Chapter 4

Plasma Polymerization for Tissue Engineering Purposes

Gaelle Aziz, Rouba Ghobeira, Rino Morent and Nathalie De Geyter

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.72293

Abstract

The ability of non-equilibrium plasmas to modify surfaces has been known for many years. And a promising way to perform surface modifications without altering the bulk properties is plasma polymerization since this technique is versatile and can be applied to a wide range of materials. Plasma polymer films usually show good biocompatibility when compared to classical biomaterials. The possible biomedical use of plasma polymers motivates the study of their behavior during storage and in aqueous environment. Therefore, it is of major importance to understand the change of properties of these plasma polymers over time and when in contact with certain fluids. Recently, plasma polymer gradients (surfaces that display a change in at least one physicochemical property over distance) have attracted significant attention from the biomedical filed where the interaction of cells with a material surface is of major interest. This chapter discusses biomaterial functionalization via plasma polymerization focusing on their use in the biomedical field as well as their aging and stability behaviors. Plasma polymer gradients as valuable tools to investigate cell-surface interactions will also be reviewed.

Keywords: biomaterial, plasma polymer, surface gradient, stability, aging

1. Introduction

1.1. Tissue engineering

Tissue engineering (TE) was first expressed at the NSF "National Science Foundation" workshop in 1987 by Dr. Fung. TE was later described as the application of engineering and life sciences to better understand the correlations between the structure and the function of tissues as well as the development of replacements for the restoration, preservation and/or enhancement of tissue functions [1].

But it was not until 1993 that Langer and Vacanti gave TE the classical definition of: "an interdisciplinary field that applies the principles of engineering and the life sciences toward the development of biological substitutes that restore, maintain, or improve the tissue function [2]." Various, more or less similar TE definitions can be found in the literature. Moreover, since this is a relatively new field, specific definitions are not always given and may stretch from decellularized matrices to cellular implants.

In tissue engineering, biomaterials must possess appropriate surface properties for better cell-material interactions. In addition, biomaterials should possess appropriate bulk properties to function properly in a bio-environment. Therefore, a suitable approach is to select a biomaterial having good bulk properties and enhance its surface properties using a preferential surface treatment [3, 4]. In this way, one can obtain an "ideal" biomaterial with selective surface properties that are decoupled from its bulk properties and avert the need to develop completely new materials which is quite costly and time-consuming.

In the past few decades, tailoring materials surface properties has been extensively performed using various modification techniques such as chemical treatments and etching, ozone treatment, UV radiation, and plasma treatments [5–10].

Plasma surface treatments are most promising due to the speed and uniformity of modification, their chemical flexibility and positive environmental impact [11, 12]. Various types of plasma surface modification technologies have been used to modify materials by incorporating a variety of functional groups on their surfaces; this is done to improve the surface energy, wettability, adhesion, and bioactive response [13, 14].

1.2. Plasma: a brief introduction and historical background

Plasma is defined as the fourth state of matter in the sequence: solid, liquid, gas, and plasma. The transition between these different respective states can occur by increasing the temperature of the material under consideration.

This state of matter was first described in 1879 by Crookes as "a world where matter may exist in a fourth state." Later, in 1928, this state of ionized gas was eventually given its name "plasma" by Irving Langmuir, when he introduced it in his studies of electrified gases in vacuum tubes [15]. Plasmas can be natural such as lightning, polar light and the stars or man-made. Therefore, without being aware, every person has faced various forms of plasma. Man-made plasma can be generated in laboratories by combustion, flames, lasers or controlled nuclear reactions. But, in the field of plasma polymerization, most plasma are generated and sustained using an electrical discharge.

Plasma is generally formed when gas atoms are subjected to a high enough thermal or electrical energy. Subjected to energy, gas atoms become ions by releasing some of their electrons. Radicals are then created by electron-molecule collisions and bond breaks in molecules. Some excited species will also be created by energy adsorption which will generate photons. This unique mixture of electrons, ions, radicals, photons and neutrals constitute the so-called plasma [16, 17].

Plasmas are classified as thermal "equilibrium" and non-thermal "non-equilibrium" based on the relative temperatures of electrons, ions and neutrals.

In a non-thermal or cold plasma, the electron temperature (≈ 10,000°C) is much higher than the gas temperature (< 200°C), whereas, in a thermal or hot plasma, the electron temperature is very close to that of the heavy particles.

Plasmas used in the field of plasma polymerization are usually cold plasmas since it involves heat-sensitive materials [18, 19].

2. Plasma modification of surfaces

The ability of non-thermal plasmas to dramatically modify surfaces properties has been known for over 25 years. Plasma treatments allow surface modification of polymeric materials without altering their bulk properties [20–22]. These plasma processes can be categorized into 3 major types of reaction: plasma activation, post-irradiation grafting (briefly discussed in the next sections), and plasma polymerization (the focus of this chapter).

2.1. Plasma activation

In plasma activation, surface modification is done by exposure to non-polymer forming plasmas. The active species in the plasma can bombard the polymeric surface and break covalent bonds thus leading to radical formation. These radicals can subsequently react with other species in the plasma to form functional groups. In this way depending on the selected plasma gas, different functional groups such as carbonyl, carboxylic acid, hydroxyl, and amine functional groups can be added on the surface thus making it more hydrophilic [23–25]. It is believed that radical species rather than ions or electrons are most important in this type of modification [26].

2.2. Plasma polymerization

Observations of organic compounds formed in a hydrocarbon based plasma discharge dates back to 1874 [27]. These deposits were considered to be undesirable by-products. However, in the 1960s [28–30], studies of plasma polymerization started and were completed by considerable advances in polymer science [31]. Plasma polymerization was defined as "the formation of polymeric materials under the influence of plasma" [31]. Nevertheless, the real potential of plasma polymerization was not uncovered until only the past two decades. Nowadays, plasma polymerization is known as a very valuable surface modification technique.

During the process of plasma polymerization, high energy electrons as well as UV will ionize the precursor molecules [32–34]; this leads to radicals which are highly unstable and reactive species that will interact and bond with one another and deposit on the substrate thus forming a coating on its surface. Plasma will also lead to bond breaks on the substrate surface thus creating radicals. These will interact with the precursor's radicals acting as anchor sites which enhances the plasma polymerized coating stability.

During plasma polymerization, two processes occur simultaneously: - ablation (removal of surface molecules) and – polymerization (surface monomer deposition). These two processes are in competition and their interaction and co-existence in plasma is well known [35].

Plasma polymerization is very complex and versatile since various parameters such as discharge power, treatment time, precursor type and concentration can affect the physic-chemical characteristics of the deposited coating. Moreover, different reactive species can be formed in the plasma depending on the used dilution gas (e.g., helium, argon, air or nitrogen), which also affects the characteristics of the coating [25, 36–38].

Advantages of plasma polymerization include the following:

1) Ultra-thin film formation
2) Good adhesion to the substrate material and deposition is independent on the structure or type of the substrate
3) Relatively good chemical stability and physical durability of the coatings
4) Various precursors can be chosen which leads to a vast array of surface functionalization (monomers used do not have to contain a double bond for the polymerization to proceed)
5) Many process parameters can be used thus providing great diversity of surface modifications
6) The obtained coatings are more or less uniform

Nevertheless, plasma polymerization also presents several disadvantages:

1) System dependency
2) Scaling up and converting it into a continuous process could present some technical challenges
3) The specific roles of each plasma component are difficult to separate and analyze
4) It is hard to predict the exact surface characteristics of the deposited plasma polymer especially when complex molecules are used
5) Coating multi-functionality can also be an issue
6) Everything in the coating range of the plasma can become part of the coating

However, despite its disadvantages and focusing on its numerous advantages, plasma polymerization has rapidly developed during the past decades and is now used for various applications.

Plasma polymers

Plasma polymers are markedly different from conventional polymers. Conventional polymers have a well-defined structure of repeating units that corresponds to the used monomer. Whereas, plasma polymers are crosslinked, randomly structured deposits obtained from the fragmentation and recombination of monomers within an electric discharge.

During plasma polymerization, the active species fragments the organic precursor (monomer), thus creating radicals that can recombine both in the plasma and on the substrate surface forming a crosslinked so-called plasma polymer coating/film on the substrate surface.

As to the film chemical structure, during this process, partial loss of functional groups occurs (fragmentation) in a system/process dependent way. Moreover, not all radicals will react and some will be trapped in the plasma polymer network [41]. As a consequence, the elemental composition of plasma polymers differs from that of conventional polymers prepared from the same monomer. For example, the elemental composition of conventionally polymerized polyethylene $(C_2H_4)_n$ is equal to that of the monomer (C_2H_4); however, in plasma polymerized ethylene the hydrogen concentration is lower (radical formation by -H bond scission) and oxygen is incorporated in the plasma polymer (by reaction with the formed radicals).

