Plasma-synthesised carbon-based coatings for cardiovascular applications

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Abstract

Current cardiovascular stent platforms interact poorly with the human vasculature and still rely on drug therapy to avoid early failure due to blood clotting. A drug-free coating technology that could fully integrate an implanted stent through a combination of hemocompatibility and differential regulation of endothelial cells and smooth muscle cells would present a clear advantage over existing clinical approaches. Plasma discharges have been used as a coating technology in a wide range of applications over the last decades. Carbon-based thin films prepared by different plasma deposition methods are usually regarded as biocompatible materials as they are able to prevent the adhesion and activation of platelets and preferentially promote the adsorption of albumin over fibrinogen. However, the available literature seldom addresses entirely the aspects of biocompatibility and the challenging mechanical demands of such materials for stent coating. Recent advances suggest that plasma enhanced chemical vapour deposition can be used to prepare carbon-based thin films that allow for the linker-free immobilization of bioactive molecules. If successfully applied to a stent these coatings could represent a step towards stent specific biofunctionalization. This review examines the feasibility of using plasma discharges for the synthesis of carbon-based biocompatible materials for cardiovascular implantable devices, particularly stents.

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Keywords: Plasma deposition; Carbon coating; Functionalization; Biocompatibility; Cardiovascular; Stent

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1. Introduction

Every year more than 6 million people undergo surgery around the world for the insertion of medical implants such as hip and knees prostheses, pacemakers, heart valves and coronary stents [1,2]. In the US alone around 1 million orthopaedic implants are inserted annually for knee and hip replacements [3] and these numbers are expected to rapidly increase to around 3 million by the year 2030 [4]. The increasing life expectancy of the population elevates demands for medical implants with a superior life span that can resist corrosion and wear, especially in load bearing and blood and/or tissue contacting applications. Additionally, insertion of exogenous materials into the body triggers immune responses in the areas surrounding the implant which can compromise implant performance and even necessitate revision surgery for implant replacement or extraction. Novel surface technologies that could mediate interactions between the implant and the host to prevent adverse reactions would clearly benefit the biomedical devices industry. In particular, there is a great demand for a universal technology capable of producing tailored biofunctional interfaces to simultaneously address a number of specific tasks depending on the application. Modulation of the surface properties such as hemocompatibility, protein adsorption / immobilization, differential attachment, proliferation and differentiation of cells are highly desirable. This review investigates the potential of using plasma-based tools as a universal coating technology for implantable medical devices, with particular focus on coronary stents. The deposition of biofunctional surfaces on coronary stents is a particularly interesting and complex case study as it combines: (i) physical–chemical, (ii) mechanical, (iii) geometric, (iv) blood clotting and (v) cellular response considerations.

1.1. Current coronary stent platforms

The World Health Organization [5] estimates that cardiovascular diseases represent 30% of total deaths worldwide, with coronary heart complications, including atherosclerosis, leading the list with a total of 7.3 million deaths every year. The same organization predicts that these numbers will rise up to 8% within the next 15 years, exacerbating an already serious problem. In the last three decades, percutaneous coronary interventions became widely used in the treatment of cardiovascular diseases and have been established as the first-line technique for revascularization of the coronary arteries. Among implantable biomedical devices, coronary stents are now the dominant vascular implant in percutaneous coronary interventions [6] with around 800,000 stents implanted annually in the US alone [7]. Stents are expandable cylindrical meshes used to re-establish the normal blood flow in blocked atherosclerotic coronary arteries, resupplying ischemic tissue. However, despite their widespread use, current stent platforms have only sub-optimal biocompatibility, interacting poorly with vascular cells and promoting blood clot formation.

The first commercially available stent platform was based on metallic alloys such as stainless steel (SS). Despite the extraordinary results in reducing the rate of abrupt vessel closure, one of the major pitfalls of coronary angioplasty, post-procedure complications related to stent implantation started to arise. The most common was in-stent restenosis, triggered by an inflammatory response due to blood vessel intima injury during stent deployment. Damage to the endothelium led to the migration and over-proliferation of underlying smooth muscle cells (SMC), causing vessel re-narrowing in a process called neointimal hyperplasia [8]. In order to overcome the draw-backs of bare metal stents, drug eluting stents (DES) were introduced. DESs are grafted with layers of biodegradable [9–11] or non-biodegradable [12–14] polymers, in which a pharmacological agent is loaded and can be locally released within a given period of time. By incorporating anti-proliferative agents, drug-eluting stents were successful in inhibiting the over-proliferation of SMC, hence reducing the effects of hyperplasia. However, an unintended consequence of DES was a substantially increased risk of long-term stent thrombosis, termed late stent thrombosis (LST) [15]. LST is mainly associated with reduced endothelialisation of the stent, leading to a delayed healing of the vessel and over-exposure of the stent struts to the blood. Another possible reason for late thrombosis could be associated with adverse inflammatory responses to the drugs and the grafted polymer used in these stents. Extensive research has been undertaken to address the issues of in-stent restenosis and late-stent thrombosis, either through the development of more biocompatible polymer-based coatings [16,17] or by integrating specific antibodies with the ability to recruit and immobilize endothelial progenitor cells [18–20], thus promoting endothelialisation. The incorporation of nitric oxide (NO) donors in the stent design has also been considered as an encouraging alternative platform to reduce in-stent restenosis and LST. NO is an exogenous signalling molecule that participates in important biological processes and possesses several vasculoprotective properties [21]. It has been shown that the administration of different NO donors inhibits the proliferation and migration of SMC [22,23], enhances the proliferation of endothelial cells [24], prevents platelet aggregation [25] and adhesion [26] to the vascular endothelium and reduces intimal hyperplasia following vascular injury [27]. The delivery of NO through eluting stent platforms has been achieved by incorporating NO donors in a polyurethane polymer which was then coated onto the stents [28] or by incorporating the NO donor with paclitaxel [29] (an anti-proliferative agent extensively used in the prevention of restenosis). However, no significant improvements in preventing...
restenosis were shown so far relative to the controls in different in-vivo animal trials.

