.[127,132] CA measurements have been conducted with different solutions, i.e., water, 0.1 M HCl, and 0.1 M NaOH.[127,132] The CAs do not change during air aging at −20 and 25 °C over 12 weeks. At 50 °C, the HCl and NaOH CAs increase for the first 2 d and then remain stable for the remaining aging period. The convergence of the advancing CAs with 0.1 M HCl and 0.1 M NaOH implies movement of the polymer segments that contain carboxylate groups near the surface at the higher temperature.

In the case of plasma grafting, the aging behavior of grafted acrylic acid coatings depend on grafting density.[99] As shown in Figure 10, the grafts form domains that restrict the movement of hydrophilic segments at high grafting density. The absence of acrylic acid domains and interfacial tension of grafted acrylic acid creates the impetus for the polar chains to move into the interior at low grafting density.

Aging Behavior of Plasma-Prepared Hydroxylated Surfaces

Water plasma-treated PTFE and FEP samples demonstrate the stability of CAs during aging in air whereas water plasma-treated PP does not,[48] which again emphasizes the difficulty in establishing general rules for effects and aging of plasma treatments on different substrates. However, the compositions, depth profiles, and aging behavior of plasma-

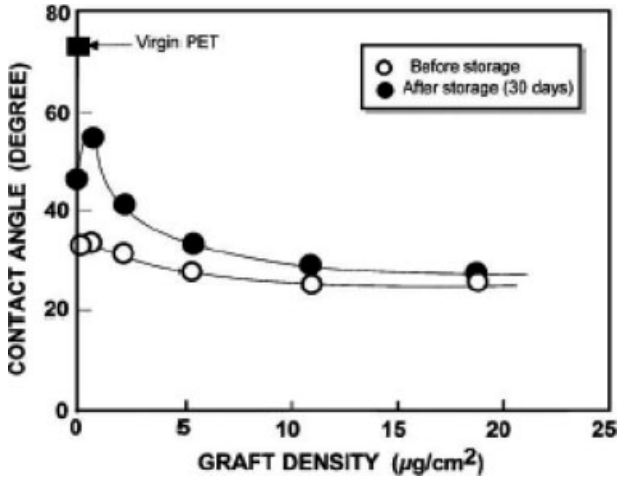


Figure 10. Variation of CA with grafting density of acrylic acid on PET films. Reproduced with permission from ref. [99]

treated samples may vary considerably for a given plasma and substrate combination as plasma conditions are changed. Water plasmas, for example, can be surface modifying or etching depending on pressure and power.[179]

Little research has been carried out on the aging behavior of hydroxy plasma polymers but surface restructuring has been reported for plasma polymers with hydroxylated surfaces.[147]. By analysis of high resolution electron energy loss spectroscopy (HREELS) spectra propargyl alcohol plasma polymer shows a higher percentage of OH than allyl alcohol plasma polymer, though XPS analysis leads to the opposite conclusion.[147] HREELS has a shallower depth of analysis of only 1–2 nm in comparison to XPS (10 nm), and hence the apparent contradiction can be reconciled by invoking surface restructuring. The lower degree of crosslinking in allyl alcohol plasma polymer allows migration of OH groups from the surface into subsurface regions and this reorientation reduces the interfacial enthalpy of the surface. This model of surface restructuring is supported by CA measurements, which show higher values for allyl alcohol compared to propargyl alcohol plasma polymers.[117] The depth of analysis of CA measurements is thought to be 0.5 to 1 nm.[173]

