# Nanoengineered plasma polymer films for biomedical applications

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## Abstract

This forward looking concise review describes recent advances in the field of nanoengineered plasma polymer films. These types of coatings are relevant in many fields of application and have gained substantial research and technological interest over the last decade. The review starts with an introduction of plasma polymerization as a technique for preparation for nanometer thin polymer-like coatings. This is followed by the examples of the use of nanoengineered plasma polymer coatings in applications relevant to biomedical devices. Applications in antibacterial coatings and drug delivery vehicles are discussed. Significant section of this paper is dedicated to cell guidance surfaces which have an extensive range of applications ranging from coatings for medical devices to research tools that can help unraveling complex biological questions and vehicles for the growing field of cell therapies. The vision of the authors about the future directions of the field have also been presented, including a section on novel oxazoline based coatings that carry great promise for advances in the biomaterial and biomedical fields. Copyright © 2018 VBRI Press.

**Keywords:** Plasma polymers, antibacterial coatings, drug delivery, nanotechnology, medical devices, cell therapies, surface modification, thin films.

## Introduction

Plasmas, the forth state of matter, have intrigued, puzzled and fascinated humans for millennia. Plasmas are everywhere around us. Plasmas make 99 per cent of the visible universe. Plasma are the lightning, the auroras and the sun core. Plasmas can be wild and uncontrollable but when their energy is confined, they become useful tools in numerous applications. Processes such as plasma etching and deposition revolutionized the semiconductor industry and made possible production of high speed computer processors which we are all enjoying today. Several new applications of plasmas such as in plasma medicine [**1**] and plasma nanoscience [**2**] are currently a hot topic of research and hold promise to revolutionize many fields.

Surface engineering has been an area where plasma processes made and continue making substantial impact. The capacity to preserve valuable bulk properties but to alter the properties at the surface contributes substantial added value to numerous products in fields ranging from medicine to membrane filtration and electronics. An important aspect of plasma processing is the deposition of organic thin films. In most cases the deposition of such films is carried out under low pressure in reaction chambers such as these described by Whittle *et al.* [**3**] The origin of the field of plasma polymerization could be traced back to the work of Linder and Davies in the 1930s who were the first to report polymer deposits on electrodes [**4, 5**]. However, purposeful deposition of organic films from plasma started in the 1960s with the work of Goodman [**6**] and Yasuda [**7**], followed many others who have made and are still making significant contributions to this field.

Plasma polymers are a unique class of materials. They differ from conventional polymer by their irregular structure which makes it difficult to identify repeating units. For this reason, it is often argued about the use of the term "polymer", many insisting on classification as "organic films deposited from plasma". Although the latter may be more appropriate, the term "plasma polymers" is widely used and this is how these coatings will be referred to in this article. Plasma polymer are typically highly crosslinked and if deposited under appropriate conditions can be resistant to many solvents. This makes them valuable in many industry and research applications. An important characteristic of plasma polymers is that they can be deposited on practically any type of substrate material. This compare plasma polymers favorably to other techniques for preparation of very thin coatings such as layer-by-layer (L-b-L) or self-assembled monolayers (SAMs) which require a specific substrate such as charged and metallic surfaces respectively [**8, 9**].

 

The substrate independent nature of the process in due to the complex interplay between physical, mechanical and chemical bonding between coating and substrate. While plasma species such as ions, radicals and electrons bombard the material substrate, nanoscopic defects are formed on the surface which contribute to mechanical bonding. At the same time, this bombardment generates surface charges and reactive chemical species such as radicals which provide physical and chemical bonding, respectively. To test the substrate-independent nature of the deposition process, we conducted a set of experiments where a gold coated glass slide was placed in the plasma chamber together with an analogous surface where the gold layer was modified with a thiol monolayer [**10**]. In this way, we presented to the plasma a metallic (gold) and an organic (thiol) surface. The goal of this strategy was to ensure absolutely identical deposition environment (same type of substrate and same run) and capacity to use the same thickness measurement technique. Comparison of the thickness of the resultant coating showed that plasma polymer films deposited faster on the organic substrate. However, this was the case only in the first moments of deposition. Once few nanometers of coating were deposited, the rate of film deposition had become substrate independent [**10-12**]. These seminal studies demonstrated that from practical perspective plasma polymer coatings can be considered substrate independent.

Another valuable feature of plasma polymers is the versatility of coating chemical and physical properties that can be achieved. Practically any compound that is volatile enough to be introduced in the reaction chamber through the vacuum or carrier gas can be readily deposited as a thin film. Even precursors such as ethanol that are difficult to polymerase by conventional means can be deposited in polymeric like films [**13**]. Furthermore, plasma polymerization allows to achieve surface chemistries that are not achievable by conventional means. For example, conventionally, oxazolines are polymerized through a ring opening reaction. As a result, the oxazoline rings are consumed in the polymerization process. When coatings based on oxazoline precursors are deposited from plasma some of the ring structures are preserved on the surface of the resultant coatings which provides unique opportunity to conduct binding reactions of biomolecules, nanoparticles and various ligands that carry carboxyl acid groups in their structures [**14, 15**]. In addition, coatings deposited from plasma have valuable properties for application on biomedical devices such as excellent biocompatibility, reduction of inflammatory response and capacity to reduce bacterial colonization [**14-16**].

Amongst many other practical applications, plasma polymers secured a prominent place in the biomaterial and biomedical fields. Examples of commercial products that are being facilitated by plasma polymers are the Ciba Vision NIGHT & DAY contact lances which allow continuous use for 30 days, wounds care products such as MySkin™ and R&D tools such as the BD PureCoat cell culture places. In the following, recent progress in the development of advanced nanoengineered plasma polymer coatings for the purposes of biomedical applications coming from our group and others will be briefly summarized.