Hence, the material obtained from plasma polymerization is very different than that obtained by conventional polymerization of the same monomer [39].

2.3. Post-irradiation grafting

Surface modification via polymer coatings is also frequently done by surface grafting methods which are often referred to as "plasma-induced graft (co)polymerization." This is a two-step process. In the first step, the surface is exposed to air plasma or subjected to an ozone treatment which creates peroxide groups at the surface. Other non-oxygen containing plasma can also be used (e.g., Ar or He plasma) followed by atmosphere or O_2 exposition; created radicals will then form peroxides and hydroperoxides. In the second step, the formed functionalities are used to initiate a polymerization reaction by contact with the monomer molecules. Each functionality being a potential initiating site, the number of created (hydro)peroxides has a significant effect on the surface grafting density.

3. Cellular response to surfaces

For the cells, the surface is the most important part of the material. Cell-biomaterial interactions depend on the surface energy, chemical composition and surface morphology [40, 41]. Moreover, cell growth, spreading and viability were shown to be closely linked to their adhesion on the surface [42]. Consequently, suitable surface properties contribute to better cell adhesion and subsequent proliferation. It is well established that for numerous cell types surface wettability is a paramount factor that influences cell adhesion, with this being more favorable on hydrophilic surfaces compared to hydrophobic surfaces. **Figure 1** shows micrographs of fibroblast cells cultured on untreated (hydrophobic) and argon plasma treated (hydrophilic) UHMWPE substrate. As seen in this **Figure 1**, cells are significantly more spread on the hydrophilic treated surface compared to the hydrophobic untreated one [43]. Additionally, surface charge has also been shown to have a significant influence on cell adhesion [44, 45].

A promising way to achieve optimal surface attributes (e.g., surface wettability and charge) is plasma polymerization which has already been used successfully to enhance cell adhesion on various substrates.

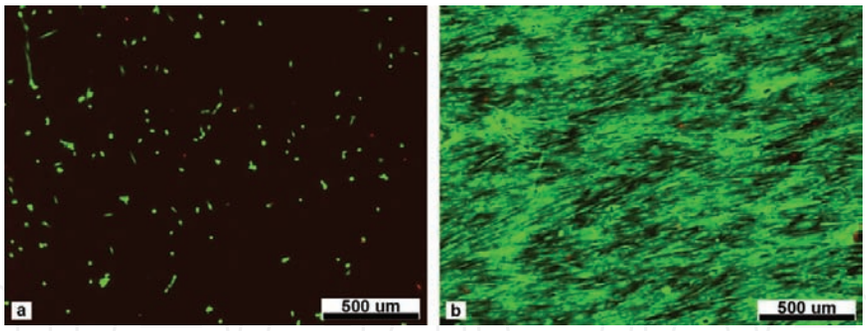

Figure 1. Overall cell morphology of fibroblasts cultured on (a) untreated and (b) argon plasma-treated UHMWPE.

In this section, previous works on the bio-application of plasma polymers and their interactions with cells will be reviewed.

Biological applications of plasma polymers

Plasma polymerization is a convenient way to introduce desired functional groups on a surface. Plasma polymers are frequently used to immobilize biomolecules and enhance cell adhesion. NH_2 and COOH based plasma polymers are most commonly used since these groups are known for their good chemical reactivity. Moreover, in aqueous solution at physiological pH value, amino/carboxyl groups can introduce a positive/negative charge to the surface thus increasing its affinity for biological components [46–48]. For example, DNA [49, 50], heparin [46], glucose oxidase [51], and collagen [52] have been immobilized on amine or carboxyl based plasma polymers. Hydroxyl and aldehyde groups have also been used to bind heparin [53] and collagen/albumin [54], respectively. However, plasma polymers with these groups are less extensively investigated due to their lower reactivity.

For the effect of plasma polymers on cell attachment and proliferation, various studies on different substrates using numerous plasma media and cell types have been performed. A summary of some of these studies is presented in **Table 1**.

Furthermore, plasma polymer films were used for bacterial adhesion and biofilm prevention by coating the surface with a suitable antibacterial agent (e.g., silver nanoparticle).

Xiaolong et al. [55] produced PET fabrics with antibacterial properties by depositing a plasma polymer organosilicon film where silver nanoparticles were incorporated. A similar study was also conducted on PET meshes by plasma polymerization of acrylic acid followed by incorporation of Ag nanoparticles [56]. Results showed excellent mesh antibacterial properties with a decrease of more than 99.7% in bacterial concentration compared to an untreated mesh. In another study, Degoutin et al. [57] used plasma to graft acrylic acid onto nonwoven polypropylene and the carboxyl groups were used to immobilize an antibiotic "gentamicin." Results showed a 99% efficacy against *E. coli* bacteria.

These results and discussions strongly support the idea that polymer coatings represent a very promising way to modify a biomaterial surface in order to adapt it to a specific biomedical application.

Substrates	Plasma media	Cell lines	Observations	Refs
Si, PS, PET	Acrylic acid	Fibroblast	Enhanced fibroblast adhesion	[48]
PET, TCPS	Acrylic acid	3 T3 murine	Improved cell adhesion	[58]
PET	Acrylic acid	Smooth muscle cells	Immobilization of proteins and cell growth	[52]
Glass coverslips, PLGA	Acrylic acid	Caco-2	Mammalian cell sheet formation	[59]
PET	C_2F_4	3 T3 fibroblast	Cell adhesion, growth and proliferation	[60]
PET	C_2F_4	NCTC 2544, 3 T3 fibroblast and MG-63	Good cell adhesion and proliferation	[61]
Glass	Acrylic acid	Leukemia cells	Lower cell growth (60% reduction)	[62]
PCL, PLA	Allylamine, C_2H_4/N_2	Osteoblast, 3 T3 fibroblast	Increased cell metabolic activity and improved cell colonization in the core region of the scaffold	[63]
Titanium alloy	Allylamine and ethylene diamine	MG63	Improved cell adhesion, function and spreading	[64]
LUX tissue culture dishes	Acrylic acid	Rat osteosacroma cells	Improved cell adhesion	[65]
PET	Acrylic acid and allylamine	Human neuroblastoma cells	Improved cell adhesion, differentiation and maturation	[66]
PEEK	Acrylic acid	MC3T3-E1	Improved cell adhesion, spreading and proliferation	[67]
PET, Si	C_2F_4	CVEC	Enhanced endothelial cell response; increased cell attachment, spreading and viability	[68]
PCL, PLLA	O_2, acrylic acid, allylamine	MC3T3-E1	Improved protein adsorption and cell attachment	[69]
Ti	Allylamine	MG63	Improved adhesion and cell functions	[45]
PET	Allylamine	Human skin fibroblast	Improved cell attachment, viability and metabolic activity	[70]
PS	Isopropyl alcohol	Fibroblast	Enhanced cell attachment and proliferation	[71]

Table 1. Summary of some of the studies on plasma surface modification of materials and their effect on cell adhesion and growth.

4. Effect of aqueous environments on plasma polymers

For biomedical applications, the effect of water on the plasma polymer films is of particular importance.

Immersed in a solvent, plasma polymers can be subject to numerous processes such as:

- delamination from the substrate
- detachment of oligomers
- swelling
- reaction with the solvent

However, not many studies focus on the physic-chemical changes that happen to the plasma polymer films after exposure to aqueous environments.

Plasma polymer stability behavior depends on the type of the polymer. Muir et al. [72] studied the penetration of water into the films and characterized the swelling of allylamine (Aam) and heptylamine (HA) plasma polymers. When immersed in water, the plasma polymerized Aam film (ppAam) was found to swell by 5% and to contain 3% water whereas the ppHA film did not appear to swell but contained 5% water. The swelling characteristics of other plasma polymers have also been reported [73–75].

Moreover, the degree of swelling strongly depends on the plasma process parameters. Zhang et al. [73], demonstrated that ppAam deposited at 20 W only shows a small degree of swelling while ppAam deposited at 5 W shows a large degree of swelling. This is due to the fact that at low powers the plasma polymer contains a large number of oligomers which are not covalently bound to the film; these oligomers can thus be readily extracted in the solvent. In fact, when studying the morphology of ppHA, Vasilev et al. [76] found that pores of several nanometers in diameter were formed after ppHA has been immersed in water for 24 h (see **Figure 2**). And the dimension of the pores was found to depend on the deposition conditions with larger pores obtained at lower powers (see **Figure 3**). This was attributed to oligomer water extraction after low molecular weight fragments were detected in the water. This results in the formation of gaps in the film and leads to ruptures of the polymer chains thus forming the observed porosity.