Other aging studies on OH plasma polymer have been carried out by Gengenbach et al.,[73] Whittle et al.,[116] Swaraj et al.,[150] and Oran et al.[149] The former work showed that the aging behavior of methanol plasma polymer surfaces depended on the discharge power.[73] While the CAs of a 60 W methanol plasma polymer do not show much variation over 22 d, the plasma polymer created under 10 W plasma power shows a rapid increase within the first 7 d. It is likely that the higher degree of crosslinking generated by the higher discharge power prevents surface reorientation. Whittle et al. found no noticeable changes in allyl alcohol plasma polymer in terms of O/C ratios, percentage of C–O and C=O bonds during 330 d of air aging.[116] This result is probably caused by the high discharge power used to create this surface. Similar results have been reported when using NEXAFS, SSIMS, and XPS to study the aging of allyl alcohol plasma polymers.[149,150] Plasma polymers deposited under high discharge power conditions show an invariant[150] or increasing[149] O/C ratio, while those deposited at low discharge power register a drop in O/C ratios with aging time.[149,150] The increase of oxygen is caused by auto-oxidation of radicals in the formation of plasma polymers while the decrease of oxygen is caused by migration or reorientation of hydroxy groups into the bulk polymer. The higher crosslink density created by higher discharge power prevents the migration of hydroxy groups.

The study of the aging of hydroxy plasma polymers is hampered by the absence of a suitable marker for XPS, such as N in aminated surfaces, and evidence that derivatization may also report other groups in these multi-functional polymers.[25] However, the same mechanisms of surface restructuring, entropy, and others (Figure 7) are expected to be operational.

Aging Behavior of Aldehyde Plasma Polymers

The aging behavior of aldehyde plasma polymers depends on the monomer and excitation frequency.[163] Aging is affected by a balance between incorporation of polar groups that result from post-plasma oxidative processes, an incorporation that increases the surface hydrophilicity, and surface reorientation, which reduces the hydrophilicity of the plasma polymerized surfaces. In the case of ethylbutyraldehyde plasma polymers, the aging is dominated by incorporation of polar groups for the first 5 d regardless of excitation frequency.[163] This observation is similar to the aging behavior of heptylamine plasma polymers.[73] This decrease of CAs suggests that ''similar amounts of radicals had been trapped in the plasma polymers at different excitation frequencies''. For nonylaldehyde (NA) plasma polymer, no changes in CAs are detected for 100 d when stored under ambient conditions. This observation is similar to that for 1,3-diaminopropane plasma polymers. It has been speculated that the invariant nature of the NA and 1,3-diaminopropane plasma polymer surfaces is a result of coincidental compensatory effects of oxidation and surface restructuring.[73] For capronaldehyde plasma polymers deposited at 275 and 375 kHz, CAs decrease initially and then stabilize, which is similar to ethylbutyraldehyde plasma polymers. However, for capronaldehyde plasma polymer deposited at 125 kHz, the CAs increase with storage over the first few days. At a frequency of 175 kHz, CAs do not change on storage. Gong and Griesser have postulated that the capronaldehyde plasma polymer deposited at a higher frequency has more trapped radicals, which results in more extensive oxidation post-deposition[163] and thus more polar groups that increase surface hydrophilicity.

In the lower frequency regime, fewer radicals also result in a reduced crosslinking density, which allows movement of hydrophobic groups to the surface and thereby increases air/water CAs.

Cell Colonization on Plasma-Treated and Plasma-Polymerized Surfaces

Aminated Surfaces

Early studies on the cell colonizing ability of plasma-prepared surfaces have focused on oxygen-containing surfaces, perhaps because of the success of plasma-oxidized PS as a commercially successful product for tissue culture labware. Once Primaria tissue culture ware became available, nitrogen-containing surfaces also became of interest. Several studies, reviewed below, focused on generating amine-rich surfaces, although XPS analysis of Primaria shows an N 1s peak whose main component has a BE of 400.0 eV,[41] which is characteristic of *amide* groups. Accordingly, an interesting question is whether amine or amide groups are better for cell colonization surfaces.

Nakayama et al.[26] have concluded that the presence of amine groups rather than the total nitrogen content of the surface is key to promoting cell attachment, ruling out any influence of surface texture on cell attachment because of comparable roughness regardless of treatment conditions. However, they have used only a 24 h cell attachment assay, which may not be predictive of longer-term colonization. Many other studies have used longer cell colonization times.