## Antibacterial coatings

Coatings that are capable of protecting a medical device from the attachment and colonization by bacteria can greatly benefit patients and medicine. Infections are still a major issue causing morbidity and mortality to patients, and adding substantial cost to healthcare. Hospital acquired infection (HAI) are documented to affect nearly two million patients in the USA, which is associated with 100,000 deaths and more than 30 billion dollars in added healthcare costs. Nearly half of the HAI are associated with medical devices and these are the most costly and complicated infections to treat. For example, infection rates with some medical devices such as mechanical heart valves can be four per cent and cost for treatment may exceed US$50,000 per case. Catheters are other devices that are often infected. Bloodstream infections associated with intravenous catheters are estimated to result in more the 28,000 deaths annually in the USA. Urinary catheters have nearly 100 per cent infection rate if used for longer that one week.

It is now well understood that the infection begins with the attachment of individual planktonic bacterial cells to the surface of the device. These cells then proliferate, produce extra cellular matrix polymers such as sugars and proteins and in this way form communities called biofilms. The formation of biofilm is crucial for the survival of bacteria. The biofilm provides to bacteria cells unique signaling pathways that are not available to planktonic bacteria, protects the cells from the immune system and enormously (up to 1000 times) increase the dose of antibiotics required to clear the infections. Furthermore, once matured, biofilms release planktonic bacteria that infect other sides of the host and has a serious contribution to development of antibiotic resistant species.

After all this being said, it is clear that in the context of medical devices it is of vital importance to prevent the initial states of bacterial adhesion to the surface of the device. This was understood some decades ago and now well accepted. So, what if we can somehow prevent bacteria for attaching to the surface of the device? This question brought the concept of antibacterial coatings i.e. coatings that have the purpose to protect the device surface from bacterial adhesion and colonization. Antibacterial coatings became an intense and growing area of research over the last two decades [**17, 18**]. Amongst the various technologies for preparation of antibacterial surfaces are these facilitated by plasms polymers summarized in a following review [**19, 20**].

 

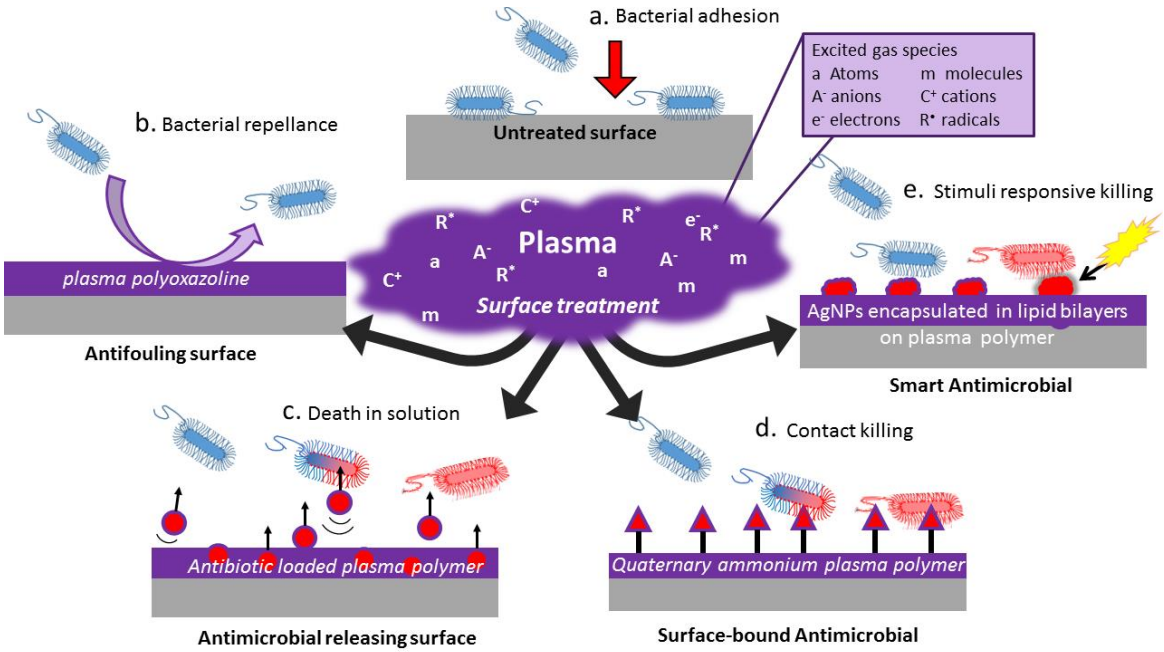


**Fig. 1:** a. A schematic showing plasma surface treatment facilitating the generation of the four classes of antibacterial strategies: b. antifouling surface, c. antimicrobial-releasing surface, d.surface bound antimicrobial and e. smart, stimuli responsive antimicrobial surface.

Based on the mechanism of action, we distinguish four classes of antibacterial coatings **(Fig. 1)**. The first class are coating that bacteria do not like to attach to; the second class are these coatings that kill bacteria upon contact; the third class are coatings that release antibacterial agents that kill bacteria in the vicinity of the material; and the forth class are coatings which would release antibacterial agents only upon the presence of some external stimuli. In our research, we have developed prototypes of all these classes of coatings. An example of the first class **(Fig. 1b)** are the oxazoline based coatings which were first reported by our group [**14, 21**]. We found that bacteria would attach in small numbers to these types of coatings but will not proliferate and will not develop a biofilm. The mechanism behind this phenomenon is still a mystery but the finding provides excellent opportunities for developing medical device coating technologies. Individual bacteria which do not proliferate and are not protected in biofilm will be an easy prey to the immune system. Combined with other interesting properties, which will be discussed later in the paper, plasma deposited coatings from oxazoline precursors present exciting opportunities for the future of medical device technology. Plasma polymers have also been used to facilitate immobilization of hydrophilic polymers such as polyethylene glycols (PEG) which is know to resist protein and bacteria adhesion [**22**]. Jacob and co-workers also reported plasma deposited coatings based on essential oils with antibacterial properties [**20, 23**].