Förch et al. [77] found that for ppAam, the roughness of the polymer film increased from 0.85 to 1.26 nm after soaking in water which was attributed to the swelling of the film in water;

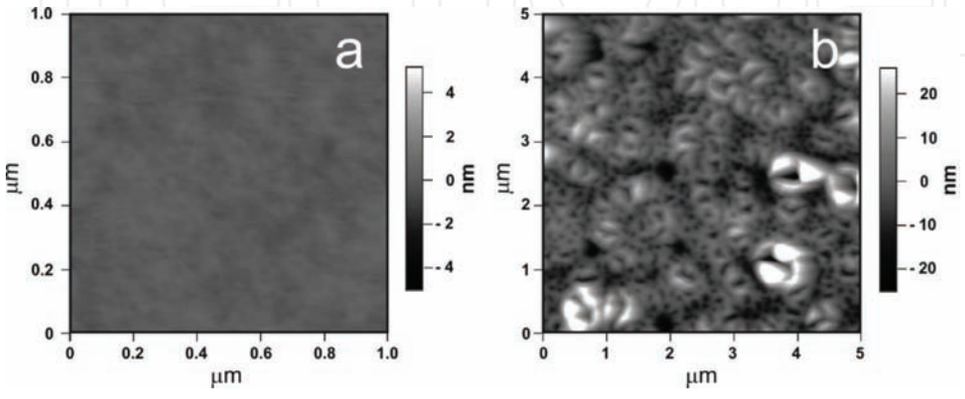

Figure 2. AFM topographic images of HA plasma polymer films deposited with a power of 20 W: (a) as deposited, (b) after immersion in water for 24 h.

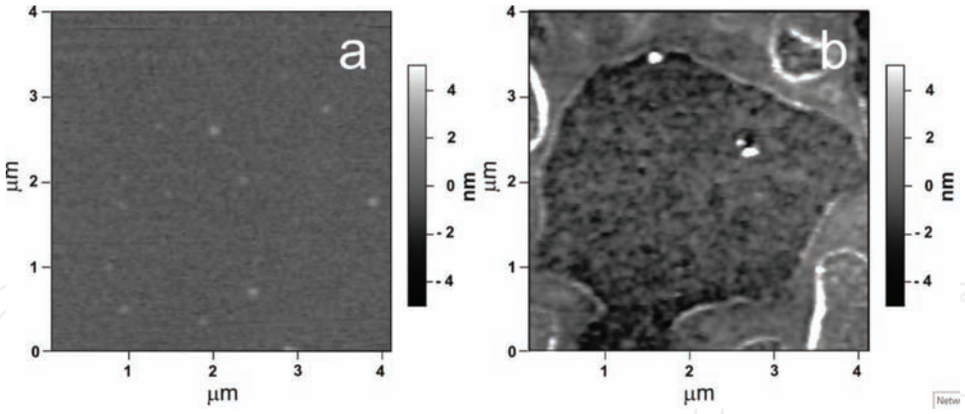

Figure 3. AFM images of HA plasma polymer films deposited with a power of 50 W: (a) as deposited or (b) after immersion in water for 24 h.

whereas, Tarasova et al. [78] used XPS to study the changes in surface chemistry of ppAam and ppHA after immersion in water for up to 24 h. Results were similar to the ones obtained after these plasma polymers were stored in air, both undergoing rapid oxidation; amine and imine groups were converted to amides with an increase of C—O and C═O groups.

In order to improve plasma polymers stability, studies on the interaction of plasma polymer with the aqueous environment as a function of plasma deposition parameters have been conducted. Optimizing these parameters was shown to be very important in reducing the induced changes [77, 79]. Moreover, substrate pretreatment for cleaning or activation was also shown to prevent the delamination of the polymer film [77].

However, enhancement of plasma polymer stability is still insufficiently studied and more effort is still needed for a precise stability evaluation and quantification.

5. Surface aging

It is widely known that the enhancement in surface wettability obtained after plasma activation processes changes with storage time. This phenomenon is referred to as aging or hydrophobic recovery and is due to the tendency of a surface to minimize its surface energy by reverting to its original structure. This leads to a loss of surface polar functional groups that re-orientate to the bulk [18]. Therefore, in the case of plasma activation, in order to avoid the adverse effect of aging, it is advisable to only use freshly prepared samples.

On the other hand, plasma surface grafted polymers and plasma polymerized films show much less modifications after storage in ambient air and are thus considered comparatively stable in time. However, research on plasma polymers show that they are susceptible to oxidation upon storage in air [31]. Since these coatings have shown great potential for many applications including biomedical ones, several studies have been done to better understand this so-called aging process and therefore further evaluate the relevancy of plasma polymers. And since most products are usually stored for a certain period before they are used, the film properties at the time of use are usually considered more important than immediately after treatment.

Major advancements in the understanding of oxidative reaction mechanisms that occur during plasma polymer aging have been made by Gengenbach et al. [80–83].

This was done using XPS, FTIR spectroscopy and contact angle goniometry characterization techniques which allowed significant perception of the eventual surface compositional changes.

Their studies included detailed oxidation investigations of hydrocarbon based plasma polymers [80], fluorocarbon coatings [81], nitrogen coatings [82] and other plasma deposited films [83]. Results showed that the aging process was due to the reaction of ambient oxygen with the residual radicals present in plasma polymers; ESR spectroscopy showed that the free radicals detected in freshly deposited plasma films slowly disappear upon storage in air. Results also showed that, the kinetics, mechanisms and formed oxidative products during aging depend on many factors, such as the structure of the film, the type of functional groups and the mobility of the surface.

6. Plasma polymer gradients

After accomplishing significant advancements in the biofunctionalization of surfaces by different chemical and physical homogeneous modifications, a growing research interest is being shifted toward the development of gradient surfaces presenting graded wettability, chemistry, biomolecule density and nanoparticle distribution [84]. This interest stems from the fact that many essential and poorly understood biological activities are driven by such gradients. For instance, chemotaxis mediate a number of physiological processes such as leukocyte recruitment to the infection site, guiding of neuronal and glial cells during nervous system development or regeneration and cancer metastasis. Moreover well-ordered gradient distribution of specific functional groups, extracellular matrix components, signaling biomolecules and even topographical cues induce particular cell type proliferation, migration and differentiation [85–87]. Besides their biological importance, gradients are also powerful for high throughput screening in several applications such as biomaterial development, tissue engineering and sensors, in the sense that a single sample designed with a gradient surface is used to procure multiple data points. This reduces dramatically the number of samples and cells, eliminates inaccuracies triggered by sample reproducibility and speeds up the analysis [84, 88, 89].

Different approaches are commonly adopted to create surface gradients such as self-assembled monolayers (SAMs), grafting on hydrogels and Boyden chambers and filters. Several limitations are associated with these traditional methods including the substrate dependency (e.g., gold-coated surface is required for SAMs), the short term gradient "shelf-life," the restricted chemistries that can be obtained and the long experimental timing [46, 90, 91]. As an alternative, applying high energy source plasmas that are associated with many advantages such as the absence of solvent, the specificity and the substrate independency has shown great successes in the generation of gradient surfaces. In 1989, Witt el al. were one of the first groups if not the first, to generate wettability gradients on polyethylene, polystyrene, polydimethylsiloxane, and polytetrafluoroethylene by a radio frequency (RF) plasma activation operating in oxygen, ammonia and sulfur dioxide atmospheres. A special gradient apparatus consisting of an aluminum box