In contrast, using a number of N-containing plasma polymers in conjunction with XPS data that show conversion by oxidative post-plasma aging of a large fraction of the amine groups into amides within the period between sample fabrication and cell assays, Griesser et al concluded that *amide* groups appear to be the main surface chemical groups that promote cell attachment to N-containing plasma polymers in their 7 d cell colonization studies.[51] This conclusion is supported by aging studies and XPS analyses[70,73,78] that demonstrate that amine groups appear with an N 1s BE of ≈399.3 eV, whereas amides give rise to a contribution at a BE of 400.0 eV. It appears likely that some earlier work had been marred by incorrect XPS N 1s BE assignments and failure to recognize post-plasma oxidation processes. While they do not rule out some influence of amine groups on cell interactions, it is noted that there was similar cell culture performance on amine-containing hydrocarbon and fluorocarbon surfaces, despite the fact that amine groups are substantially more acidic on fluorocarbon surfaces (ammonia plasma-treated PTFE and FEP) than for alkylamine plasma polymers because of the electron withdrawing effect of adjacent fluorine atoms. One would have expected that such a difference in protonation of amines would affect cell colonization.

Anchorage-dependent cells colonize synthetic surfaces by integrin-mediated docking onto a layer of adsorbed adhesive glycoproteins. Therefore, understanding the influence of surface chemical groups on cell colonization requires not only detailed surface analysis, but also study of the adsorption and denaturation of such proteins on specific surface chemistries. Ertel et al.[136] have shown that while a N-containing plasma polymer promotes fibronectin adsorption, it does not initiate cell attachment, an observation that appears to contradict an earlier study that reported that fibronectin coatings promoted cell adhesion.[180] However, this apparent inconsistency can be resolved by postulating that the adsorbing fibronectin molecules denature on the plasma polymer surface such that the cell adhesion motif is no longer recognizable. Thermanox substrate, a commercial tissue culture coverslip that contains nitrogen, also does not promote cell proliferation.[181]

The molecular interfacial mechanisms of cell attachment to N-containing plasma polymers have been elucidated by Steele et al.[182] who show, by studies with growth media selectively depleted of specific proteins, that such plasma polymers are able to stimulate cell attachment to their surfaces via two alternative, separate pathways. Adsorption of either fibronectin or vitronectin promotes endothelial cell or fibroblast cell attachment and growth, a dual mode of cell support not found in some other, non-N-containing surfaces. It thus appears that both these proteins could adsorb without significant denaturation onto the amine and amide plasma polymers of that study. This is an interesting difference to the finding by Ertel et al.[136] discussed above that suggests fibronectin denaturation on the surface of that study. The reasons for such observations are not fully understood but there is the possibility that the greater hydrophilicity of the plasma surfaces used by Griesser et al. facilitate protein adsorption without denaturation. However, it is noted that an increase of hydrophilicity as such does not translate to better cell adhesion, as observed by Harsch et al. with flame-treated polysiloxane.[88]

The importance of protein denaturation is also emphasized by the finding that substantial adsorption of vitronectin and fibronectin occurs onto perfluoropolymer substrates, to extents comparable to those on amine and amide plasma polymers, yet the attachment of endothelial cells is negligible on the perfluoropolymer surfaces, in contrast to the good colonization on the plasma polymers (unpublished data). These findings emphasize that the extent of denaturation of adhesive glycoproteins may be more important than the adsorbed amounts.