A new generation of bioabsorbable stents [30–32] have been prompting increasing interest from the scientific community. In theory, these stents can be temporarily used has a treatment for atherosclerosis as they are dissolved in the body after a period of time, leaving behind a structurally normal and recovered blood vessel. Although promising, some issues regarding their mechanical properties, which are paramount for a proper stent deployment, radio-opacity and control over the rate that they are absorbed in the body still need to be addressed [33].

Currently used DES platforms still carry an ongoing risk of thrombosis requiring patients to be on pharmacological dual anti-platelet therapies, which can result in bleeding and is not feasible for patients requiring additional surgery. A drug-free coating technology, with all the necessary mechanical and biological properties for enhancement of the clinical performance of coronary stents, would provide a clear benefit over current stent platforms. In particular, the coating should be able to resist cracking and delamination during and after stent deployment in order to avoid the contact of the struts with the blood stream. It should also be multi-functional, promoting the adhesion and proliferation of endothelial progenitor cells, for re-endothelialisation, while also inhibiting the over-proliferation of smooth muscle cells to avoid hyperplasia.

1.2. Plasma activated biofunctional stents

Waterhouse et al. [34] reported the feasibility and safety of delivering a prototype plasma-activated coating (PAC) in vivo. This coating was deposited using hydrocarbon-based radio-frequency plasma discharges and prepared with energetic ions and a graded interface between the stainless steel surface and the PAC coating to provide sufficient adhesion. Following some initial optimisation of mechanical and biological properties the coating was applied to a stent and implanted in rabbit iliac arteries. Results showed that PAC had sufficient adhesion, undergoing stent crimping and expansion, and performed equivalently in terms of re-endothelialisation when compared to bare metal stent platforms.

Plasma discharges have been extensively used as a coating technology in a vast number of applications. The extreme versatility of plasma-based systems could be fostered to provide a universal coating platform for biocompatible and multi-functional cardiovascular implants. A considerable number of publications have reported the viability of using plasma-based technologies for the manufacture of biofunctional coatings for cardiovascular implants. These works were recently reviewed by Wise et al. [35]. Efforts have been made to deliver mechanically robust coatings with sufficient adhesion strength to metallic substrates. Wear and corrosion resistance due to fluid permeation after prolonged exposures to body fluids has also been a concern. With regards to coating biocompatibility, it is still not clear which physical and/or chemical properties play the most important roles. Bonding configuration, chemical composition, presence of dopants, surface roughness and wettability have been regarded has important factors predominantly affecting blood protein adsorption and cellular response such as platelet adhesion and activation.

Previous studies [36,37], also showed that carbon-based polymer-like coatings produced by plasma-enhanced chemical vapour deposition (PECVD) with enhanced ion-bombardment possess superior biological qualities over conventional coating alternatives (e.g. chemical vapour deposition, physical vapour deposition and wet techniques such as solvent dipping). Importantly, these coatings also allow the covalent immobilization of biomolecules [38]. The possibility to conjugate bioactive molecules to biomedical implants is particularly interesting. The development of more biocompatible stents may be achievable through a specific and controlled functionalization via the stable immobilization of bioactive proteins or peptides that can interact with the vasculature, especially by promoting endothelialisation, inhibiting the proliferation of smooth muscle cells and reducing the formation of thrombus.

2. Plasma synthesised carbon-based coatings

Among the four fundamental states of matter, (i.e. solid, liquid, gaseous and plasma), plasma is the most abundant in the Universe. For instance, the stars, interstellar and inter-galactic space, nebulae, lightning and auroras borealis are all in the form of plasma. The plasma state is achieved when enough energy is supplied to a gas, or a gaseous mixture, so that a fraction of the atoms (or molecules) that constitutes that medium becomes ionised. In contrast to a gaseous medium, formed only by non-charged species (atoms and/or molecules), the plasma medium is comprised not only of non-charged but also by charged species (ions and electrons) and photons. Therefore, the electrical and thermodynamical properties inherent to a plasma medium are far more complex and subtle than in the gaseous state. The complex interaction between positively and negatively charged particles gives rise to complex phenomena and collective behaviours, which are unique to the plasma and may vary depending on the conditions in which the plasma is produced and sustained.

Plasmas can also be produced in laboratory conditions. Humankind has been studying and developing different ways to produce and control plasmas for its own benefit for more than 130 years. Plasma discharges have found a large number of applications from illumination, metallurgy, micro-electronics, environment, materials engineering, analytical spectroscopy, medicine, etc [39]. The success of man-made plasma discharges is a result of the unique physical and chemical characteristics inherent to this medium, as well as the extraordinary flexibility achieved so far in controlling a large window of working conditions. Plasma can be artificially produced in a wide range of size scales (from micrometre up to several metres), geometries (planar, cylindrical, spherical, etc.), densities (from 10^7 up to 10^22 particles per cubic meter), pressures (from a few mTorr up to atmospheric pressure), temperatures (from negative values up to 10^8 °C) and lifetimes (from femtoseconds up to years).