with a translating cover and two aluminum plates serving as electrodes, was designed for this purpose. During the treatment, the cover is retracted with a constant velocity automatically controlled by a microprocessor driving the stepping motor. This movement linearly increases the plasma exposure time over the sample length. As a result, water contact angles (WCA) increased along the length of the sample thus ensuring the presence of a wettability gradient. Moreover, a wide range of wettability gradients is obtained by varying the gas, the radio frequency power, the cover retraction velocity and the plasma exposure time. This study highlighted the high flexibility of the plasma treatment to generate gradients with defined length and magnitude and pointed out, by using several substrates, that the process is substrate independent [92]. Therefore, a steep rise in literature focusing on the generation of gradients by plasma activation followed. However, the different plasma activation methods that were described are mainly limited to the production of wettability gradients with a relatively restricted control over the chemical group specific incorporation. Other concerns include the aging effect of the treated surfaces due to the reorientation of the incorporated groups away from the surface when the environment is thermodynamically unfavorable and the roughening of the surface due to the plasma etching effect [92, 93]. Consequently, the interest was shifted toward the generation of polymer gradients via plasma polymerization to be able to control more precisely the functional group nature and densities, the gradient stability and the gradient shape. Nevertheless, it was until 2003 that the first method enabling the deposition of controllable horizontal plasma chemical gradients was described by Whittle et al. [93] and was subsequently adopted as it is or with some amendments by several other groups [91, 94]. In their study, Whittle et al. created hydrocarbon/carboxyl and amine/carboxyl functionality gradients on glass substrates over a distance of 11 mm. Instead of using the traditional cylindrical plasma reactor, a RF glow discharge T-shaped reactor presenting a drawer as sample holder was used [93, 95]. As a first step, an amine coating was deposited on the whole glass substrate by performing a plasma polymerization using allylamine (Aam) monomers as precursors and a continuous power of 10 W while the drawer was fully extended. An underlayer presenting a good adhesion was thus formed for the subsequent gradient deposition. In the second step, the power was decreased to 5 W and a plasma polymerization was performed while the drawer was slowly closed at a constant velocity of 1 mm/min along with a controlled change in the plasma composition over time. This was performed by introducing acrylic acid (Aac) as the second monomer while decreasing instantaneously the flow rate of Aam by 4 cm^3 stp/min. For the deposition of hydrocarbon/carboxyl gradients, the same procedure was followed but with the use of octa-1,7-diene instead of Aam precursors. The obtained plasma polymerized surfaces were characterized by X-ray photoelectron spectroscopy (XPS) and chemical derivatization of acid functionalities using trifluoroethanol. A gradual increase in the concentration of acid functionalities was observed in the case of hydrocarbon/carboxyl gradients and an increase of acid and amine functionalities was attained in opposite directions in the case of the amine/carboxyl gradients. These findings demonstrated the power of this first-hand methodology to successfully generate plasma polymer gradients that can subsequently allow the grafting of a broad range of biochemical entities in a spatially structured manner [93]. Surface engineers waited around 3 years after the study of Whittle to begin their investigations regarding the grafting of biomolecules and the cell-biomaterial interactions when a plasma polymer gradient is implicated. Moreover, several other methods generating plasma polymer gradients were

described with a distinctive focus on amine and carboxylic acid being the most 2 extensively considered functionalities in the subsequent literature of gradient plasma polymerization. In what follows, an overview on the achievements of these carboxylic acid and amine plasma gradients in several tissue engineering and biomedical applications will be given.

6.1. Surface plasma polymer gradient of carboxylic acid functionalities

In 2006, Parry et al. [91] performed a plasma copolymerization of Aac and octadiene (OD) based on the mechanism described by Whittle et al. [93] but with a modification of the setup in a way allowing the production of 20 similar gradients at a time. Up to 20 substrates could thus be placed in the redesigned RF plasma reactor and moved under a slot by an automated stepper motor in 250 μm paces at a rate of 750 μm/min. Simultaneously, a controlled composition of the monomer mixture is sent to the chamber via two computer-regulated valves. A thorough characterization of the surface gradient was executed by angle resolved x-ray photoelectron spectroscopy (ARXPS) that showed in great details how acid functionalities changed on different positions of the gradient and highlighted the presence of vertical changes especially when it comes to the plasma polymer thickness. An assay investigating the passive adsorption of immunoglobulin G (IgG) as a function of the acid surface density was supplemented to the study to be, to the best of our knowledge, the first reported biological assay done on plasma polymer gradients. ARXPS measurements showed that IgG was by far more absorbed on the OD gradient end and that IgG amount decreased gradually as the concentration of Aac increased thus creating an IgG gradient [91]. In 2009, Walker et al. [96] also deposited a gradient of OD/Aac on coverslips using the plasma deposition/masking method of Whittle but this time with a renovated protocol permitting the generation of submillimeter-scale gradients instead of millimeter scale length. In the updated method, OD was constantly fed to the reactor as the slot moves across the substrate surface, then it was brusquely turned off and a pulse of Aac was launched. The scale length and density of the carboxylic groups were thus tailored by varying the pulse width of Aac. The obtained gradient surface was used to immobilize the intercellular signaling molecule delta-like-1 Dll 1, a factor enhancing stem cells self-renewal and preventing cell differentiation which is an issue to be considered when developing cell therapy technologies. Since tiny changes in surface properties can considerably affect the stem cell behavior either by enhancing the commitment path toward their differentiation to particular cell types or by maintaining and stabilizing the stem cell pluripotent phenotype, concentration-based factor and chemical group gradients are highly expedient to study stem cells. Instead of directly grafting Dll 1 factor on the generated gradient, a mouse monoclonal antimyc-tag (9E10) antibody is covalently coupled, then Dll 1 is immobilized on the antibodies thus avoiding the alteration of its biological activity by separating it from the solid surface. A visualization of the Dll 1 gradients was performed by binding a rabbit anti-Dll-1 antibody and then introducing a colloidal gold-conjugated secondary antibodies. Several Dll 1 gradients with different slopes and end points were obtained depending on the plasma Aac pulse width adopted during the plasma polymerization (**Figure 4**). During the same year, the first cell tests on plasma gradients were performed by Wells et al. using mouse embryonic stem (ESC) cell lines E14 and R1 in order to examine their pluripotency [97]. OD/Aac gradients were deposited on coverslips using the same setup described by Parry et al. [91]. The degree of cell spreading was studied in function of COOH concentration. Alkaline

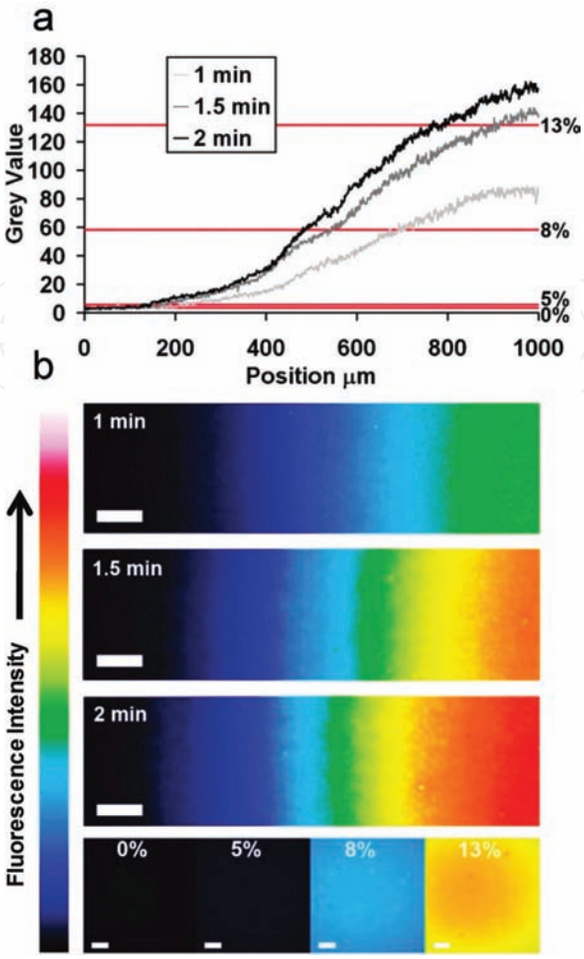

Figure 4. (a) Densitometry results of 9E10 antibodies immobilized on the gradient surface (3 different Aac pulse durations) and visualized by FITC-conjugated secondary antibodies. Horizontal lines show the results of homogeneous surface treatments (b) false color heat maps of the 9E10 antibody gradients. Homogeneous surfaces are presented for comparison. Scale bars: 100 μm.

phosphatase staining showed that cell capacity of self-renewal is preserved when the cell spreading is still below 120 μm^2 [97]. In 2012, in an attempt aiming to make a sweeping statement about this result, Harding et al. [98] used polyethylene oxide (PEO) that is well-known in the biomaterials field to limit protein adsorption and thus cell adhesion and spreading, together with Aac to produce two counter gradients. A RF apparatus consisting of a cylindrical glass chamber was used for the plasma copolymerization. As a first step, an OD layer then an Aac layer were deposited on the substrate since a unique Aac deposition resulted in the coating dissolution in water. Then a mask 12 ˚titled in respect to the surface was employed to deposit a PEO-like gradient by using the monomer diethylene glycol dimethyl ether (DG) as a precursor. A successful fabrication of AA-DG plasma polymer gradient was revealed by XPS, profilometry and infrared microscopy mapping. The gradient could be easily altered by changing the plasma process parameters. Mouse ESC were cultured on the gradient surfaces, then immunocytochemical stainings of the stem cell markers Oct4 and alkaline phosphatase were performed. Results showed a low cell adhesion and colony formation on the DG rich end and an increased colony size and decreased stem cell marker expression on the COOH rich end, thus supporting the hypothesis stating that cellular spreading influences

the fate toward cell differentiation or self-renewal. The same method using a tilted mask was then applied by Wang et al. [99] in 2014 to create the same Aac-DG gradients but also Aac-OD gradients by firstly depositing OD uniformly then using the tilted mask to deposit Aac. Attachment and differentiation of rat bone marrow mesenchymal stem cells (rBMSCs) into adipogenic and osteogenic lineages were investigated on both gradients. After 24 h of cell culture, a gradient in cell density was observed on the substrate with a decreased cell adhesion on DG and OD rich ends. The obtained cell density gradient vanished on Aac-OD gradient after 6 days but not on Aac-Dg gradient, thus suggesting the long-term efficacy of the later gradient. Cell colonies containing bone nodules were detected on this gradient especially on the Aac rich ends but not on the DG rich end. Moreover, proteins and calcium were not secreted on the DG end implying that osteogenic differentiation is influenced by local cell densities. However, the induction of the cells toward an adipogenic lineage showed that this differentiation is cell density insensitive.

