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Review

Overview on the Antimicrobial Activity and Biocompatibility of Sputtered Carbon-Based Coatings

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Abstract: Due to their outstanding properties, carbon-based structures have received much attention from the scientific community. Their applications are diverse and include use in coatings on self-lubricating systems for anti-wear situations, thin films deposited on prosthetic elements, catalysis structures, or water remediation devices. From these applications, the ones that require the most careful testing and improvement are biomedical applications. The biocompatibility and antibacterial issues of medical devices remain a concern, as several prostheses still fail after several years of implantation and biofilm formation remains a real risk to the success of a device. Sputtered deposition prevents the introduction of hazardous chemical elements during the preparation of coatings, and this technique is environmentally friendly. In addition, the mechanical properties of C-based coatings are remarkable. In this paper, the latest advances in sputtering methods and biocompatibility and antibacterial action for diamond-based carbon (DLC)-based coatings are reviewed and the greater outlook is then discussed.

Keywords: diamond-like carbon; biomedical applications; antibacterial; biocompatibility

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1. Introduction

Nanotechnology has been a great contributor to progress in the efficient development of new materials with several applications, including biomaterials [1]. Among nanomaterials, carbon-based nanomaterials are attractive because they can be applied in a vast number of emerging and even existing applications, especially in electrochemistry [2,3], mechanics [4], tribology [5], and optics [6,7]. Another main field where carbon nanomaterials have been intensively used and developed is in the biomedical field [8–11], i.e., in biosensors [12,13], drug delivery [14,15], tissue engineering [16], and photothermal therapy [17–19]. Besides biocompatibility, carbon nanomaterials have a set of outstanding properties that allows them to be used in the aforementioned applications, namely, a wide variety of morphologies, excellent mechanical behavior, high thermal conductivity, good corrosion resistance, excellent photoluminescent properties, high transparency, and structural stability [1,5,20–22].

Carbon is one of the most abundant elements on our planet. This element can bond to itself in unique architectures to form nanomaterials that can be grouped in 0D (fullerenes), 1D (nanotubes), 2D (graphene), and 3D (diamond-like carbon) architectures [1,5,23].

Nanodiamonds and thin diamond-like carbon (DLC) films have shown some diamond-like properties that make them attractive to various industries. DLC has high hardness,

insulation, wear resistance, chemical stability, gas barrier properties, anti-burn characteristics, infrared permeability, biocompatibility, and a low coefficient of friction. Moreover, the growth of these types of films can be performed at low temperatures (~200 °C) [24]. Consequently, the range of applications is huge, including use in cutting tools (drills) [25–27], automotive parts (piston rings, clutch plates, pumps, injectors) [28–31], optical components (lenses) [32], oxygen barrier films for plastic bottles [33,34], sanitary equipment (faucets) and bathtub mirrors [35], decorative items, windows [36,37], electrical and electronic equipment (hard disks, integrated circuits) [38,39], and molds (injection molding) [40–43]. Due to the excellent wear resistance and low friction properties, automotive applications are growing exponentially. The application of DLC in the medical field has also been growing due to its high biocompatibility and biocidal response, thus becoming a promising material in orthopedic, cardiovascular, and dental applications [1,44–50].

Response against a wide range of microbes and biocompatibility are two crucial parameters for the success of carbon-based materials in biomedical applications. Several studies have focused on these issues; however, since biomedical devices may be introduced in several different biological environments, some controversial conclusions have been drawn related to antimicrobial and biocompatibility features. In this sense, this work will focus on the biological response of DLC coatings providing the last findings. Both the DLC structure and the most common preparation process are also highlighted, which indeed can be considered as sputtering (a PVD technology). A brief discussion on the biomedical applications of this carbon-based material is also presented.

The search strategy and selection criteria used in this review were based on PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-analyses). Searches were performed in the PubMed-MEDLINE, Scopus, and Web of Science databases. Publications were limited by date (until 15 March 2021) and journal type (original research, review articles, proceedings papers, and book chapters). The search query included the following term combinations: "diamond-like carbon AND (biocompatibility OR "cell adhesion" OR "cell growth")" and "diamond-like carbon" (all fields) and "biomedical" (all fields) AND ("physical vapor deposition" OR "chemical vapor deposition" OR "DC/RF sputtering" OR "plasma-enhanced chemical vapor deposition (PECVD)" OR "pulsed laser deposition" OR "filtered cathodic arc (FCA)" OR "plasma immersion ion implantation" OR "direct ion beam (IB)" OR "electron cyclotron resonance plasma chemical vapor deposition (ECR-CVD)" OR "ternary phase diagram")). From this first selection, other specific criteria related to orthopedic, cardiovascular, and dental applications were applied. Duplicate references were excluded. The titles and abstracts of the remaining articles were read and articles related to the theme/objective of this review were selected. Articles that were irrelevant in view of this reading were excluded, namely, articles whose topic was far from DLC coatings produced from PVD. In addition, a patent database was consulted using the same specific criteria used in the aforementioned reference databases. The results obtained were subjected to the same analysis, and a sparse number of records were obtained for the purpose of this manuscript.