Depending on the type of application, many different techniques of plasma generation can be used. For instance,
in surface modification and deposition applications it is desirable to achieve high levels of purity in the coatings. The use of a vacuum chamber, where the system is pumped down to very low pressures, allows control of the plasma composition and, therefore, also the coating composition. Magnetic fields and high voltage sources can also be used to gain control over trajectory and energy of the charged species that reach the surfaces to be coated. In the last decades for instance, different types of plasma discharges have proven their potential for biomedical applications, for a wide range of tasks such as sterilisation, cell proliferation, treatment of oral pathogens and healing of burn wounds [40]. Low pressure and low temperature plasmas have become widely used for surface modification in different applications, including the deposition of thin-films on implantable biomedical devices. Organosilicon and hydrocarbon precursors are commonly used for the creation of hydrogenated silicon films and amorphous hydrogenated carbon films (frequently named diamond-like carbon films) for a wide range of applications. The rich composition of the plasma medium, i.e., electrons, ions, atoms, radicals, molecules and photons, is achieved through efficient dissociation and ionization of precursor and carrier gas atoms and molecules, which takes place via many different kinetic mechanisms and energy transfer channels. The resulting precursor ions and radicals then diffuse towards the substrate, initiating a set of surface chemical reactions and ultimately leading to the deposition and growth of a coating on the substrate surface.

PECVD, for instance, is a popular and extremely versatile plasma-based technology for the deposition of thin films [41]. The plasma is generated at low temperatures (typically ambient temperature) inside a vacuum chamber and is typically sustained by capacitively coupling radio-frequency (rf) power, usually at a frequency of 13.56 MHz, through a matching network so as to minimise power losses within the external electrical circuit. The rf power can be coupled to the plasma either in continuous or in pulsed regimes [42]. A pulsed power modulation regime is frequently used in plasma polymerization representing an extra degree of freedom in the deposition process. Samples can also be electrically biased to accelerate the plasma ions towards the growing film. The applied bias can be either continuous DC [43], radio-frequency [44] or pulsed [45]. Control over the chemical composition of the coatings is achieved by choosing different gases (or gaseous mixtures) to generate the plasma. The coatings deposited by PECVD, especially when bias is used to increase the energy of the depositing species, are characterised by superior adhesion to the substrates, high cross-linking, improved biocompatibility and chemically active and functional surfaces. This deposition technology also allows a level of control over different properties of the films, such as roughness, wettability, hardness, density, flexibility and composition without changing the properties of the underlying substrate. This control is achieved by tuning the different process parameters—a difficult task given the complexity of the plasma and the use of reactive gaseous mixtures. Therefore, the process optimization for the deposition of thin-films with desired features is time-consuming and requires further characterization and understanding of the plasma medium.

It is paramount, therefore, to understand how changes in the process parameters, e.g. power coupled to the plasma, pressure, temperature, sample bias and different gas ratios affect the plasma physical quantities and how sensitive the coating properties are to those. Process monitoring via different plasma diagnostic techniques using electrostatic probes [46], optical emission spectroscopy (OES) [47] and mass spectroscopy [48] can ultimately assist in modulating key features in the final coating. For instance, the radiation emitted by the plasma due to electronic, vibrational and rotational atomic and molecular transitions can be analysed by means of OES and infrared spectroscopy. The plasma optical signature retains pertinent information about the most populated atomic and molecular states within the plasma. Such information can be used to derive the relevant channels of creation and destruction of those populations. Electron and ion temperature, density and their transport in the discharge can also be understood through electrical diagnostics, using for instance Langmuir probes and quadrupole mass spectrometers. The electron energy distribution function (EEDF) can also be obtained using the former diagnostics. Important macroscopic parameters can be determined by appropriate integrations of the EEDF [49] in energy space. These include, for instance, electron mobility, diffusion coefficients, drift velocity, characteristic energy, power absorbed by the plasma, plasma conductivity and all the excitation / de-excitation, dissociation and ionization coefficients related with the plasma kinetic mechanisms involving collisions between electrons and other populations. There is also a great demand for simulation models capable of describing such discharges and their operation. Plasma self-consistent modelling with the necessary complexity to describe the main plasma kinetic mechanisms, the transport of species and plasma / surface interactions are seldom available. Complex hybrid simulation tools [50] comprising state-of-the-art collisional–radiative models [51], plasma fluid models [52] and plasma / surface interactions could be developed to address fundamental investigations, reactor design and optimization to ultimately provide stabilization and reproducibility of the fabrication process. Research combining plasma modelling, plasma experimental diagnostics and coating characterization techniques will yield further important insights on how discharge parameters affect the coating properties.

2.1. Diamond-like amorphous carbon and polymer-like carbon-based thin films

The exceptional physical, chemical and tribology properties of plasma synthesised diamond-like carbon (DLC) have prompted an increasing volume of research since they were first synthesized in the form of thin films in 1971 [53]. The potential use of DLC as a biocompatible coating for medical implants in a wide range of applications has been extensively investigated in the last 2 decades. A considerable number of review papers covering these investigations are now available [54–58]. The use of DLC coating for orthopaedic prostheses...
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characterization techniques were extensively reviewed by DLC thin films [67]. The designation DLC is commonly attributed to a variety of amorphous carbon-based materials that may contain different sp² and sp³ carbon bond ratios as well as different hydrogen contents. Depending on the bonding configuration and the hydrogen content, DLC materials are usually sub-classified into: (i) tetrahedral amorphous carbon (ta-C) for higher sp³ contents (typically >70%) and low levels of hydrogen; (ii) amorphous carbon (a-C) if the ratio sp³/sp² ranges between 0.4 and 0.7 and (iii) hydrogenated amorphous carbon (a-C:H) for hydrogen containing materials which are typically characterised by higher sp³/sp² ratios but they are soft because many of the sp³ bonds are hydrogen terminated. The properties of DLC thin films, their mechanisms of deposition and their characterization techniques were extensively reviewed by Robertson [62] and McKenzie [63].