Using co-polymerization of allylamine with octa-1,7-diene a series of coatings have been prepared with the aim of quantifying the critical density of amines needed for optimum keratinocyte cell adhesion.[43] However, it was, as usual, difficult to achieve selective chemistry from plasma. Results show an increase of cell adhesion with an increase of N content, albeit lower than the cell adhesion on

the positive control of collagen-1 coated samples. In another effort to correlate the density of amine surface groups with cell proliferation, Harsch et al. have increased the off time of pulsed plasma polymerization to reduce scrambling during the fragmentation of the monomers.[88] A duty cycle of 3 ms on and 45 ms off produced twice as high an amine density as a duty cycle of 3 ms on and 5 ms off. The substrates that have higher densities of amines show a better dispersion of spinal cord cells for the first 24 h but there are no noticeable morphological differences after 2 weeks.[88] One possible explanation for this initial increase of cell growth in the first 24 h may relate to the amount of protein adsorbed. At a physiological pH of 7.4, protonation of surface amines would lead to a positive charge that attracts the negatively charged adhesive glycoproteins, although the often invoked assumption that amine plasma polymers carry a positive surface charge needs to be experimentally verified. Chatelier et al.[183] have found that the pK_a of the surface amine groups on heptylamine plasma polymer is much lower than for amines in solution, at between 5 and 6, because of dielectric effects that arise from the much less polar plasma polymer substrate, which results in a low degree of protonation at a physiological pH of 7.4.

Using a mixture of N_2 and H_2 for plasma treatment, Mirenghi et al. emphasize the importance of a high percentage of N on plasma-treated PET for cell adhesion with human umbilical vein endothelial cells.[184] Similar results have been reported by Wertheimer's group for the adhesion of growth plate chondrocytes and human U937 macrophages on N_2–C_2H_4 plasma polymer.[68] (Human U937 macrophages do not adhere to existing cell culture materials.) The latter study also reports the presence of a critical concentration of nitrogen to adhere different types of cells lines, i.e., 25% N and above for human U937 macrophages and 19% and above for growth plate chondrocytes.[68] Although both reports acknowledge the importance of identifying the chemical states and groups of the N atoms, they do not provide the relevant data. Surface texture is not expected to influence the results with a N_2/H_2 plasma-treatment time of 5 min, but this assumption needs to be checked in light of the fact that even short plasma-treatment times can cause etching, and the numerous studies that have shown considerable effects of surface topography on cell colonization. The former in another study[181] did not verify the presence of surface amine groups, assuming their presence based on other researchers' results when using NH_3/H_2 plasma. However, this may be erroneous because the resultant surface chemistry can, from the same plasma gases, vary considerably with plasma conditions and reactor geometry, among other things, as well as aging.

In addition to the observation of cell adhesion, fibrinolytic and prothrombic studies have been carried out to assess the ability of seeded endothelial cells on N-rich surfaces to secrete prostacyclin, tissue plasminogen activator, and plasminogen inhibitor-1.[185] Similar positive results are reported for NH_3-treated PTFE with and without a fibronectin coating, compared with control samples of untreated PTFE, fibronectin coated on untreated PTFE, and NH_3 plasma-treated PTFE with and without gel or collagen coatings. It is concluded that surface amines are important with and without fibronectin coating, but unfortunately the assumption that ammonia plasma creates mainly amine groups on PTFE has not been checked. The surface analytical data of Gengenbach et al.[169] indicate that this is not so, amines being unstable next to the strongly electronegative atom F and short lived because of complex sets of reactions. It is unfortunate that many studies of bio-interfacial interactions make unwarranted and at times incorrect assumptions about surface chemical compositions instead of performing appropriate surface analytical studies.

Beyond assays with isolated cells, Tseng and Edelman have used a circulating system for flow of blood over a butylamine plasma polymer on expanded PTFE (ePTFE).[186] Their results confirm that amine and amide groups enhance endothelial cell seeding on ePTFE vascular grafts under constant and pulsatile flow conditions. The role of serum is not discussed, but it is shown that endothelial cells are equally adherent to collagen-coated native ePTFE as to collagen-coated plasma polymerized ePTFE under different flow conditions investigated for the duration of 5 d, which suggests that protein adsorption in some cases can mask surface chemical differences.