2. Diamond-Like Carbon

Diamond-like carbon (DLC) films are amorphous carbons with variable sp^2, sp^3 carbon, and -H bonds. Depending on the bond contents, DLCs can be named as follows: ta-C and a-C for hydrogen-free DLCs with high and low C-C sp^3 bonds, respectively; ta-C:H or a-C:H if the DLC films for hydrogenated with high or low C–C sp^3 bonds, respectively (Figure 1); in the crystalline state, sp^2 occurs as graphite and sp^3 occurs as a diamond form [23,51]. The presence of sp^2 and sp^3 leads to an amorphous structure, as in the case of DLC films, where the ratio of sp^3/sp^2 ranges between ~10% and ~90%. This amorphous combination was first confirmed by Aisenberg and Chabot in an attempt to fabricate diamond, in that case, using a carbon ion beam [52]. The denomination of DLC films includes several types of carbon-based films, which mainly depends on the proportion between sp^2 and sp^3 bonded carbon and doping elements, such as metal/carbide (Ag, Cu,

Co, W, . . .) or hydrogen [24]. Hainsworth et al. [53] showed a systematic terminology in this regard (Table 1). A hydrogen-free amorphous carbon film with prevailing sp^2 bonds is denoted as a-C, whereas the hydrogenated film is termed as a-C:H. In the case of the films where sp^3 has a significant fraction (about 70%), in the absence of hydrogen, it is denoted as ta-C, and if the film has hydrogen, it is ta-C:H. The DLC films doped with metal elements are termed Me-DLC. In order to organize the existence of regions in the several compositions of DLC, Robertson et al. proposed a ternary diagram where the axes denote the sp^3 bond, sp^2 bond, and hydrogen contents [54].

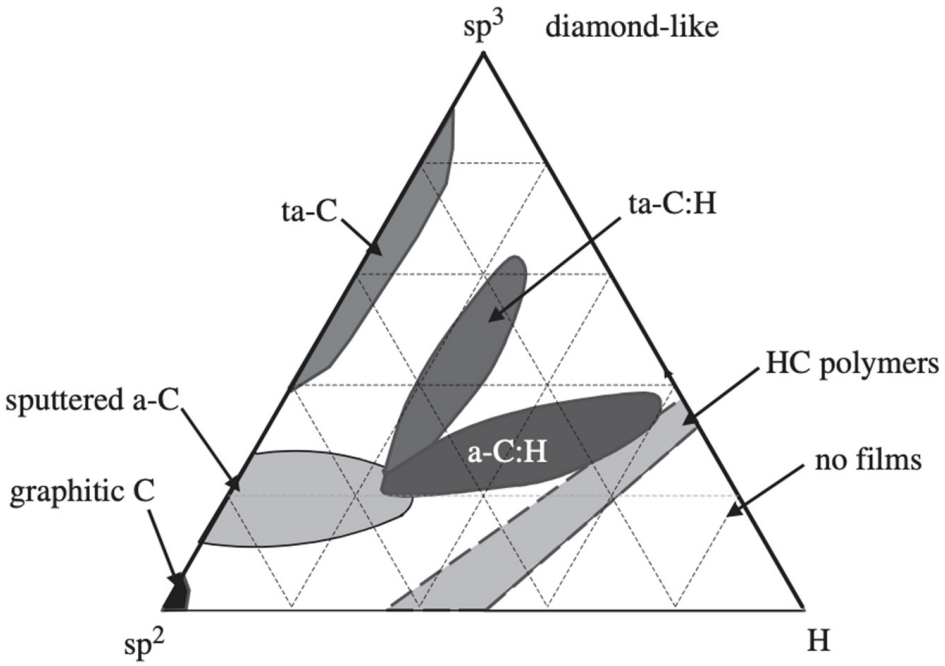

Figure 1. Ternary phase diagram of DLC films according to their sp^2, sp^3, and hydrogen contents. Reprinted with the permission from [55]. Copyright 2004 The Royal Society.

Table 1. Terminology applied to different types of DLC films (a denotes amorphous and ta denotes tetrahedral amorphous).

Term	Hydrogen	sp^2	sp^3	Metal Doping
a-C	No	Main		
a-C:H	Yes	Main		
ta-C	No		Main	
ta-C:H	Yes		Main	
Me-DLC	No			Yes

Different types of metallic and non-metallic elements can be integrated into the DLC matrix to improve the tribological characteristics due to the lack of reactivity of this coating. These elements are Si, F, B, Ti, Al, Mo, Co, Fe, Ni, Cu, W, Zr, Ag, Au, and N. Some of them can improve adhesion or decrease residual stresses, which may open new opportunities for materials composed of different chemical elements. Depending on the type and concentration of the third element added [17], the hydrophobicity and electrical properties are the DLC properties that experience the greatest changes [24,35]. In biological terms, the metals that are more interesting regarding their antimicrobial features, including antiviral features, are Ag [49,50,56–59], Au [60–66], and Cr [67–69].