Highly hydrogenated soft a-C:H thin films are usually denominated by hydrocarbon plasma polymers [64–66]. These films are characterised by a polymer-like structure which is deposited on the substrate material. The atomic arrangement of this structure is strictly dependent on the type of carbon precursor (monomer) and the process parameters used during the deposition. Carbon-based polymer-like deposits are usually highly cross-linked and may contain large concentrations of imprisoned radicals. The mechanisms of plasma polymerization are still not fully understood and several models have been proposed in the last decades and these were also recently reviewed [67].

Carbon-based materials can be prepared as a coating in the form of a film using a wide range of plasma-based deposition techniques. Different properties of the DLC and polymer-like carbon coatings can be tuned by setting adequate deposition parameters. For instance, ta-C thin-films prepared by arc vapour deposition [68,69] are characterised by extreme hardness (up to 80 GPa), great wear and corrosion resistance and chemical inertness. These properties make ta-C potential coating candidates for load-bearing biomedical applications [70]. However, the high internal compressive stress characteristic of thick ta-C films still remains the primary source of coating failure in orthopaedic implants [69]. The exposure of the underlying metallic substrate due to poor adhesion and delamination presents one of the major obstacles to the commercial success of DLC coatings.

a-C films can be prepared using a variety of plasma-based deposition technologies. For instance, a-C materials are usually made by plasma magnetron sputtering (PMS) where a graphite target is exposed to argon plasma [71,72]. Carbon atoms and ions are sputtered from the graphite target and are then deposited onto the substrates. Substrates to be coated are usually electrically biased [73] which allows control over the kinetic energy of the carbon ions reaching the samples. Control over the bias voltage is characteristically used to deliver films with different physical structures, surface topographies and sp³/sp² ratios.

a-C:H are commonly prepared by PMS [74–76] or by plasma enhanced plasma vapour deposition (PECVD) [77,78]. In the first case, a reactive mixture of argon and hydrogen is injected in the PMS reactor. Hydrogen atoms bind with the sputtered carbon atoms leading to the deposition and growth of a hydrogenated carbon thin film. The concentration of hydrogen gas during the deposition process can also be adjusted to yield films with different hydrogen contents, hence with diverse properties as well. Highly hydrogenated a-C:H have reduced internal stress [55] resulting in improved film adhesion to the underlying substrates [79]. Hydrogen also behaves as an etching element and increasing the hydrogen concentration in the plasma was reported to promote surface smoothness of the films [62]. Control over the chemical composition of the DLC coating by PECVD is achieved by choosing different gases, or gaseous mixtures, to generate the plasma. While argon is commonly used as the background gas, different hydrocarbon precursors (acetylene, methane, ethylene, ethane, butane, etc.) are used to tailor different chemical and physical structures of the a-C:H films. For instance, increased hydrogen contents and smoother surfaces can be created by using methane (CH₄) instead of acetylene (C₂H₂) as the precursor gas [80]. Also, the incorporation of hydrogen in CH₄ plasmas was shown to reduce the coating friction and wear when deposited on steel substrates [81]. The incorporation of dopants, such as nitrogen, phosphorous, fluorine or silicon can also be easily performed by choosing the appropriate gaseous mixture. This feature is particularly interesting since the combination of these elements with DLC was shown to be paramount in tailoring the mechanical and biological performance of the a-C:H coatings. It is now generally accepted that sample bias is a fundamental process parameter in the production of DLC coatings. Films with variable sp³ fraction [82], residual stress [83], substrate adhesion [84], refractive index [85] and hemocompatibility [86] can be prepared by adjusting solely the sample bias while maintaining constant the other process parameters. Importantly, sample bias promotes the creation of radicals in the coating’s surface and bulk, allowing for a further functionalization of the coated materials with bioactive proteins, peptides and other biomolecules [36].

2.2. Improving the mechanical stability of carbon-based coatings

a-C and a-C:H made by PMS and PECVD are generally characterised by a reduced internal stress and improved substrate adhesion when compared to ta-C films. However, and despite their excellent biocompatibility, these materials still can’t fulfil all the requirements necessary for a permanent and successful translation to real medical implants. These coatings are particularly sensitive to wear and corrosion, especially when deposited on metal substrates and exposed to saline solutions or biological fluids. It has been observed that the prolonged exposure of the coatings to fluids reduces the interfacial strength, ultimately leading to delamination and spallation at the coating / substrate interface [61,87,88]. Chandra et al. [88] proposed that failure of DLC coatings after exposure to fluids, such as water and phosphate buffered saline (PBS), was related with the permeation and perforation
of the solution through porosities and failures localised at the coating surface.

A great amount of research has been undertaken to address the issue of coating failure due to poor: (i) substrate adhesion and (ii) wear and corrosion resistance. A potential solution for the first would be to increase the interfacial strength between substrates and carbon-based coatings through the addition of interlayers [87–93]. The deposition of silicon-based thin layers between metallic substrates and DLC coatings, prepared by rf—PECVD, can significantly increase the adhesion strength of DLC to stainless steel (SS) substrates. SiNₓ interlayers have been reported as particularly effective for the enhancement of both adhesion and coating corrosion resistance of samples incubated in saline solution up to 2 years [87]. The deployment of an a-Si:H to a-C:H graded interface (also prepared by rf—PECVD) was shown to increase the adhesion strength and corrosion resistance of DLC on Ti alloys [88]. The addition of 90 nm thick Si interlayers prior to DLC deposition on CoCrMo substrates resulted in a substantial enhancement in the adhesion strength when compared to non-interlayered samples [92].