Coatings that kill bacteria upon contact **(Fig 1.d)** have attracted attention because they we considered safer than those releasing antimicrobial agents. Plasma polymers have been utilized to support such strategies on several occasions [**24, 25**]. Most common are quaternary ammonium compounds (QACs). These molecules are known to damage the bacteria cell membrane causing the death of the cell. In a recent study we evaluated the surface concertation of QACs required to kill bacteria.[**26**] For the purpose of this study we prepared surface density gradient of glycidyltrimethylammonium chloride by time dependent covalent immobilization onto amine group rich plasma polymer coatings. We determined that there is a threshold surface concentration of $NR_4^+$ groups equivalent to 5 At% nitrogen and surface potential of +120 mV required to inactivate attached bacteria. This finding is important because it points to the minimum surface density of QACs needed to provide effective protection to medical devices. Another important message from this work is that once placed in biological media the coatings become covered by constituents of the medium which practically inactivate them. This limits the application of contact killing coatings as they can protect the device only for the period before placement in a biological fluid. Nanoough surfaces can also have contact killing properties. Our contribution to this area was to examine the role of surface chemistry on bacteria killing efficacy of silica nanograss [**27**]. As a hybrid between contact killing and low fouling coatings can be attributed also those produced by chlorinated precursors [**28, 29**]. However, the usefulness of these coatings in medical device technology should be interpreted with care since they may be cytotoxic to mammalian cells and tissue.

The most extensively explored and arguably the most effective low fouling surfaces are platforms which release antimicrobial compounds. **(Fig 1.c.)** [**19, 30, 31**]. We were the first to use plasma polymers to release antibiotics. The strategy was based on 'sandwiching' levofloxacin between two plasma polymer layers [**30**]. The first layer had the purpose to control the properties of

 

the substrate making the technology applicable to any type of material. The role of the second layer, deposited on top of levofloxacin particles, was to control the antibiotic release rate via plasma polymer film thickness. This strategy was inspired by earlier work where we revealed that upon appropriate condition of deposition plasma polymers can become porous thus allowing the transport of small molecules through the coatings [**32**]. Subsequently we used this discovery to generate nanocavities within plasma polymer films of controlled dimensions via nanoparticles templating. [**33**] We also utilized nanoporous materials as reservoir of antibiotics and controlled their release rate via the amount of plasma polymer deposited over the pores [**31**]. This strategy will be discussed in greater detail later in this article. We were also the first to develop plasma polymer facilitated platforms for nitric oxide release obtained either by using the intrinsic chemistry of the precursor or porous platforms [**34, 35**].

As a branch of the releasing type strategies should also be considered these that use silver nanoparticles. This is because silver oxidizes and upon immersion in aqueous medium silver nanoparticles dissolve leading to release of silver ions. These silver ions are the actual species that kill bacteria and not the silver nanoparticles themselves. The enormous interest in silver based antibacterial technologies over the last 20 years is triggered by the spreading phenomenon of antibiotic resistance [**18,36,37**]. We have developed several strategies for preparation of silver based antibacterial coatings. In a study published in Nano Letters in 2010, we first loaded amine based plasma polymer films of around 100 nm thickness with silver ions by immersion in $AgNO_3$.[**38, 39**] The choice of plasma polymer film chemistry was based on the knowledge that amine groups have the capacity to complex silver ions. In a subsequent step, the loaded silver ions were reduced to silver nanoparticles. This work was one of the first to demonstrate that by tuning the rate of release of silver ions the coatings can be tuned to completely inhibiting bacterial growth but allowing normal function of mammalian cells, which is an ideal situation for application on medical device surfaces. The hypothesis behind the coatings design was that mammalian cells can tolerate greater amount of silver because of their larger size and more complex physiological apparatus compared to bacterial cells. This hypothesis was later confirmed by a number of other researchers and summarized in the following review article.[**40**] Recently, we compared our silver nanoparticle loaded plasma polymer coatings with a commercial dressing having physically deposited silver layer. We determined that our coatings had at least the same antibacterial efficacy but this was achieved by at a fraction of the amount of silver used in the commercial dressings.[**41**] We have developed another strategy for fabrication of silver nanoparticles based antibacterial coatings by combining functional plasma polymers and the electrostatic immobilization of appropriately surface modified silver nanoparticles.[**42-48**] The method results in antibacterial coatings with excellent efficacy and also allows for control of the nanoparticles number density on the surface. In this way we were able to deliver the appropriate amount of silver which allows strong antibacterial action but also ensures the biocompatibility of the coatings. Furthermore, we were able to demonstrate that the coatings did not cause adverse inflammatory response, which is essential when medical devices are concerned.[**42, 44**] Others have also explored the possibility to load plasma polymers with silver. One technique pioneered by Favia and d'Agostino involves the simultaneous deposition of plasma polymers and sputtering of silver ions which then form clusters within the coatings.[**49**] Silver clusters have been also incorporated in diamond-like carbon (DLC) coatings.[**50**] The latter approach is interesting because of the benefits offered by the mechanical hardness of the DLC films.

The last class of antibacterial coatings are those that release antibacterial agents upon influence of external stimuli. **(Fig.1.e)** These external stimuli could be temperature, pH, salt concentration or the bacteria themselves.[**17**] It would be fair to say that so far plasma polymers have been insufficiently explored in such advanced applications and only very few examples exist. In an elegant approach Jenkins and co-workers have used maleic anhydride based plasma polymer coatings to immobilize lipid vesicles containing $NaN_3$.[**51**] The concept behind this approach is based on the knowledge that pathogenic bacteria express toxins and lipases capable of damaging the cell membrane. Thus, when pathogenic bacteria are present in the vicinity of the coating their lipases and toxins trigger the releases of antibacterial agents which leads to the self-destruction of the pathogens. The same strategy can be applied sensing of infiltrating pathogens by using fluorescent dyes.[**52**] The only drawback of this approach is the gentleness and fragility of lipid vesicles which may limit practical application. In our work, we prepared silver nanoparticles that were capped by a lipid bilayer.[**42**] These lipid encapsulated nanoparticles were immobilized on solid surfaces pre-modified with an amine group rich interlayer. We also synthesized nanocapsules containing polyhexanide which were responsive enzymatic degradation by hyaluronidase, an enzyme expressed by *Staph Aureus* [**53**]. The field of responsive and smart antibacterial technologies is rapidly growing because it allows the delivery of antimicrobial toxins only when they are needed. On one hand this allows for reducing systemic toxicity which is inevitable when drugs are delivered by conventional means. On another hand it provides enormous opportunities in sensing technologies which signal when infection or infiltrating pathogens are present.