In an attempt to correlate *in vitro* protein adsorption with *in vivo* implantation outcomes, Tang et al. have found that the high density of fibrinogen not elutable by sodium dodecyl sulfate (SDS) correlates to high numbers of adherent phagocytes explanted from a Swiss Webster mice model.[187] Their N-containing plasma polymer with an amine density of 20% elicited the highest amount of surface enzyme activity and adherent phagocytes, but surprisingly the chronic fibrotic response to these surfaces does not show any difference to other surfaces which had 2–3 times reductions in fibrinogen not elutable by SDS. These results suggest that the surface functionalities may not be critical for the long term response to implantation.

An absence of response to differences of chemical groups has also been observed by Sefton et al.[188] using NH_3 plasma-treated poly(ether urethane) (PEU), by subjecting the samples to a range of tests that include blood coagulation and complement activation with different markers. Surprisingly, NH_3-treated PEU shows a similar behavior to a range of materials that include untreated nylon, untreated Silastic, fluorinated PEU, poly(ethylene imine)-treated PEU, and H_2O plasma-treated PEU. These results suggest that either ineffective NH_3-plasma treatment results in poor anti-thrombogenicity or the surface groups may not be important in interactions with blood and cells. Deconvolution of XPS spectra show that 19.5% of C 1s carbon is in the form of C–O or C–N bonds for NH_3-treated PEU, though the presence of amine has not been

determined directly. Previously, it has been shown that allylamine plasma polymers are more thrombogenic than LDPE.[71]

Oxygen-Containing Surfaces

Plasma treatment of PS is well known to produce surfaces with excellent cell compatibility. Studies by Ertel et al.[136] with a variety of oxygen-containing surface chemistries produced by plasma polymerization show that higher oxygen contents result in enhanced growth of bovine aortic endothelial cells over 3 d. However, this has to be a consequence of the presence of specific chemical surface groups because the amount of oxygen as such did not correlate with higher cell growth on other surfaces, such as untreated PET which has 28% surface O.[136] The oxygen consisted mainly of hydroxy and carbonyl groups[189] and thus Ertel et al. considered carbonyl groups to be important for cell adhesion.[190] On the other hand, alcohol monomers also produce plasma polymers with good cell colonization ability,[51] though the multifunctionality of their surface chemical composition makes it difficult to ensure that hydroxy groups are important for cell attachment. Daw et al.[93] have also found that increasing oxygen content is important but not the sole criterion. The O/C ratio of tissue culture PS is 3.8 times that of acrylic acid/octadiene plasma polymers, but the former has inferior cell attachment compared with the latter.

The oxygen content does not correlate with the adsorption of the cell adhesion that promotes protein fibronectin[136] nor the lack of affinity of the surface for the cell attachment that inhibits proteins albumin and IgG.[189] However, increasing oxygen results in tighter binding of albumin and IgG on the surface. The tight binding of proteins might provide a stable platform for cell growth, but tight binding of proteins is not sufficient, as shown by the failure of cell colonization on plasma fluoropolymers.[191,192] It has been hypothesized that the presence of oxygen-containing surface groups might induce endothelial cells to secrete endogeneous fibronectin,[193] but Steele et al. have since shown that adsorption of the cell adhesive glycoprotein vitronectin is the key factor in promoting cell colonization on these surfaces where fibonectin is of low effectiveness.[182]

As for the effect of oxygen content, a high concentration of carbonyl groups as such is not sufficient, as shown by poly(vinyl methyl ketone) which does not show high proliferation of cells.[190] Since no specific mechanism of cell attachment can be attributed to the presence of ketones, it has been speculated that the multifunctionality of plasma polymers combined with a dense network of crosslinks contribute to good cell colonization.[189] The influence of ketones has been further analyzed using plasma copolymerization of methyl vinyl ketone/octa-1,7-diene.[95] In the absence of carboxy groups, carbonyl groups do not promote the growth of osteoblast-like cells.[95]