6.2. Surface plasma polymer gradient of amine functionalities

In addition to COOH functionalities, NH_2 groups were also shown to be very powerful in influencing a wide range of particular cell type performances such as adhesion, proliferation, migration and differentiation. Therefore, when the research community started investigating surface gradients, a distinctive focus was directed toward the production of amine gradients and their use in several biomaterial and tissue engineering applications [100]. To the best of our knowledge, all COOH plasma polymer gradients described so far were only deposited on flat substrates, however some amine plasma polymer gradients were deposited on 3D scaffolds. For instance, in 2006 Barry et al. [101] thought of generating an amine gradient on poly(D,L-lactic acid) 3D porous scaffolds in order to solve the common problem of the highly disproportionate cell colonization on the scaffold periphery in comparison to the hardly accessible scaffold center that remains poorly colonized and supplied by nutrients. This issue was solved by plasma polymerizing hexane, known to be resistant to cellular adhesion, on the periphery of the scaffold while generating an amine plasma polymer coating on the central surface. To do so, a first plasma polymerization step using Aam monomers as precursors was performed, then a second polymerization using the cell-repellent hexane was achieved at lower deposition rate. XPS measurements throughout the whole scaffold showed that when the second hexane polymerization step is absent, a decrease in amine functionalities is observed toward the center. However, when hexane polymerization is introduced, the nitrogen concentration is reduced by 1 to 2% in the periphery thus creating a reversed gradient. After seeding 3 T3 fibroblasts on the treated scaffolds, X-ray micro-computed tomography and scanning electron microscopy revealed a uniform cell distribution throughout the whole scaffold with well spread cells in the center associated with a high production of extracellular matrix (ECM) components. The use of hexane and Aam to create amine gradients was also considered by Zelzer et al. [102] in the subsequent year, but this time on flat glass coverslips. The idea behind the study was to compare between mammalian cell interactions on gradient and on uniformly treated surfaces. A T-shaped borosilicate RF reactor was used to plasma polymerize uniformly an amine coating on glass coverslip using Aam as precursors. Afterward, a poly-hexane was deposited on the poly-Aam coated surface after placing a mask either directly or making

use of a spacer clamping the mask at a distance of 0.04 mm from the surface. The direct positioning of the mask resulted in steep gradients while the use of a spacer gave more shallow gradients. Wettability gradients were detected by WCA measurements showing a gradual decrease from 93° to 66°, thus correlating with the gradual increase of N/C ratio. NIH 3 T3 fibroblasts cultured on the gradients surfaces were preferentially adhered and proliferated on the N-rich end with a gradual cell density decrease toward the poly-hexane rich end. Surprisingly, experiments performed on uniform surfaces revealed significant differences in cellular behavior compared to the gradient surfaces, leaving question marks on the use of gradients for high throughput screening. The cell signaling and the protein synthesis might be different between gradient and uniform surfaces since the cell neighboring environment differs. Several subsequent studies involving amine plasma polymer gradients and their general results are summarized in **Table 2**.

Authors (year)	Plasma reactor/ monomers used	Gradient formation method	Surface chemical properties	Biological assay/ cell type	Bioresponsive properties
Robinson et al. [46]	Cylindrical RF reactor/ Aam- OD	Moving slot with a simultaneous change in the monomer mixture composition	Gradual increase in N/C ratio over a distance of 14 mm	Adsorption of heparin that mimics the heparan sulfate proteoglycans found in all tissue types	-Gradual increase in heparin adsorption parallel to the increase in N/C ratio. -Heparin functionality not correlated with the continuous increase in heparin adsorption
Robinson et al. [103]	T-shaped RF reactor/ Aam- OD	Moving slot with a simultaneous change in the monomer mixture composition	Gradual increase in N/C ratio on washed and unwashed samples highlighting the stability of plasma polymer gradient surfaces	——	——
Harding et al. [87]	T-shaped RF reactor/ Aam- OD	Moving slot with a simultaneous change in the monomer mixture composition	Gradual increase in N/C ratio over a distance of 12 mm	D3 murine embryonic stem cell line culture	-Maximum cell adhesion on the N-rich end -Inverse increase in stem cell marker expression toward the lower N/C ratio. -Correlation between the presence of stem cell markers and the formation of more multilayered and compact cell colonies.

Authors (year)	Plasma reactor/ monomers used	Gradient formation method	Surface chemical properties	Biological assay/ cell type	Bioresponsive properties
Mangindaan et al. [100]	RF reactor/ Aam	Use of a mask with a 1 mm gap on a polypropylene substrate	-Wettability gradient with WCA varying from 15° to 90°. - Gradient over 1 cm of nitrogen content from 5.8 to 16.0% and amine content from 1.98 to 4.03 per 100 carbons.	L-929 fibroblast culture	Continuous increase in the cellular density with more than 2-fold density on N-rich end
Delalat et al. [104]	RF reactor/ Aam-OD	Moving slot with a simultaneous change in the monomer mixture composition	Gradient over 12 mm of nitrogen content from 0 to 12.0%	Mouse embryoid body cell culture	-Highest cell adhesion on the gradient central regions -Increased cell proliferation toward the Aam end. -Cell differentiation toward mesodermic and ectodermic lineages on high nitrogen content regions -No correlation between amine content and endodermal differentiation
Liu et al. [105]	RF reactor/ Aam-OD	Moving slot with a simultaneous change in the monomer mixture composition	-Wettability gradient with WCAs varying from 90° to 70° -Gradual increase in N/C ratio over a distance of 12 mm - Unchanged surface topography	-Adsorption of fluorescein isothiocyanate-labeled bovine serum albumin (BSA) and rhodamine-labeled fibronectin (FN) -Human adipose-derived stem cell culture	-Gradual decrease in the amount of adsorbed BSA from OD toward Aam sides. -Gradual increase in the amount of adsorbed FN from the OD toward the Aam sides. -Increased cell adhesion and spreading toward the Aam side -No difference in cell performances in the absence of serum -Increased osteogenic cell differentiation toward the Aam side -Decreased adipogenic differentiation toward the Aam side.

Table 2. Overview of literature on amine gradient obtained by plasma polymerization and not discussed in the text.

http://dx.doi.org/10.5772/intechopen.72293

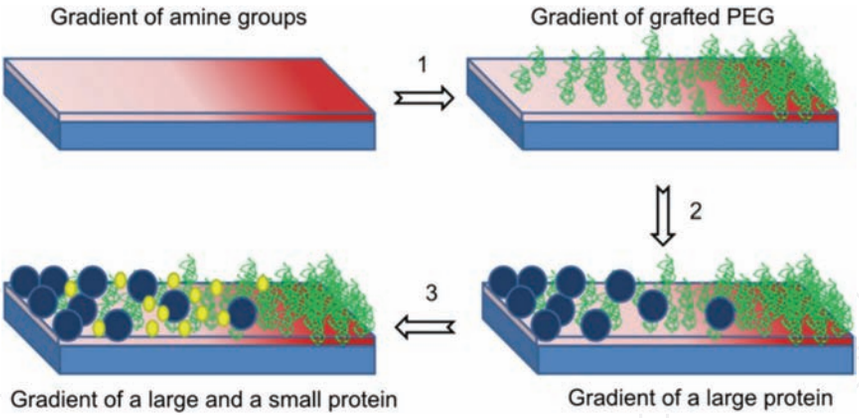

Figure 5. Schematic representation of the creation of two-protein gradient. Step 1. PEG grafting on the amine plasma polymer gradient to generate a PEG density gradient. Step 2. Large proteins adsorption. Step 3. Small protein adsorption.