***Drug delivery vehicles by plasma techniques***

Controlled and targeted drug delivery has been a subject of extensive research for a member of decades. This is because there is a pressing medical need for reliable and scalable drug therapies that provide controlled and sustained release, greater bioavailability as well as

 

targeted and triggered delivery. There are only limited examples where plasma polymers have been used to create drug delivery platforms. It would be fair to say that our group was one of the pioneers in this field. In an early example we have sandwiched on a solid substrate drugs particles, formed after drying of solution cast drug, between two plasma polymer coatings.[**30**] The first, plasma polymer layer had the purpose of giving the substrate a particular wetting characteristics in order to be able to apply the technology to any type of substrate material. In the case of medical devices these carrier substrates could be metallic, ceramics, polymers or composite. The second layer, deposited on top of the drug, had the role of controlling drug release rate. We demonstrated that the thickness of this overlayer can be conveniently used to control the rate of drug release, however, this could be also achieved by controlling the coating hydrophobicity and physicochemical properties. This work was inspired by our earlier fundamental studies where we showed that choosing appropriate deposition conditions we can control the porosity of the plasma polymer films.[**32, 33**]

We also utilized porous platforms to prepare drug delivery vehicles. In the first study of its kind we loaded vancomycin in nanoporous alumina prepared by anodization.[**31**] We demonstrated that by controlling the amount of plasma polymer on top of the loaded nanoporous substrates we were able to efficiently control the release rate of the drug. In a follow-up publication we demonstrated that the strategy is applicable to a range of drug and also to proteins.[**30**] More recently we reported using the same platform for delivery of biologically active monoclonal antibody rituximab.[**54**] The bioactivity of the antibody was demonstrated in a culture of CD20-positive Daudi cells. It was interesting to see that the porous alumina platform was capable of protecting the biological molecules from the harsh plasma environment. These studies were inspired by our earlier research which demonstrated that plasma polymers start growing from the rims of the pores and slowly close them.[**55-57**] This allowed us to control the opening of the pores and by this the rate of exposure of the loaded drug to the solvent and in the same time the movement of dissolved drug molecules out of the pores.

Another plasma inspired techniques for drug delivery that we developed consists of coating of free drug particulates. Off-the-shelf drug particles are placed on a vibrating bed which forces a continuous motion of the particles. Upon deposition, the particles are thus uniformly coated with plasma polymer. We were the first to demonstrate the encapsulation of antibiotics into a plasma polymer shell.[**58**] We could control the thickness of the shell which allowed us to regulate the release rate of the drug. We measured the Minimum Inhibitory Concentration (MIC) of the antibiotic before and after releaser from the plasma polymer capsules. The efficacy was unchanged, which indicated that in the case of ampicillin, the plasma environment did not affect the activity of the compound. Using similar technique, we have coated particles of various materials and sizes, including porous silica particles loaded with drugs. [**59-61**]

This capacity to encapsulate particles of pure drug by plasma polymers is a significant advance in the field of drug delivery. The process is fast, solvent free and consists of a single step. Also, it allow for a much greater drug loading compared to existing wet processes which typically consist of a member of dissolution and drying steps. The fact that no solvents are used offers significant technological advantages since the process reduces cost, time and the necessity for treatment of waste solvents. Currently, we are further developing this exciting plasma polymer based encapsulation technology. We are not only using capsules based on hydrophobic plasma polymers to control the release rate of soluble drugs but also hydrolytic capsules to improve dissolution and bioavailability of poorly soluble compounds. Other areas of interest is decorating functional plasma polymer capsules with ligands in order to achieve a targeted delivery of the drugs to the zone of interest and to create responsive capsules that would deliver the drug only when it is needed.

## Cell guidance surfaces

Before discussing the state of art in this area we should first define the term “Cell guidance surfaces”. Cell guidance are these surfaces which are capable of

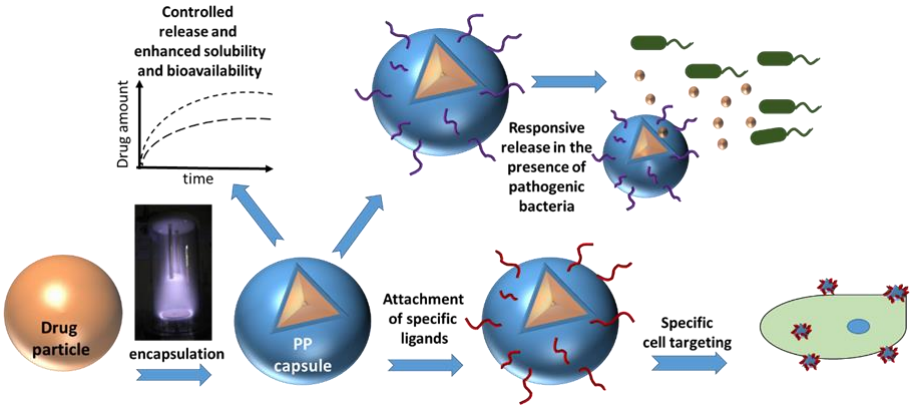


**Fig. 2:** Plasma encapsulation of drug particulates and the opportunities in various future directions.

 

controlling the function and behavior of biological cells. This can be cell adhesion and proliferation, differentiation and expansion in the case of stem cells, or inflammation and infections. The applications of cell guidance surfaces are in numerous areas such as biomedical device, tissue engineering scaffolds, bioreactors, the growing area of cell therapies and cell capture based diagnostic devices.

In order to create functional cell guidance surfaces it is essential to have the capacity to tightly control surface properties. These include surface chemical and physical properties, nanotopography, nanomechanics and ligand density, just to name a few. The field of plasma surface nanoengineering offers opportunities to control all these properties with very high precision. As discussed earlier in this article, plasma polymers can be deposited on any type of substrate materials. This is an enormous advantage since medical devices can be made from the four classes of materials i.e. metals, ceramics, polymers and composites. Furthermore, plasma polymers can be deposited on complex shapes. Combined with clever nanoengineering, plasma polymerizations become a powerful utility for tailoring the surface properties of biomedical tools and devices. In the following, we will give some examples of plasma polymer facilitated cell guidance surfaces. Surfaces that control infection also fit in this category but will not be addressed in this section since they we discussed earlier in the article.