A most interesting series of studies by Short et al. has investigated the importance of surface hydroxy and carboxy groups, separately, for the culture of keratinocytes and osteoblasts. The approach used a mixture of two vapors for plasma polymerization, which enables creation of a range of surface densities of the desired groups, assessable by detailed surface characterization. Using co-polymerization of octa-1,7-diene and allyl alcohol, maximum cell attachment occurs on a pure allyl alcohol plasma polymer for which XPS shows 25% of the total C 1s signal to be in the C–O component.[154] This component has been interpreted as hydroxy groups, although ether, peroxy, and hydroperoxy groups have the same BE and post-plasma oxidation is known to produce peroxides.[166] While the increasing OH concentration correlates well with the increase in the non-dispersive component of the surface energy determined by CAs, the dispersive component does not vary much.[154] These finding show that cell attachment increases with surface energy and concentration of OH, but the generalization of such findings may not necessarily extend to other surface chemistries.

The same group has used the plasma co-polymerization of octa-1,7-diene and acrylic acid to form an analogous series of coatings that vary in carboxy density.[43] Maximal keratinocyte cell adhesion occurs on surfaces with a low concentration of COOH (2.3% of the total C 1s peak) and surface energy.[43] Similar results have been reported by Daw et al. when using osteoblasts on plasma co-polymers of acrylic acid and octa-1,7-diene.[93] Optimal cell attachment occurs at 3.0–5.0% COOH. Further increases in COOH concentration results in reduced cell adhesion.[43,93] Gupta et al.[135] have since performed similar studies with human bladder smooth muscle cells and bone marrow stroma cells (BMSC), which proliferate on 3.8% carboxy plasma co-polymers of octadiene and acrylic acid. The toxicity of carboxy groups at high concentrations was identified early.[194] It may relate to a high negative surface charge. However, self-assembled monolayers (SAMs) with a high density of COOH terminal groups show similar cell attachment density and proliferation as acrylic acid/octa-1,7-diene plasma copolymers.[91,92] These studies do not investigate the adsorption and possibly varying extents of denaturation of adhesive glycoproteins, which might help elucidate the molecular interfacial mechanisms that underlie the efficacious nature of the plasma co-polymer carboxy surfaces.

The influence of surface hydroxy groups has investigated by several groups that use various surface fabrication techniques. Although there are earlier cell adhesion studies with hydroxylated substrates, Curtis et al. provides one of the more comprehensive cell adhesion studies in the presence of serum with various acid etching and ozonization techniques on PS substrates.[194] By isolating the different groups with acetylation and alcohol esterification, they have found that hydroxy rather than carboxy groups are

responsible for the adhesion of leukocytes and BHK cells. Again, however, assumptions have made implicitly about selectivity and efficiency of derivatization reactions. They also suggest that hydroxy groups play the same function as fibronectin in cell adhesion and, as such, the absence of fibronectin will not affect cell proliferation if hydroxy groups are present. This hypothesis has been disproved by Steele et al.[182] who demonstrated that fibronectin was not the only cell adhesive glycoprotein present in serum and vitronectin adsorbed efficiently on oxygen-containing plasma surfaces to stimulate cell colonization.

With various polymers that have been Ar plasma-treated to varying degrees, Tamada and Ikada have found that maximum cell attachment occurs at air/water CAs of $\approx 70^{\circ}$.[195] Although Ar plasma is expected to create various oxygen-containing groups by post-plasma oxidation processes, derivatization indicates that hydroxy groups dominate these plasma-treated substrates, although it can be queried as to whether the method would also have derivatized hydroperoxide groups, which are known products of reaction between carbon-centred radicals and atmospheric oxygen.