Further enhancement of DLC adhesion strength to metallic substrates can be fostered by creating a graded interface between the metal surface and the carbon layer. Graded metallic interfaces were previously reported and performed on SS substrates prior to the deposition of carbon-based plasma polymers (a-C:H ) using a low pressure PMS system [94]. A SS target is bombarded by energetic argon atoms coming from the plasma, which ultimately leads to the sputtering of metallic ions from the target and their subsequent deposition on the substrate surface. If a hydrocarbon precursor (such as acetylene) is gradually added to the plasma during the sputtering process, it is possible to create a metal-carbon graded interface. This graded interface is characterised by an increasing carbon to metal content from bottom to top with the top layer being exclusively composed of DLC or a carbon-based plasma polymer. The adhesion strength of DLC coatings to SS substrates prepared with graded layers was reported to be greater than 26 MPa. This value represents an improvement of 260% when compared to coating deposited directly on the same substrates [94].

2.3. Biocompatibility

Biocompatible coatings for cardiovascular implants should be inherently non-thrombogenic and able to recruit and promote the proliferation of endothelium cells while inhibiting the attachment and overgrowth of smooth muscle cell (SMC) layers. They should fully integrate into the human vasculature by prompting healing responses to prevent implant rejection and severe immune responses post-implantation. Implant hemocompatibility can be achieved by preventing the adsorption and further denaturation of undesirable blood proteins that mediate the pathways of blood clot formation. The attachment, on the coating surface, of bioactive molecules that could prevent platelet adhesion and activation and that could be capable of further inducing favourable local cellular responses would represent a great advantage.

Although plasma synthesised carbon-based coatings were shown to retain good hemocompatibility in various in-vitro essays, in-vivo investigations to access their capacity for endothelialisation, SMC inhibition and hemocompatibility are seldom reported. Good results of these coatings in the great majority of such in-vitro tests were explained by the higher affinity of the surfaces to preferentially adsorb albumin over fibrinogen. However, in-vitro tests poorly simulate the harsh conditions of the human vasculature such as the high flow regimes of blood in arteries. High flow regimes can lead to desorption of adsorbed or weakly attached proteins. For this reason covalent immobilization is preferred over physical adsorption. Recent advances [36] showed that PECVD prepared carbon-based coatings with high concentrations of long-lived unpaired electrons can be used for the covalent attachment of various biomolecules in their bioactive form. These advances could establish a new era of truly bio-functional and -compatible cardiovascular implants.

2.3.1. Adsorption of blood proteins

The biocompatibility and particularly the hemocompatibility of a blood-contacting surface are strongly affected by the affinity of the surface to adsorb specific blood proteins. The time scale on which blood proteins interact with and are adsorbed on a coating’s surface is several times smaller than that of any of the interactions involving other blood components. For this reason, local cellular response to a foreign body implanted in a blood vessel will be in part governed by the nature of the proteins adsorbed on its surface. For instance, it is known that adsorbed fibrinogen (Fib) enhances the adhesion and activation of platelets [43], triggering the coagulation pathway. Furthermore, Fib is also a fundamental element in the coagulation cascade since it is cleaved into fibrin to form an irreversible thrombus. On the other hand, albumin can inhibit the adhesion and activation of platelets, preventing the formation of a clot.

The mechanisms governing the adsorption of different blood proteins on carbon-based surfaces are not yet fully understood. The subject has driven increasing research in the last two decades and although some advances have been achieved, important questions remain unanswered. Difficulties arise in determining exactly which physical and/or chemical properties of the surfaces rule protein adsorption. Available data suggest surface roughness and surface energy, hydrogen and nitrogen fraction, sp³ content and electrical band-gap as possible candidates. However, these properties are generally interlinked and their decoupling is largely limited by the deposition technique used to prepare the surfaces.

It has been suggested that the adsorption of blood proteins on a DLC surface is related to the charge (electrons) transfer between the proteins and the surface [56,95]. Protein adsorption and further denaturation occurs if the Fermi energy level of the protein is such, that electrons from its valence band are transferred to the conduction band of the coating material [95]. Therefore, hemocompatible coatings should be made with electron energy band gaps greater than those of proteins that are responsible for the formation of thrombus—such as
fibrinogen, which has a band gap of 1.8 eV [96]. On the other hand, adsorption of albumin would be favourable as it can inhibit the activation of platelets on the surface of the film.