In the field of medical devices, controlling inflammation and foreign body response is of paramount importance.[**62, 63**] In current medical practice, these biological process are controlled by the means of various drugs. However, capacity to engineer device surfaces in a way that allows for intrinsic control over inflammatory pathways would be an enormous advance in the field of medicine.[**63**] Recently, we started interrogating the role of surface nanotopography on the response of inflammatory cells. We were able to generate model surfaces of controlled nanotopography in terms of vertical magnitude and lateral spacing. To achieve this, we combined functional coatings deposited from plasma and electrostatic self-assembly of nearly monodispersed gold nanoparticles of desired diameters. The surfaces prepared is this manner had the targeted nanotopography, however, the surface chemistry was a mix of the chemical functionalities of the plasma polymer coating and the gold nanoparticles. This is a problem that is often neglected in other studies but it is important to resolve in order to be able to discriminate between the roles of surface chemistry and nanotopography in guiding physiological processes. To tailor the outermost surface chemistry plasma polymer became an indispensable tool. By depositing a 5 nm thin plasma polymer coating we were able to tailor the outermost surface chemistry to nitrogen/amine, carboxyl acid and pure hydrocarbon rich, the three most abandoned chemical functionalities in the body. We found that the expression of pro-inflammatory cytokines from primary macrophages and the number of adhered cells was significantly alerted by surface nanotopography and chemistry.[**62, 64**] Alteration in neutrophil response was also affected.[**64**] We were also further able to interrogate how inflammasome components ASC and AIM2 modulate the acute phase of biomaterial implant-induced foreign body responses.[**62**] We use the same type of model substrata to examine collagen I and III deposition from primary human dermal fibroblasts, major contributor to fibrous capsule formation around implanted medical devices. Surface nanotopography and chemistry was again found to be strong modulator.[**65, 66**] These studies demonstrate that surface nanotopography and chemistry are potent tools to mediate inflammatory processes and foreign body response.

To evaluate the role of surface properties on the response of biological cells we often use surface gradients – surfaces where one or more surface parameters change in a gradient manner. Surface gradients are interesting for at least two reasons. Firstly, these surfaces offer excellent platform to develop tools that allow to mimic physiological processes that are known to be guided by gradients but are difficult to study *in vivo* due to the complexity of the environment. Some of the important process that are naturally driven by gradients are the embryonic development, neuronal differentiation, immune function, vascular remodeling and wound healing, atherosclerosis, chronic inflammation, cancer metastasis and others. Bacteria are also known to migrate towards food and run away from toxins. By using gradients that mimic the physiological environment these complex processes could be studies in the laboratory with conventional analytical techniques. The second reason for using gradients is because these materials are useful screening tools for investigating that effect of a great range of surface properties on biological phenomena with a single substrate. This advantageous since prevents errors that can occur when using multiple samples, requires less cells and in this way reduces variability associated with using multiple passages, speeds up analysis and lowers costs.

Plasma facilitated techniques make possible generation of surface gradients of a wide range of surface properties. We typically produce surface chemical gradients by copolymerization of two precursors with different chemistry.

This is done in a system design similar to this first reported by Whittle *et al.* [**67**] which utilized plasma deposition of precisely controlled precursor ratios through a slot in a mask placed over a moving substrate. We extended these original studies to greater range of chemistries and different substrate materials [**56, 68-72**]. For example, we created combinations of pure hydrocarbon and amines, amines and acids, hydroxyls and aldehyde, and others. **(Fig 3.c)** [**26, 56, 68-74**] These surface gradients proved to be very useful to studying the effect of surface chemistries on cellular behavior and for the immobilization of various ligands and nanoparticles. For example, recently, using pure hydrocarbon to amine gradients we were able to demonstrate that ERK1/2 is an important downstream signaling pathway of surface

 

chemistry directed adipose-derived stem cell fate and demonstrate the selective $CaCO_3$ deposition by SaoS-2 cells. [**75, 76**] We were also able to produce gradient of pH tunable wettability and surface potential which are of interest for various applications**. (Fig.3.a)** [**69**]

The chemical gradients are flexible starting platform for generations of density gradients of various ligands.[**72, 73, 77, 78**] In an early example, we used gradient of amine functional groups to covalently immobilize aldehyde terminal PEG. In this way we were able to generate PEG density gradients and to control the adsorption of two proteins, thus creating a gradient of two proteins on a single substrate.[**68**] More recently we utilized gradients of increasing aldehyde surface group concentration to covalently bind proteins and growth factors. Using this approach we were able to determine the optimal surface density of neural growth factor (NGF) required to drive the differentiation of embryoid body cells into neural lineages.[**72**] The chemical gradients are also useful for the covalent or passive binging in a surface density dependent fashion of other entities such as nanoparticles, small and polymeric molecules.[**26, 56, 74, 79**]

An area that we are very interested in is the role of surface nanotopography in biological responses. To address this poorly understood scientific question we often use surface gradients of nanotopography. To create such gradients we utilize functional plasma polymers and appropriately functionalized gold nanoparticles. The role of gold nanoparticles is to produce surface roughness at the nanoscale. We chose gold nanoparticles because we can synthesize them nearly monodispersed, in this way ensuring control over the height of nanotopography. Gold nanoparticles, in the size rage we use, are chemically inert and thus do not interfere with cellular processes. One method for generation of nanoparticle density gradients consist of controlling the number of attached nanoparticles by the density of available binding sites via chemical gradients, mentioned above.[**56, 79**] Another method that we developed in our laboratory consists of the immersion with a desired speed of a functional plasma polymer modified substrate into solution of gold nanoparticles of controlled size**.(Fig.3.b)** Using this method we were able to prepare a range of nanoparticle density gradients and utilize them to study the role of nanotopography on cell attachment and proliferation, stem cells differentiation and inflammatory responses.[**62, 64, 80, 81**] In all cases surface nanotopography was found to be a significant modulator of cellular behavior. We also used nanotopography gradient prepared in such manner to investigate physical processes. In a recent paper, we were able demonstrate that the classical Wenzel and Cassie theories cannot correctly account for wetting phenomena at the nanoscale.[**82**] Furthermore, we derived and experimentally substantiated a predictive equation that is capable of accurately predicting the behavior of a water droplet on nanorough surfaces. This equation is now known as the Vasilev-Ramiasa equation.[**82**]