Aldehyde plasma surfaces, prepared by plasma polymerization of acetaldehyde, have been found to support the attachment and growth of epithelial cells to an extent comparable to tissue culture PS.[158] Some improvement is obtained when immobilizing collagen, other proteins, and oligopeptides onto acetaldehyde plasma polymer surfaces,[157] but on the other hand, these *in vitro* results do not translate well to the *in vivo* situation of epithelial overgrowth in an animal model, where the plasma surface is clearly inferior to the coatings comprising immobilized biological molecules.[196]

Platelet adhesion studies have also been carried out on oxygen-containing plasma polymers. When using chemical grafting to create a carboxylated surface on an amine surface, it has been thought that defect spots are responsible for higher platelet activation.[107] However, carboxylated surfaces created by plasma polymerisation of acrylic acid show less thrombogenicity than untreated PE.[71]

Thus, studies have generally found that many cell types attach and grow relatively well on many plasma polymers and also often on plasma-treated surfaces. However, most studies have used isolated, suspended cells in culture, and evidence is emerging that success in such *in vitro* evaluations is not a good predictor of the *in vivo* performance. Successful cell colonization *in vitro* indicates that cell-adhesive glycoproteins can adsorb onto the plasma-prepared surfaces without substantial denaturation (of the cell adhesion epitope at least), but the *in vivo* situation comprises much more complex biological milieu, as well as a wound healing response. An interesting question is whether other proteins out-compete cell-adhesive glycoproteins in competitive adsorption to the biomaterial surface, or whether cell sheets and tissue react to the presence of (adsorbed protein layers on) synthetic materials in more complex ways. While a number of studies (beyond the scope of this review) have probed single protein adsorption onto plasma-prepared surfaces, unfortunately very few data exist on competitive protein adsorption on plasma-treated and plasma-polymerized surfaces, and the available data are insufficient to arrive at secured conclusions.

Conclusion

Methods are well established for the plasma fabrication of aminated surfaces, by ammonia or N_2/H_2 plasma treatments, or by the plasma polymerization of allylamine or alkylamines. These surfaces have found wide use for the immobilization, by solution chemical methods, of subsequent layers such as PEOs and polysaccharides. Multi-step chemical reactions that result in the covalent immobilization of biologically active molecules have also been applied successfully. However, in many cases the amine surfaces have a rather limited shelf life, with post-plasma oxidation reactions and surface adaptation leading to the disappearance of amine groups from the surface. While this means that covalent interfacial reactions have to be performed within a short time after plasma fabrication, the aging effects do not adversely affect cell compatibility, with the resultant surfaces that contain amide groups, showing a good ability to support cell colonization, though the effectiveness seems to depend on the process vapor and the plasma conditions.

Plasma-fabricated surfaces that contain carboxy groups have also been well documented. The plasma co-polymerization of acrylic acid with an alkane appears to be the most reliable route towards such surfaces. Results with CO_2 plasma treatment appear more dependent on the substrate and less stable. The aging of plasma co-polymerized carboxylate surfaces appears not to be a problem, probably because carboxylate groups are a product of post-plasma oxidative reactions anyway. Such co-polymer surfaces have shown an excellent ability to support the colonization of some human cell lines of clinical interest. Immobilization of proteins onto plasma-carboxylated surfaces also is well established.

Fewer studies are available on hydroxy and aldehyde surfaces prepared by plasma methods. Hydroxy surfaces can be prepared by water plasma treatment or the plasma polymerization of alkyl alcohol vapors. Water plasma treatment suffers for many polymer substrates, in the same way as amine surfaces, from surface adaptation that leads to the movement of surface modification effects into the polymer. Both hydroxy and aldehyde surfaces have been used for the covalent immobilization of biologically active molecules, and both types of surface chemistries support cell colonization quite well.

Aging is a widespread phenomenon that often has not been recognized, particularly in some of the earlier studies on the use of plasma-fabricated surfaces for bio-interfacial

applications. It can prevent covalent interfacial reactions, masked by physisorption of proteins. Moreover, as the surface chemistry varies upon aging, it is essential to assess aging effects, and if they are present, one must characterize the surface chemistry at the time biological tests are performed in order to be able to correlate chemical surface composition with biological responses. Aging effects can be more important than the initial chemistries of the plasma surfaces.

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