It has also been reported that surface roughness and wettability may influence the adsorption of blood proteins onto the surfaces [80,97–99]. Albumin for instance was described to have greater affinity for more hydrophilic and rougher ta-C surfaces, made by filtered arc deposition, when compared to smoother and less hydrophilic a-C:H thin films prepared by plasma-enhanced chemical vapour deposition (PECVD) [80]. However, different chemical compositions (e.g. hydrogen content) and bonding configurations between the different samples may have also influenced protein adsorption. Using spectroscopic ellipsometry (ES) [97] suggested that different a-C:H coatings made by plasma magnetron sputtering can adsorb human serum albumin (HSA) and human fibrinogen (Fib) differently. In this work the surface concentration ratio (proportional to the thickness of the adsorbed protein layer) HSA/Fib~1 was higher for samples prepared with floating potential whereas surfaces made using a negative bias presented a ratio HSA/Fib~0.4. Later the same authors [85] showed that a-C:H coatings made under the same conditions presented a different behaviour with respect to HSA and Fib adsorption. The HSA/Fib thickness ratio was found to increase on samples prepared with bias, which were identified by AFM as being rougher. Berlind et al. [99] studied HSA adsorption on different amorphous, graphitic and fullerene-like surfaces also using spectroscopic ellipsometry. They found that amorphous structures, i.e. with smoother surfaces, adsorbed more HSA when compared with graphitic and fullerene-like thin films. However, the authors could not correlate a higher HSA adsorption exclusively with the roughness of the thin films. This was because the different films also presented different chemical structure, wettability and water-binding affinities which are important parameters that also influence protein adsorption. Available data suggests that blood protein adsorption is a complex phenomenon and there is still no clear consensus on its drivers. Additional investigations are therefore necessary to understand the basic phenomena of protein/surface interactions and how different features in the surfaces can regulate differential protein absorption.

2.3.2. Surface functionalisation with immobilised bioactive molecules

Recent studies found that rf-PECVD carbon-containing plasma polymers prepared with bias have the ability to covalently immobilise proteins in their bioactive form [36,38]. Organic precursor plasma discharges can be used to deposit hydrophilic plasma activated radical-rich a-C:H-like coatings (PAC) if sufficient sample bias, usually up to ~1 kV, is provided [100]. Here, the ions formed in the plasma are accelerated with increased energy provided by the high voltage bias towards the growing carbonised film on the substrate surface. Ion impact promotes the formation of regions of carbon extended states such as π conjugated sp² carbon bonds with a high degree of cross-linking. These regions contain stable unpaired electrons that can move and diffuse towards the coating surface. Free-radical content, measured by electron paramagnetic resonance spectroscopy, was found to be proportional to the bias voltage and thickness of the deposited film [94]. Colorimetric assays using immobilised horseradish peroxidase catalase as a probe have been employed to confirm the capability of PAC coatings to retain the bioactivity of the immobilised protein layer [38,45,101]. A variety of polyanino acids have also been found to be covalently attached to PAC surfaces via chemical bonding with the unpaired electrons on the film’s surface [36].

Metallic substrates such as stainless steel when coated with PAC alone are significantly more hemocompatible, a property revealed by its ability to considerably reduce the formation of human thrombus in different in-vitro assays. Blood compatibility is an essential characteristic for biocompatible cardiovascular stents and although necessary, it does not address endothelialisation and restenosis. A stable immobilization of bioactive biomolecules on the coating surface could represent a leap forward towards the true biofunctionalization of cardiovascular implants such as arterial stents. Plasma-made radical-rich coatings for stents can be envisaged, immobilising the appropriate biomolecule or biomolecule cocktails, to impart appropriate local biological responses to achieve full vascular integration of stents (see Fig. 1).

The advances discussed above suggest that chemical conjugation of proteins through the reactions of unpaired electrons with protein amino acid side chains is possible while preventing protein denaturation. It was shown that PAC surfaces could be used for the immobilization of tropoelastin in a bioactive conformation on flat stainless steel substrates [37]. Tropoelastin, being a precursor of elastin, is an important vascular protein since it participates in the correct structuring and functionalization of the whole vascular system. Elastin provides fundamental rheological properties to blood vessels, facilitates endothelialisation and constitutes (together with collagen) the sub-endothelia layer, the lamina, interfacing the intima and the media. As an essential component of the lamina, elastin fibres play an important role in avoiding infiltration of smooth muscle cells towards inner layers of the blood vessel while promoting and regulating the growth and proliferation of luminal endothelial cells to form a healthy endothelium. Covalently immobilised tropoelastin and specific domains of tropoelastin were shown to drastically enhance the hemocompatibility of PAC coated SS substrates in in-vitro assays using human whole blood [102]. Endothelial cell attachment and proliferation were also improved when compared to uncoated samples.

More recently, PAC surfaces functionalised with bioactive plasmin revealed superior human blood compatibility and endothelialisation [103] when compared to SS controls. Plasmin is an enzyme, derived from plasminogen, present in the blood that cleaves fibrin clots in a process called fibrinolysis. When conjugated with PAC, plasmin is able to prevent the formation and deposition of fibrin complexes reducing the probability of clot development. Hemocompatibility and good in-vitro proliferation rates of endothelial cells suggest that stents functionalised with PAC immobilised plasmin could prevent early thrombogenicity while promoting endothelialisation for stent vascular integration.
PAC conjugated with constituents of high density lipoproteins were also recently studied for hemocompatibility, endothelialization and smooth muscle cell proliferation [104]. A low concentration of high density lipoproteins in the blood was previously associated with restenosis and thrombosis after stent deployment and coronary angioplasty. PAC was shown to covalently immobilise constituents of HDL while retaining its bioactivity. Stainless steel PAC coated substrates functionalised with HDL strikingly reduced the formation of human blood clots in-vitro. Moreover, HDL coated surfaces encouraged endothelialisation and prevented the over-proliferation of smooth muscle cells.

3. Translation of carbon-based coatings to coronary stents

Significant efforts have been made to develop biocompatible coatings for metallic cardiovascular implants and especially for coronary stents. Although plasma discharges are perceived as a promising solution to fulfil this task, a great deal of research still needs to be carried out. Despite the increasing volume of investment and research, there is still no available plasma-based coating technology for stents proved to successfully address all the aspects of biocompatibility in humans.