Since the biological systems in vivo are much more complex than in vitro assays, some authors considered a closer mimicking of the real systems by designing, instead of one dimensional or single protein gradients, 2 protein and 2 dimensional gradients. For instance, in 2009 Vasilev et al. [94] created an Aam-OD gradients on SPRchips or on silicon wafers based on the method described by Whittle et al. [93]. Afterwards, polyethylene glycol (PG), known to be resistant to protein adsorption, was grafted on the amine gradient thus generating a PEG density gradient. The obtained density gradient was then benefited to control the deposition of 2 proteins, namely the large protein fibrinogen and the small protein lysozyme, by differential passive adsorption. A first incubation with the larger protein led to its adsorption on low PEG density regions, then a second incubation with the small lysozyme led to its adsorption only where there is still a "room" for it to adsorb since the previous fibrinogen adsorption passivated gradually the surface. As a result, 2 reversed gradients of 2 proteins could be designed and the method could be generalized to other pairs of small and large proteins (**Figure 5**). In 2013, Mangindaan et al. [90] designed a 2 dimensional amine gradient by performing firstly a plasma polymerization of Aam on a propylene membrane while a mask is placed on top with a gap distance of 1 mm. Subsequently, the same procedure is repeated but after rotating the sample by 90°. WCA measurements showed that both gradients were well controlled by varying the plasma treatment exposure time in each step. L-929 fibroblasts seeded on the treated surfaces adhered and grew proportionally with the amine content on the 2 dimensional gradient with a predominant effect of the gradient created during the initial plasma deposition.

7. Conclusions

From the work presented in this chapter it is clear that plasma polymer coatings are very useful tools for biomaterial surface modification. However, despite the numerous advantages of these coatings for biomaterial advancements, their aging and stability remain an issue that requires further investigations and considerations. More attention and focus on these aspects can make

plasma polymerization become one of the most used and important surface modification techniques. Plasma polymer gradients are also very promising for biological applications and many advances in the area of plasma uses can be made by developing such coatings.

Acknowledgements

This research has received funding from the European Research Council (ERC) under the European Union's Seventh Framework Program (FP/2007-2013)/ERC Grant Agreement n. 335929 (PLASMATS).

Author details

Gaelle Aziz*, Rouba Ghobeira, Rino Morent and Nathalie De Geyter

*Address all correspondence to: gaelle.aziz@ugent.be

Department of Applied Physics, Research Unit Plasma Technology (RUPT), Faculty of Engineering & Architecture, Ghent University, Ghent, Belgium

References

[1] Nair LS, Bhattacharyya S, Laurencin CT. Nanotechnology and tissue engineering: The scaffold based approach Nanotechnologies for the Life Sciences. 2006;**1**:4-23

[2] Langer R, Vacanti JP. Tissue engineering. Science. 1993;**260**(5110):920-926

[3] Gupta AK, Gupta M. Synthesis and surface engineering of iron oxide nanoparticles for biomedical applications. Biomaterials. 2005;**26**(18):3995-4021

[4] Jayagopal A, Stone GP, Haselton FR. Light-guided surface engineering for biomedical applications. Bioconjugate Chemistry. 2008;**19**(3):792-796

[5] Abenojar J, Torregrosa-Coque R, Martínez MA, Martín-Martínez JM. Surface modifications of polycarbonate (PC) and acrylonitrile butadiene styrene (ABS) copolymer by treatment with atmospheric plasma. Surface and Coatings Technology. 2009;**203**(16):2173-2180

[6] Kaczmarek H, Kowalonek J, Szalla A, Sionkowska A. Surface modification of thin polymeric films by air-plasma or UV-irradiation. Surface Science. 2002;**507**:883-888

[7] Arenholz E, Svorcik V, Kefer T, Heitz J, Bäuerle D. Structure formation in UV-laser ablated poly-ethylene-terephthalate(PET).AppliedPhysicsA:MaterialsScience&Processing.1991; **53**(4):330-331

[8] Habibi Y. Key advances in the chemical modification of nanocelluloses. Chemical Society Reviews. 2014;**43**(5):1519-1542

[9] Xue C-H, Li Y-R, Zhang P, Ma J-Z, Jia S-T. Washable and wear-resistant superhydrophobic surfaces with self-cleaning property by chemical etching of fibers and hydrophobization. ACS Applied Materials & Interfaces. 2014;**6**(13):10153-10161

[10] Shi X, Xu L, Le TB, Zhou G, Zheng C, Tsuru K, et al. Partial oxidation of TiN coating by hydrothermal treatment and ozone treatment to improve its osteoconductivity. Materials Science and Engineering: C. 2016;**59**:542-548

[11] Puleo D, Kissling R, Sheu M-S. A technique to immobilize bioactive proteins, including bone morphogenetic protein-4 (BMP-4), on titanium alloy. Biomaterials. 2002;**23**(9):2079-2087

[12] Biederman H. Plasma Polymer Films. London: World Scientific; 2004

[13] Gogolewski S, Mainil-Varlet P, Dillon J. Sterility, mechanical properties, and molecular stability of polylactide internal-fixation devices treated with low-temperature plasmas. Journal of Biomedical Materials Research Part A. 1996;**32**(2):227-235

[14] Chen TF, Siow KS, Ng PY, Majlis BY. Enhancing the biocompatibility of the polyurethane methacrylate and off-stoichiometry thiol-ene polymers by argon and nitrogen plasma treatment. Materials Science and Engineering: C. 2017;**79**:613-621

[15] Wolf RA. Atmospheric Pressure Plasma for Surface Modification. Hoboken, New Jersey, United States: John Wiley & Sons; 2012

[16] Christophorou LG, Olthoff JK. Fundamental Electron Interactions with Plasma Processing Gase. Berlin, Germany: Springer Science & Business Media; 2012

[17] PL_INTL. Plasmas international. Perspectives on plasmas. 2004. Available at www.plasmas.org/what-are-plasmas.htm [Accessed on 2 August 2017]

[18] De Geyter N. Plasma Modification of Polymer Surfaces in the Subatmospheric Pressure Range; 2007-2008

[19] Yasuda HK. Fundamental aspects of ionized gas. In: Plasma Polymerization. Cambridge, Massachusetts, United States: Academic Press; 2012

[20] Alves CM, Yang Y, Carnes D, Ong J, Sylvia V, Dean D, et al. Modulating bone cells response onto starch-based biomaterials by surface plasma treatment and protein adsorption. Biomaterials. 2007;**28**(2):307-315

[21] Novotná Z, Rimpelová S, Juřík P, Veselý M, Kolská Z, Hubáček T, et al. The interplay of plasma treatment and gold coating and ultra-high molecular weight polyethylene: On the cytocompatibility. Materials Science and Engineering: C. 2017;**71**:125-131

[22] Lee JH, Park JW, Lee HB. Cell adhesion and growth on polymer surfaces with hydroxyl groups prepared by water vapour plasma treatment. Biomaterials. 1991;**12**(5):443-448

[23] Morent R, De Geyter N, Desmet T, Dubruel P, Leys C. Plasma surface modification of biodegradable polymers: A review. Plasma Processes and Polymers. 2011;**8**(3):171-190

[24] Desmet T, Morent R, Geyter ND, Leys C, Schacht E, Dubruel P. Nonthermal plasma technology as a versatile strategy for polymeric biomaterials surface modification: A review. Biomacromolecules. 2009;**10**(9):2351-2378

[25] De Geyter N, Morent R, Leys C, Gengembre L, Payen E. Treatment of polymer films with a dielectric barrier discharge in air, helium and argon at medium pressure. Surface and Coatings Technology. 2007;**201**(16):7066-7075

[26] Van Dyke LS, Brumlik CJ, Liang W, Lei J, Martin CR, Yu Z, et al. Modification of fluoropolymer surfaces with electronically conductive polymers. Synthetic Metals. 1994;**62**(1):75-81

[27] Os MT. Surface Modification by Plasma Polymerization: Film Deposition, Tailoring of Surface Properties and Biocompatibility. Universiteit Twente; 2000

[28] Goodman J. The formation of thin polymer films in the gas discharge. Journal of Polymer Science. 1960;**44**(144):551-552

[29] Stuart M. Dielectric properties of cross-linked polystyrene film formed in the glow discharge. Nature. 1963;**199**:59-60

[30] Bradley A, Hammes JP. Electrical properties of thin organic films. Journal of The Electrochemical Society. 1963;**110**(1):15-22

[31] Yasuda H. Plasma Polymerization. London, UK: Academic Press; 1985

[32] Bax DV, McKenzie DR, Weiss AS, Bilek MM. The linker-free covalent attachment of collagen to plasma immersion ion implantation treated polytetrafluoroethylene and subsequent cell-binding activity. Biomaterials. 2010;**31**(9):2526-2534