Surface nanomechanics is another important regulator of cellular responses.[83] We developed a model platform where surface elastic moduli changes in a gradient manner.**(Fig.3.d)[84**] The surface is first

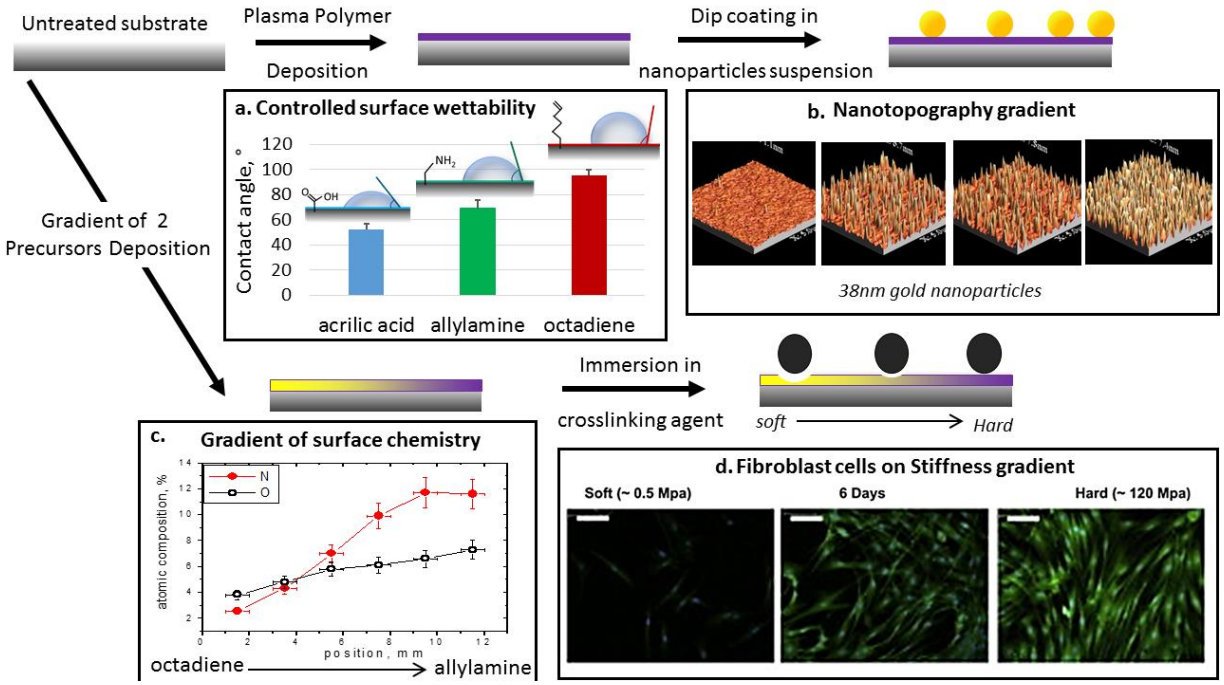


**Fig. 3.** Schematic of plasma based approaches to create homogeneous and gradient surfaces of controlled wettability, nanotopograpy, chemistry and stiffness. a. Contact angles of plasma polymer films deposited from acrylic acid, allylamine or octadiene precursors. b. AFM images of 4 different section of a gradient of nanotopography substrate prepared by adsorption of gold nanoparticles. c. XPS analysis of the atomic composition of a gradient of surface chemistry prepared from allylamine and octadiene precursors. d. Optical microscopy images of fibroblast growing on a gradient of surface stiffness.

 

functionalized with a plasma polymer layer. Next, 20 bilayers of polyallyl amine and polyacrylic acid are deposited by L-b-L technique. The compound is then immersed in a time controlled fashion in a crosslinking agent using the same approaches as we used to create nanoparticles density gradients. As anticipated, the surface elastic modulus increases towards the side of the substrate immersed into crosslinking agent for longer time. In order to correct for changes in surface chemistry and wettability caused by the crosslinking reaction (which is dehydrating in nature) we deposited a 10 nm thin outermost plasma polymer layer of amine or carboxyl acid based precursors. Using atomic force microscopy (AFM) based force measurements and the Herzian theory we were able to determine that the additional plasma polymer layer did not significantly alter the surface elastic modulus of the polyelectrolyte multilayers that were already crosslinked in a gradient manner. Adhesion and proliferation studies with primary human dermal fibroblasts demonstrated that the cells attach and proliferate faster towards the stiffer end of the surface. The result was similar regardless of the outermost surface chemistry employed, which suggests that surface stiffness is a stronger regulator to cellular behavior than the different surface functionalities used in the study.

The examples provided above are a demonstration of the versatility of plasma polymerization as a tool to create surfaces of a wide range of physical, chemical, topographical and bioactive properties. This opens numerous opportunities in the biomedical field and beyond for developing tools for research and advancing many applications.

## Future directions – focus on oxazolines

The field of plasma polymer deposition is growing and forever looking at new ways to design surfaces with unique properties. Of particular interest is our recent work towards the plasma polymerization of oxazoline precursors.[**85**] Oxazolines are currently on the forefront of biomaterials research because they are biocompatible and have demonstrated low fouling properties that surpass those of the gold standard polyethyleneglycol, due to their superior stability. However, organizing polyoxazolines into thin organic film by conventional methods is a complex exercise.[**86**] The first report of surface modification using both plasma and oxazoline was released in 2014 by Popelka *et al.* [**87**] In this work, low pressure air plasma was used to activate a low density polyethylene surface before immersing it into a dichloromethane solution of poly-2-ethyl-oxazoline produced via conventional wet cationic ring opening polymerization. The synthesis of poly-2-ethyloxazoline (PEtOx) in this case took 24h and organic solvents were required both for the polymerization and subsequent surface immobilization steps. Although surface analysis confirmed the successful binding of PEtOx onto the substrates, this study did not report on the behavior of biomolecules or organisms on the modified surfaces. Our group was the first to develop a swift approach for the direct, fast and waste free generation of polyoxazoline-like thin films.[**88**] We used plasma polymer deposition to produce robust, nanometre thin coatings from methyloxazoline precursor. By tuning the deposition conditions, we could produce plasma deposited polyoxazoline (PPOx) thin films stable in various pH and salt conditions, biocompatible and chemically reactive.[**15**] FTIR and XPS analysis demonstrated that the films are decorated with many reactive oxazoline rings available for covalent binding of carboxyl acid group functional ligands such as biomolecules. The later is important since it brings PPOx films deposited form