3.1. Challenges

Stents are three-dimensional objects with wide-ranging dimensions, aspect ratios, complex geometries and strut designs. Consequently, coating uniformity using appropriate plasma discharges such as low pressure PECVD or PMS is a non-trivial task. One of the main challenges is to avoid edge and shadowing effects on the stent struts caused either by adjacent struts or by the electrical connections used to support the stent in the discharge. rf-PECVD discharges can provide stable plasma media that uniformly surround flat surfaces during the deposition process. Also, when using sample biasing, ions are accelerated uniformly towards the full length of flat surfaces when a uniform sheath lies between the plasma and the sample surface. However, the intricate design of a stent and the use of electrical and supporting contacts give rise to the development of complex plasma sheaths that are not totally conformal to the stents. For this reason a thickness gradient in the coating across the stent outer surface is usually hard to avoid. Moreover, while the outside struts are directly coated and bombarded with energetic ions from the surrounding reactive plasma, the inner struts are exposed only to the poor plasma and less energetic ion flux inside the stent. Therefore, the physical and chemical properties of the coating are generally spatially dependent. Coating thickness, for instance, is usually thinner on the inside struts. Importantly, the bonding configuration has also been reported to be different in the outer and inner struts with the latter showing lower sp³/sp² ratios [105].

Strut shadowing is particularly accentuated when using PMS discharges as the great majority of these platforms are built with planar magnetrons providing only unidirectional sputtering. When no rotation is applied to the stent, gradients in the thickness of the coating are to be expected as thicker layers are deposited on the struts facing the magnetron. An enhancement of the coating uniformity could be achieved using, for instance, hollow cathode magnetrons (HCM) instead of planar ones. HCM discharges [106–108] use cylindrical targets that surround the samples to be coated. When the plasma is generated the sputtered material covers the outer layers of the samples facing the target. In addition, rotation can be applied to minimise shadowing of the inner struts. Using this technology could, however, still face some possible drawbacks. Shadowing would not be totally eliminated since a mechanical support to hold and rotate the stent inside the magnetron would still be required. Additionally, it is known that coating properties, such as thickness, are strictly dependent on the aspect-ratio (radius to length ratio) of the magnetron [108]. Therefore, custom-made HCM reactors would need to be built to fit specific stent dimensions, a demand that could largely decrease the cost efficiency and compromise the universality of the process.

Stent design imposes a further obstacle with regards to its surface characterization. An accurate determination of the bulk
and the surface physical (roughness, wettability, etc.) and chemical (elemental composition, atomic bonding configuration, etc.) properties using the standard characterisation and spectroscopic techniques is difficult to achieve. The biocompatibility of coatings deposited on stents is even more challenging to assess in-vitro since it is hard to reproduce and quantify cellular attachment and proliferation on non-two-dimensional flat surfaces.

Stents are subjected to extreme radial and longitudinal forces communicated by an expandable catheter balloon during deployment. Localised plastic deformation follows stent expansion and for this reason the coatings should be extremely robust to avoid cracking and spallation in the areas subjected to high strains. Fig. 2 shows examples of carbon-based coated SS stents that underwent balloon expansion. The coatings were prepared by PECVD using acetylene as a precursor gas. Micro-sized cracking and delamination occurred in the regions where strain was more accentuated which is shown by the development of plastic deformation in the strut—Fig. 2a and b. Although spallation was not propagated throughout the strut, exposure of such coating to the blood stream could aggravate delamination leading to further peeling. Fig. 2c–f show peeling and spallation examples of similar coatings following crimp and balloon expansion. Low adhesion strengths of the coatings resulted in a generalized peeling and major areas of the underlying stent material were exposed.

Given these reasons it is no surprise that the vast majority of the investigations available in the literature are merely preliminary, reporting the deposition and characterization of plasma-synthesised coatings on flat substrates. For instance, the adsorption of blood proteins, the adhesion and activation of platelets and the attachment and proliferation of smooth muscle cells and endothelial cells are typically assessed on coatings deposited on Si-wafers and flat metallic samples. Also, correlations between: (i) plasma deposition parameters, (ii) coating physical, chemical and mechanical properties and ultimately (iii) coating biocompatibility are also performed in similar circumstances. However, one should expect that coatings grown on flat samples would perform differently from actual coated stents in in-vitro and particularly in in-vivo. Only few works addressed these issues but not to their full extent.

3.2. Plasma-assisted coated stents

Polyethylene glycol (PEG) conjugated DLC coatings on nitinol flat samples revealed superior in-vitro hemocompatibility presenting higher albumin/fibrinogen adsorption rates and decreased platelet adhesion when compared with bare metal and DLC surfaces [109]. However, PEG/DLC coated nitinol stent performance in canine iliac arteries was substantially inferior revealing more developed neointimal hyperplasia areas compared to DLC stents and even to control non-coated nitinol stents [110]. The authors associated restenosis with the accumulation of a thick collagen layer formed due to over proliferation of fibroblasts on the PEG surface, a factor that had been overlooked in the previous in-vitro assays.