[33] Bax DV, Wang Y, Li Z, Maitz PK, McKenzie DR, Bilek MM, et al. Binding of the cell adhesive protein tropoelastin to PTFE through plasma immersion ion implantation treatment. Biomaterials. 2011;**32**(22):5100-5111

[34] Gan B, Bilek M, Kondyurin A, Mizuno K, McKenzie D. Etching and structural changes in nitrogen plasma immersion ion implanted polystyrene films. Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms. 2006;**247**(2):254-260

[35] Yasuda H, Hsu T. Plasma polymerization investigated by the comparison of hydrocarbons and perfluorocarbons. Surface Science. 1978;**76**(1):232-241

[36] Chiper A, Borcia G. Argon versus helium dielectric barrier discharge for surface modification of polypropylene and poly (methyl methacrylate) films. Plasma Chemistry and Plasma Processing. 2013;**33**(3):553-568

[37] Jacobs T, Morent R, De Geyter N, Dubruel P, Leys C. Plasma surface modification of biomedical polymers: Influence on cell-material interaction. Plasma Chemistry and Plasma Processing. 2012;**32**(5):1039-1073

[38] Liu Y, Su C, Ren X, Fan C, Zhou W, Wang F, et al. Experimental study on surface modification of PET films under bipolar nanosecond-pulse dielectric barrier discharge in atmospheric air. Applied Surface Science. 2014;**313**:53-59

[39] Inagaki N. Plasma Surface Modification and Plasma Polymerization. Boca Raton, Florida, United States: CRC Press; 1996

[40] Ponsonnet L, Reybier K, Jaffrezic N, Comte V, Lagneau C, Lissac M, et al. Relationship between surface properties (roughness, wettability) of titanium and titanium alloys and cell behaviour. Materials Science and Engineering: C. 2003;**23**(4):551-560

[41] Pandiyaraj KN, Kumar AA, RamKumar M, Deshmukh R, Bendavid A, P-G S, et al. Effect of cold atmospheric pressure plasma gas composition on the surface and cyto-compatible properties of low density polyethylene (LDPE) films. Current Applied Physics. 2016; **16**(7):784-792

[42] Gupta AK, Gupta M, Yarwood SJ, Curtis AS. Effect of cellular uptake of gelatin nanoparticles on adhesion, morphology and cytoskeleton organisation of human fibroblasts. Journal of Controlled Release. 2004;**95**(2):197-207

[43] Aziz G, Cools P, De Geyter N, Declercq H, Cornelissen R, Morent R. Dielectric barrier discharge plasma treatment of ultrahigh molecular weight polyethylene in different discharge atmospheres at medium pressure: A cell-biomaterial interface study. Biointerphases. 2015;**10**(2):029502

[44] Meyer-Plath A, Schröder K, Finke B, Ohl A. Current trends in biomaterial surface functionalization—Nitrogen-containing plasma assisted processes with enhanced selectivity. Vacuum. 2003;**71**(3):391-406

[45] Finke B, Luethen F, Schroeder K, Mueller PD, Bergemann C, Frant M, et al. The effect of positively charged plasma polymerization on initial osteoblastic focal adhesion on titanium surfaces. Biomaterials. 2007;**28**(30):4521-4534

[46] Robinson DE, Marson A, Short RD, Buttle DJ, Day AJ, Parry KL, et al. Surface gradient of functional heparin. Advanced Materials. 2008;**20**(6):1166-1169

[47] Xu J, Gleason KK. Conformal, amine-functionalized thin films by initiated chemical vapor deposition (iCVD) for hydrolytically stable microfluidic devices. Chemistry of Materials. 2010;**22**(5):1732-1738

[48] Detomaso L, Gristina R, Senesi GS, d'Agostino R, Favia P. Stable plasma-deposited acrylic acid surfaces for cell culture applications. Biomaterials. 2005;**26**(18):3831-3841

[49] Zhang Z, Chen Q, Knoll W, Foerch R, Holcomb R, Roitman D. Plasma polymer film structure and DNA probe immobilization. Macromolecules. 2003;**36**(20):7689-7694

[50] Zhang Z, Knoll W, Förch R. Amino-functionalized plasma polymer films for DNA immobilization and hybridization. Surface and Coating Technology. 2005;**200**(1):993-995

[51] Muguruma H, Hiratsuka A, Karube I. Thin-film glucose biosensor based on plasma-polymerized film: Simple design for mass production. Analytical Chemistry. 2000;**72**(11): 2671-2675

[52] Gupta B, Plummer C, Bisson I, Frey P, Hilborn J. Plasma-induced graft polymerization of acrylic acid onto poly (ethylene terephthalate) films: Characterization and human smooth muscle cell growth on grafted films. Biomaterials. 2002;**23**(3):863-871

[53] Yuan S, Szakalas-Gratzl G, Ziats NP, Jacobsen DW, Kottke-Marchant K, Marchant RE. Immobilization of high-affinity heparin oligosaccharides to radiofrequency plasma-modified polyethylene. Journal of Biomedical Materials Research Part A. 1993;**27**(6):811-819

[54] Siow KS, Britcher L, Kumar S, Griesser HJ. Plasma methods for the generation of chemically reactive surfaces for biomolecule immobilization and cell colonization–a review. Plasma Processes and Polymers. 2006;**3**(6-7):392-418

[55] Deng X, Nikiforov AY, Coenye T, Cools P, Aziz G, Morent R, et al. Antimicrobial nanosilver non-woven polyethylene terephthalate fabric via an atmospheric pressure plasma deposition process. Scientific Reports. 2015;**5**:10138

[56] Kumar V, Jolivalt C, Pulpytel J, Jafari R, Arefi-Khonsari F. Development of silver nanoparticle loaded antibacterial polymer mesh using plasma polymerization process. Journal of Biomedical Materials Research Part A. 2013;**101**(4):1121-1132

[57] Degoutin S, Jimenez M, Casetta M, Bellayer S, Chai F, Blanchemain N, et al. Anticoagulant and antimicrobial finishing of non-woven polypropylene textiles. Biomedical Materials. 2012;**7**(3):035001

[58] Detomaso L, Gristina R, d'Agostino R, Senesi GS, Favia P. Plasma deposited acrylic acid coatings: Surface characterization and attachment of 3T3 murine fibroblast cell lines. Surface and Coatings Technology. 2005;**200**(1):1022-1025

[59] Majani R, Zelzer M, Gadegaard N, Rose FR, Alexander MR. Preparation of Caco-2 cell sheets using plasma polymerised acrylic acid as a weak boundary layer. Biomaterials. 2010;**31**(26):6764-6771

[60] Senesi GS, D'Aloia E, Gristina R, Favia P, d'Agostino R. Surface characterization of plasma deposited nano-structured fluorocarbon coatings for promoting in vitro cell growth. Surface Science. 2007;**601**(4):1019-1025

[61] Gristina R, D'Aloia E, Senesi GS, Milella A, Nardulli M, Sardella E, et al. Increasing cell adhesion on plasma deposited fluorocarbon coatings by changing the surface topography. Journal of Biomedical Materials Research Part B: Applied Biomaterials. 2009;**88**(1): 139-149

[62] Dhayal M, Cho S-I. Leukemia cells interaction with plasma-polymerized acrylic acid coatings. Vacuum. 2006;**80**(6):636-642

[63] Intranuovo F, Sardella E, Gristina R, Nardulli M, White L, Howard D, et al. PE-CVD processes improve cell affinity of polymer scaffolds for tissue engineering. Surface and Coatings Technology. 2011;**205**:S548-SS51

[64] Finke B, Hempel F, Testrich H, Artemenko A, Rebl H, Kylián O, et al. Plasma processes for cell-adhesive titanium surfaces based on nitrogen-containing coatings. Surface and Coating Technology. 2011;**205**:S520-S5S4

[65] Daw R, Candan S, Beck AJ, Devlin AJ, Brook IM, MacNeil S, et al. Plasma copolymer surfaces of acrylic acid/1, 7 octadiene: Surface characterisation and the attachment of ROS 17/2.8 osteoblast-like cells. Biomaterials. 1998;**19**(19):1717-1725

[66] Buttiglione M, Vitiello F, Sardella E, Petrone L, Nardulli M, Favia P, et al. Behaviour of SH-SY5Y neuroblastoma cell line grown in different media and on different chemically modified substrates. Biomaterials. 2007;**28**(19):2932-2945

[67] Zheng Y, Xiong C, Wang Z, Li X, Zhang L. A combination of CO 2 laser and plasma surface modification of poly (etheretherketone) to enhance osteoblast response. Applied Surface Science. 2015;**344**:79-88

[68] Pezzatini S, Morbidelli L, Gristina R, Favia P, Ziche M. A nanoscale fluorocarbon coating on PET surfaces improves the adhesion and growth of cultured coronary endothelial cells. Nanotechnology. 2008;**19**(27):275101