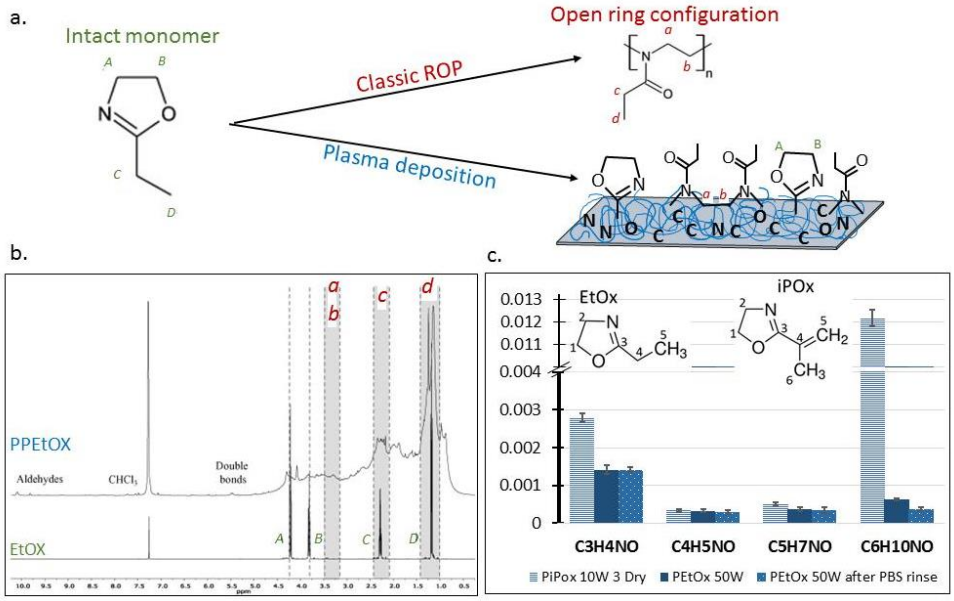


**Fig. 4.** (a) Polyethyloxazoline classic and plasma assisted generation pathways schemes. (b) $^{1}$H NMR spectra identifying protons *A, B,C* and *D* for the intact monomer, and *a, b,c* and *d*, for the open ring configuration. Bottom trace show intact 2-ethyl-2-oxazoline monomer and top trace shows plasma deposited poly-2-ethyl-2-oxazoline PPEtOx. Solvent CDCl3. (c) ToF-SIMS data showing the +SIMS CxHyNO fragment retained in PPEtOx and plasma deposited Isopropyloxazoline PPiPOx films.

 

plasma above their counterparts synthesized via conventional ring opening polymerization which consumes the oxazoline rings.[**15**] This exquisite chemistry of the plasma deposited polyoxazoline is interesting because it offers opportunities in a range of biomedically relevant applications such as sensors and devices. In our pioneering studies of PPOx film chemical reactivity, we showed that COOH- functionalised nanoparticulates and biomolecules bound irreversibly to PPOx and that surface bound proteins retain their bioactivity.[**14, 15**]

In the wake of these pioneering studies other groups began to delve into PPOx films intriguing properties. The results reported by Zanini *et al.* [**89**] strongly support our group's main original finding, specifically the partial retention of unopened oxazoline rings, as well as their reactivity toward carboxylic acid groups. Based on their quantitative 1H NMR analysis, Zanini *et al.* [**89**] concluded that for PPEtOX films deposited at 15W, 15% of the monomer was deposited with retention of the oxazoline ring, 20% of the film was polymerised via linear ring opening polymerisation while the remaining resulted from complex plasma lead fragmentation and recombinations. **Fig. 4a** illustrate the difference between the classic ring opening polymerisation (ROP) and plasma assisted pathways able to generate polyoxazolines. In **Fig. 4b**, and **Fig. 4c**, results demonstrating the partial retention of the oxazoline ring from two independent research groups using different analytical methods are represented, namely Zanini et al[89] NMR analysis and MacGregor et al[15] Tof SIMS results, respectively.

The application of PPOx in the biomedical field is of significant interest. **(Fig. 5)** Our group investigated the effect PPOx coatings on bacterial adhesion and biofilm **(Fig. 5.b)** [21] We determined that both 2-methyl-2-oxazoline and 2-ethyl-2-oxazoline plasma polymers have the capacity to resist *Staphylococcus Epidermidis* biofilm formation.[21] A decrease by up to 90% in bacterial adhesion was observed for film deposited in optimal condition leading to lesser retention of chemistry with some "self-sacrificial" properties, i.e. PPOx deposited at high deposition pressures and high powers. In good agreement with our recent report, self-sacrificial properties could explain the fair cell adhesion observed on Batth et al.[90] PPetOx film deposited at the lowest powers (0.25).

Building on their biocompatibility properties, we further used PPOx films to generate biocompatible nanoengineered platforms for the culture of several cell types including stem cells. (**Fig.5c**). Fibroblasts viability on MePPOx deposited at high powers (i.e. 50W) was even better than on biocompatible amine rich substrates, thus indicating that these coating were non-cytotoxic to mammalian cells. Plasma polymer films deposited from both ethyl and methyl oxazoline proved to reduce the expression of pro-inflammatory cytokines of bone marrow-derived primary macrophages, which could serve applications such as implant coatings (**Fig.5e**) [**88**]. Recently we have also shown that PPOx coating supported the growth of kidney derived stem cells and their differentiation into podocytes [**81, 91**].