Kim et al. [93] studied the mechanical properties of DLC coatings deposited on self-expandable nitinol vascular stents using a focused ion beam system and acetylene gas as a carbon precursor. Interlayers of Si were also deposited by magnetron sputtering between the stent surface and the DLC layer for assessment of potential enhancement of the coating adhesion strength. Stents were exposed to high stresses through a mechanism of stent contraction and expansion. DLC coatings deposited without Si interlayers were found not to withstand stress as delamination and spallation occurred at the strut/coating interface. Cracking and delamination was prevented when a-Si interlayer thicker than 0.6 nm was used. Unfortunately, there is no detailed information on the chemical, physical and biological characteristics of these DLC coatings. Similar results were found elsewhere [61] on PECVD prepared DLC coated stainless steel stents. The addition of an a-Si:H interlayer substantially enhanced the mechanical stability of the carbon coatings following stent expansion. However, the adhesion of similar coatings to stainless steel flat substrates was significantly compromised after 1 month of incubation in biofluids. Endothelial cell and platelet attachment was also studied on DLC coatings deposited on Si substrates. Superior endothelialisation and reduced platelet adhesion were found for thermally annealed a-C:H films doped with Si. Unfortunately, these results were not confirmed on stents prepared in the same conditions.

PAC coated 316L SS stents have been tested for both mechanical stability and in-vivo performance [34,111]. The inclusion of nitrogen in acetylene/argon PECVD discharges provided improvements in the mechanical properties by reducing the compressive stress and in increasing the elasticity of the coatings. Superior ability for tropoelastin covalent immobilization on 316L flat samples was observed for coatings prepared under such conditions, which was congruent with an enhancement in endothelialisation. PAC coated directly onto stents prepared in such circumstances resisted delamination and spallation following a balloon expansion with only nano-sized cracks emerging but without exposing the underlying strut. Graded interfaces between the metallic strut surface and PAC coatings can be made to further enhance PAC mechanical stability in animal trials [34]. Although PAC alone did not present significant in-vivo improvements in stent biocompatibility, further functionalization of PAC surfaces with specific strongly bounded bioactive biomolecules could overcome the limitations of current stent platforms.

4. Conclusions and future perspective

Carbon-based coatings, prepared either by plasma magnetron sputtering (PMS) or by plasma enhanced chemical vapour deposition (PECVD), are among the most promising plasma-based coatings for cardiovascular implants, particularly stents. These are generally characterised by a reduced internal stress and improved hemocompatibility. Recently, carbon-based plasma polymers were reported to prevent the adverse response to metallic substrates through linker-free immobilization of bioactive molecules via free-radicals embedded in the coating. The radicals present in the coatings, prepared by PECVD with the aid of high voltage bias, allow for the covalent binding of various types of proteins. Because the proteins maintain their bioactivity, these coatings can regulate favourable cellular
responses and trick the body to recognise the implant as a non-
foreign object. The one-step and linker-free covalent immobi-
lization of a mixture of biomolecules capable of performing
different tasks independently would be extremely advanta-
geous, fostering novel and totally drug-free biocompatible
stent platforms.

Despite the advances, considerable improvements must be
made for a successful commercialisation of biocompatible coated
stents using plasma discharges. The majority of the published
investigations in the field are still very preliminary. Plasma-based
coatings are still frequently deposited and characterised on
flat samples such as Si wafers and/or metallic flat sheets. The
complex 3D geometry of a stent sets an additional challenge.
Uniform coatings are hard to achieve due to shadowing effects
either caused by the stent itself or by the electrical connections or
mounts used to hold the stent in the plasma chamber. Further-
more, it is also expected that the physical, chemical and
tribological properties of coatings deposited on flat samples.
would not be necessarily the same to those deposited on real 3D samples. Therefore, further in-vitro and in-vivo essays are certainly needed to access plasma-coated stent performance.

Additionally, a full understanding of the fundamental processes governing the plasma medium during the deposition process is far from being achieved. The use of reactive mixtures containing hydrocarbon precursors gives rise to complex plasma chemistries. The use of sample biasing, the three-dimensional geometry of the samples and the addition of reactive dopants such as nitrogen or hydrogen represent additional challenges. The main kinetic mechanisms and the physical quantities to describe such discharges are not yet clear, demanding therefore the development of advanced plasma modelling tools capable of describing the complexity of the plasma medium. Principally, a connection between the process parameters and their effects on the final coating properties are not yet elucidated. Not even for simplest gaseous mixtures. The mechanical stability of the coating, its capability to be further functionalised and its biocompatibility needs to be thoroughly investigated as a function of changes in the

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**Fig. 3.** A proposed strategy for the development of a controllable and stable process for the production of biocompatible coatings for cardiovascular stents using plasma discharges (red arrows indicate the flow of information). This strategy involves the development of plasma hybrid simulation tools fitted with appropriate models capable of describing the main physical quantities of the plasma, plasma chemistry and transport and plasma interaction with surfaces during the deposition. Model results need to be confirmed and validated with experimental measurements obtained by means of plasma diagnostics. Ultimately, such models would be used for a complete scaling and optimization of the plasma reactor. Coated stents must be tested for mechanical stability, wear and corrosion resistance. Also, the physical and chemical properties of such coatings should be determined using appropriate characterization techniques. Coating functionalization through stable covalent immobilization of bioactive molecules is then performed. These molecules (or cocktail of molecules) should be chosen to address thrombogenicity, endothelialisation and restenosis and their efficacy should be tested in in-vivo, ex-vivo and in-vitro. Iterative feedback between plasma modelling, plasma diagnostics, coating characterization (before and after functionalization) and coating biological performance offer a strategy to identify the correct window of plasma parameters for the production of coatings with the necessary mechanical, physical, chemical and biocompatible properties.
physical and chemical properties induced by varying appropriate process parameters. Combinations of plasma discharge simulation tools, plasma diagnostics and coating characterization techniques could be used to identify the relevant window of plasma parameters for a reproducible and stable process (see Fig. 3) and ultimately offer feedback control strategies. Such an interdisciplinary endeavour will only be possible through combined efforts of scientific communities in plasma physics, materials engineering, biology and medicine.

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