[69] Myung SW, Ko YM, Kim BH. Protein adsorption and cell adhesion on three-dimensional polycaprolactone scaffolds with respect to plasma modification by etching and deposition techniques. Japanese Journal of Applied Physics. 2014;**53**(11S):11RB01

[70] Hamerli P, Weigel T, Groth T, Paul D. Surface properties of and cell adhesion onto allylamine-plasma-coated polyethylenterephtalat membranes. Biomaterials. 2003;**24**(22): 3989-3999

[71] Mitchell S, Davidson M, Emmison N, Bradley R. Isopropyl alcohol plasma modification of polystyrene surfaces to influence cell attachment behaviour. Surface Science. 2004; **561**(1):110-120

[72] Muir BW, Nelson A, Fairbrother A, Fong C, Hartley PG, James M, et al. A comparative X-ray and neutron reflectometry study of plasma polymer films containing reactive amines. Plasma Processes and Polymers. 2007;**4**(4):433-444

[73] Zhang Z, Chen Q, Knoll W, Förch R. Effect of aqueous solution on functional plasma polymerized films. Surface and Coating Technology. 2003;**174**:588-590

[74] Jeon H, Wyatt J, Harper-Nixon D, Weinkauf D. Characterization of thin polymer-like films formed by plasma polymerization of methylmethacrylate: A neutron reflectivity study. Journal of Polymer Science Part B: Polymer Physics. 2004;**42**(13):2522-2530

[75] Tamirisa PA, Hess DW. Water and moisture uptake by plasma polymerized thermoresponsive hydrogel films. Macromolecules. 2006;**39**(20):7092-7097

[76] Vasilev K, Britcher L, Casanal A, Griesser HJ. Solvent-induced porosity in ultrathin amine plasma polymer coatings. The Journal of Physical Chemistry B. 2008;**112**(35):10915-10921

[77] Förch R, Zhang Z, Knoll W. Soft plasma treated surfaces: Tailoring of structure and properties for biomaterial applications. Plasma Processes and Polymers. 2005;**2**(5):351-372

[78] Tarasova A, Hamilton-Brown P, Gengenbach T, Griesser HJ, Meagher L. Colloid probe AFM and XPS study of time-dependent aging of amine plasma polymer coatings in aqueous media. Plasma Processes and Polymers. 2008;**5**(2):175-185

[79] Aziz G, Thukkaram M, De Geyter N, Morent R. Plasma parameters effects on the properties, aging and stability behaviors of allylamine plasma coated ultra-high molecular weight polyethylene (UHMWPE) films. Applied Surface Science. 2017;**409**:381-395

[80] Gengenbach TR, Vasic ZR, Chatelier RC, Griesser HJ. A multi-technique study of the spontaneous oxidation of N-hexane plasma polymers. Journal of Polymer Science Part A: Polymer Chemistry. 1994;**32**(8):1399-1414

[81] Gengenbach TR, Griesser HJ. Compositional changes in plasma-deposited fluorocarbon films during ageing. Surface and Interface Analysis. 1998;**26**(7):498-511

[82] Gengenbach TR, Griesser HJ. Aging of 1, 3-diaminopropane plasma-deposited polymer films: Mechanisms and reaction pathways. Journal of Polymer Science Part A: Polymer Chemistry. 1999;**37**(13):2191-2206

[83] Gengenbach TR, Griesser HJ. Post-deposition ageing reactions differ markedly between plasma polymers deposited from siloxane and silazane monomers. Polymer. 1999;**40**(18): 5079-5094

[84] Goreham RV, Short RD, Vasilev K. Method for the generation of surface-bound nanoparticle density gradients. The Journal of Physical Chemistry C. 2011;**115**(8):3429-3433

[85] Menzies DJ, Cowie B, Fong C, Forsythe JS, Gengenbach TR, McLean KM, et al. One-step method for generating PEG-like plasma polymer gradients: Chemical characterization and analysis of protein interactions. Langmuir. 2010;**26**(17):13987-13994

[86] Goreham RV, Mierczynska A, Pierce M, Short RD, Taheri S, Bachhuka A, et al. A substrate independent approach for generation of surface gradients. Thin Solid Films. 2013; **528**:106-110

[87] Harding F, Goreham R, Short R, Vasilev K, Voelcker NH. Surface bound amine functional group density influences embryonic stem cell maintenance. Advanced Healthcare Materials. 2013;**2**(4):585-590

[88] Zelzer M, Alexander MR, Russell NA. Hippocampal cell response to substrates with surface chemistry gradients. Acta Biomaterialia. 2011;**7**(12):4120-4130

[89] Zelzer M, Scurr D, Abdullah B, Urquhart AJ, Gadegaard N, Bradley JW, et al. Influence of the plasma sheath on plasma polymer deposition in advance of a mask and down pores. The Journal of Physical Chemistry B. 2009;**113**(25):8487-8494

[90] Mangindaan D, Kuo W-H, Wang M-J. Two-dimensional amine-functionality gradient by plasma polymerization. Biochemical Engineering Journal. 2013;**78**:198-204

[91] Parry KL, Shard A, Short R, White R, Whittle J, Wright A. ARXPS characterisation of plasma polymerised surface chemical gradients. Surface and Interface Analysis. 2006;**38**(11): 1497-1504

[92] Pitt WG. Fabrication of a continuous wettability gradient by radio frequency plasma discharge. Journal of Colloid and Interface Science. 1989;**133**(1):223-227

[93] Whittle JD, Barton D, Alexander MR, Short RD. A method for the deposition of controllable chemical gradients. Chemical Communications. 2003;**14**:1766-1767

[94] Vasilev K, Mierczynska A, Hook AL, Chan J, Voelcker NH, Short RD. Creating gradients of two proteins by differential passive adsorption onto a PEG-density gradient. Biomaterials. 2010;**31**(3):392-397

[95] Ghobeira R, Philips C, Declercq H, Cools P, De Geyter N, Cornelissen R, et al. Effects of different sterilization methods on the physico-chemical and bioresponsive properties of plasma-treated polycaprolactone films. Biomedical Materials. 2017;**12**(1):015017

[96] Walker RA, Cunliffe VT, Whittle JD, Steele DA, Short RD. Submillimeter-scale surface gradients of immobilized protein ligands. Langmuir. 2009;**25**(8):4243-4246

[97] Wells N, Baxter MA, Turnbull JE, Murray P, Edgar D, Parry KL, et al. The geometric control of E14 and R1 mouse embryonic stem cell pluripotency by plasma polymer surface chemical gradients. Biomaterials. 2009;**30**(6):1066-1070

[98] Harding FJ, Clements LR, Short RD, Thissen H, Voelcker NH. Assessing embryonic stem cell response to surface chemistry using plasma polymer gradients. Acta Biomaterialia. 2012;**8**(5):1739-1748

[99] Wang P-Y, Clements LR, Thissen H, Tsai W-B, Voelcker NH. Screening rat mesenchymal stem cell attachment and differentiation on surface chemistries using plasma polymer gradients. Acta Biomaterialia. 2015;**11**:58-67

[100] Mangindaan D, Kuo WH, Kurniawan H, Wang MJ. Creation of biofunctionalized plasma polymerized allylamine gradients. Journal of Polymer Science Part B: Polymer Physics. 2013;**51**(18):1361-1367

[101] Barry JJ, Howard D, Shakesheff KM, Howdle SM, Alexander MR. Using a core–sheath distribution of surface chemistry through 3D tissue engineering scaffolds to control cell ingress. Advanced Materials. 2006;**18**(11):1406-1410

[102] Zelzer M, Majani R, Bradley JW, Rose FR, Davies MC, Alexander MR. Investigation of cell–surface interactions using chemical gradients formed from plasma polymers. Biomaterials. 2008;**29**(2):172-184

[103] Robinson DE, Buttle DJ, Whittle JD, Parry KL, Short RD, Steele DA. The substrate and composition dependence of plasma polymer stability. Plasma Processes and Polymers. 2010;**7**(2):102-106

[104] Delalat B, Goreham RV, Vasilev K, Harding FJ, Voelcker NH. Subtle changes in surface chemistry affect embryoid body cell differentiation: Lessons learnt from surface-bound amine density gradients. Tissue Engineering Parts A. 2014;**20**(11-12):1715-1725

[105] Liu X, Shi S, Feng Q, Bachhuka A, He W, Huang Q, et al. Surface chemical gradient affects the differentiation of human adipose-derived stem cells via ERK1/2 signaling pathway. ACS Applied Materials & Interfaces. 2015;**7**(33):18473-18482

IntechOpen