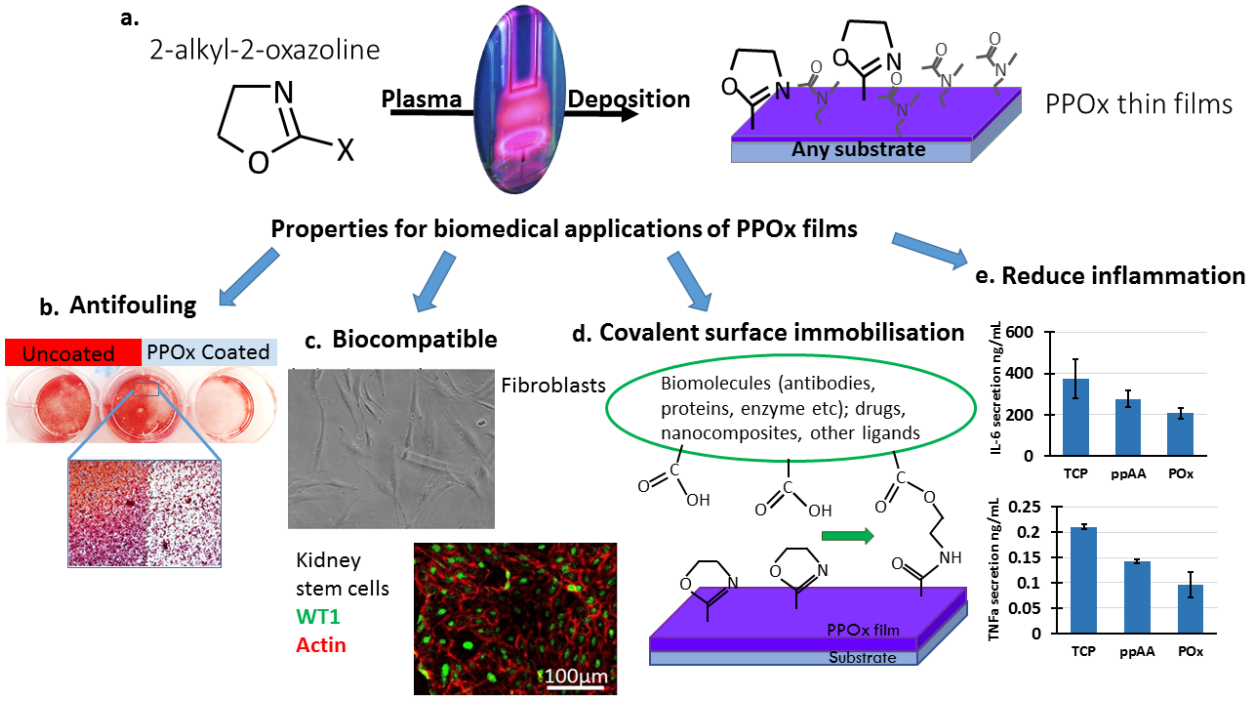


**Fig. 5.** a. Schematic of oxazoline plasma deposition pathway and resulting PPOx film composition. b. Photo of half PPOx coated tissue culture well showing high fouling on the uncoated region (left) and low fouling on the coated region (right), the bottom microscopy image is a zoom in of the coating interphase. c. Fibroblast grown on PPOx (top) and Immunochemistry images of kidney stem cells grown on PPOx. (bottom) WT1 podocyte marker in green, and red actin as counter stain. d. biomolecule covalent binding reaction scheme. e. IL-6 (top) and TNF-α (bottom) cytokine expression from bone marrow-derived primary macrophages grown on PPOx, tissue culture plates (TCP) and plasma polymerised amine surfaces. All error bars show ±SEM.

 

Looking forwards, our group is now integrating PPOx films in the development of immuno-functionalised cell-capture based diagnostic technologies,[**92**] and for the modulation of immunological responses on surfaces with controlled nanotopography [**93**]. In the cell capture platform, the role of the PPox coating is to covalently bind cancer specific antibodies which are then used to capture cancer cells from body fluid. Because of the unique way the PPOx film binds the antibodies, (**Fig.5d**) these platforms can sustain the harsh environment of natural fluids such as urine, whilst withstanding flow conditions encountered in fluidic chambers.

## Conclusion and future perspectives

We believe that by now the reader has become not only aware but also inspired by the opportunities offed by plasma polymerzation and the field of "Nanoengineered Plasma Polymer Films" overall. The capacity to modify the outermost surface of any type of material and place desired properties such as chemical, physical, topographical, mechanical or bioactive is fascinating. Furthermore, plasma polymerization allows to preserve the bulk properties of the material while the process of surface modification is fast, one step and solvent free. Surfaces can be altered to be biocompatible or biorepelling, functional or inert, wettable or non-wettable to suit a particular application. In the field of medical technology nanoengineered coatings facilitated by plasma have an enormous potential. They can be placed on stents, hip and knee implants, hearth valves and many others. They can be used as vehicles in cell therapies and drug delivery. They can be used as antibacterial coatings, to enhance tissue integration, to control inflammation, or all of these together.

These is no doubt that the field of nanoenginnered plasma polymer films will grow in the future. The number of creative approaches will also expand together with the member of potential applications. An area that has been little explored until now is the generation of stimuli responsive and smart coatings. The reason for this is that the chemical structure of plasma polymers is rather undefined and more difficult to control compared to these produced conventional polymerization. With the increased need for smart coatings and the growing number of researchers embracing plasma polymerization, it is of no doubt that imaginative approaches for direct deposition or/and by bringing in other techniques will be developed in a very near future. This will also be facilitated by revealing the complex processes occurring in the plasma and learning how to use them in our favor. When applications are concerned, an important property of plasma polymers that needs to be taken into consideration is the mechanical rigidity. This property is not trivial to measure with conventional indentation techniques since plasma polymers are usually thinner than 100 nm and often in the range of 10-20 nm. This requires the development of new techniques to measure very thin film mechanical properties, also needed in other coating methods. Collectively, nanoengineered plasma polymer coatings offer a great degree of flexibility to generate surface of a wide range of useful properties and in this way have an enormous potential to provide solution to many urgent needs in the biomedical field. However, the usefulness of these films in not limited to medical devices. Opportunities and need exist in a range of other exciting fields such as micro- and nano-fluidics and organic electronics.

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