2.1. Biomedical Applications

When such a material is implanted in the body, it must be biocompatible, which means that it must have certain characteristics, such as being chemically inert to cells and bodily

fluids. Thus, when implants are placed in a living body, the constituent materials must resist the corrosive environment and not cause inflammatory or repulsive reactions in the body or any other undesirable effects. In addition, the materials have to perform the desired function, such as feature low wear and rapid bone growth, over a long period of time, as is most commonly the case with orthopedic prostheses.

The common process for evaluating new materials for implants or other biomedical applications involves in vitro cell experiments. Although these experiments cannot determine biocompatibility in vivo, they can be a good guide for the biological response of a surface to be confirmed by in vivo experiments [70].

Several biomedical applications can be introduced in the wide list of DLC applications, as was mentioned before. Regarding the biological response of DLC, this section focuses on blood contact and orthopedic applications.

Blood contact applications can include blood pumps and heart valves. In these applications, in addition to good tribological behavior, good hemocompatibility is required. The key issue here is the composition of the implant surface to prevent the formation of thrombi that can lead to coronary heart disease. To assess this property, the adhesion of blood platelets on this surface is usually evaluated, since it is these elements that lead to thrombus formation [71–73].

Stents are also included in this type of application. A stent is a metal tube that is permanently inserted into an artery. The stent helps open an artery so that blood can flow through it. Despite the increasing number of applications worldwide, applications are limited by restenosis, occlusion, and thrombosis associated with stenting. Consequently, an ideal stent should possess the following properties: (1) ability to be crimped on the balloon catheter; (2) good expandability ratio; (3) sufficient rim radial strength and negligible recoil; (4) sufficient flexibility; (5) adequate radiopacity/magnetic resonance imaging (MRI) compatibility; (6) high thromboresistivity; (7) no restenosis after implantation; (8) non-toxicity; and (9) drug delivery capability [74]. The main side effect of artery stents lies in their metal ion release and thrombogenicity. Due to its composition, after suffering corrosion, stainless steel can release Cr, Ni, Mn, and Mo ions to blood when implanted in coronary vessels [75,76]. Thus, it is necessary to coat metal stents with suitable biomaterials that are hemocompatible, corrosion-resistant, and durable in the human blood environment.

In recent years, due to the biocompatibility already demonstrated in other cases, research on DLC as a coating for stent applications has increased [77–82]. In addition to the interest in biocompatibility and hemocompatibility, researchers have also been studying the antibacterial ability of DLC in stents, such as in the work of Carvalho et al. [59,83].

Another application that may be in contact with blood is the guidewire. Medical guidewires are used to introduce catheters, stents, and other medical devices inside the human body. A good guidewire must possess a specific inertia, high flexibility, very low coefficient of friction, and short-term biocompatibility for effective advancement through a vessel. Stainless steel is the most commonly used material for this type of wire and is coated with polytetrafluoroethylene (PTFE) or silicone overlays to achieve better lubricity and low friction. The importance of these properties is due to the need for the guidewires not to cause any trauma to the implant or vessel walls when inserted into the blood vessels. In the case of PTFE-coated stainless steel wires, these situations can occur due to non-uniformity, instability, and, above all, poor adhesion. This means these coatings may lead to the side effects of non-uniformity, instability, and poor adhesion with stainless steel resulting in the release of coated materials. With excellent self-lubricating properties and biocompatibility, DLC has attracted attention for this application [70,84,85].

The most common applications that are not in direct contact with blood are coatings for orthopedic prostheses. The main problem with prosthetic joints lies in their wear and corrosion during long-term use. Wear leads to the formation of residues (in tribology, the known third-body,) that can consequently lead to tissue inflammation, osteolysis, and finally the detachment of implants. With a sliding motion between two surfaces, the one with lower hardness suffers greater wear. Thus, in prosthetic joints, the coating

material applied to the prosthesis, which is usually metallic, must be sufficiently hard and inert to prevent wear and corrosion, and additionally have good adhesion to the substrate. As in the case of the guidewires, after long-term use, these metals usually show some problems regarding cytotoxicity, corrosion, wear, and the release of metal ions. The inertness, corrosion and wear resistance, high hardness, low coefficient of friction, and biocompatibility of DLC films have led to interest regarding their use as a biomaterial in orthopedic applications [70,77,86].

All the problems discussed here regarding the main biomedical applications may be mitigated through the surface modification of the metal by using a better biocompatible material and corrosion resistant materials, i.e., carbon-based materials, especially DLC-based ones, are coating materials that fill these expectations. Both the structure and chemical composition are crucial to achieve the desired results. The structural and chemical variations of DLC depend on the method used for its growth, the most common being sputtering, which will be briefly discussed in the next subchapter.

2.2. Preparation Method: Physical Vapor Deposition

As mentioned before, the structure of DLC is composed of C-C sp^2 or sp^3 and -H bonds. The deposition method directly affects the chemical structure present in DLCs where such different bonds can be formed.

DLC coatings can be deposited by various techniques, ranging from chemical (CVD) to physical vapor deposition (PVD) methods, the latter being the most reported in the literature over the years (as stated by the results shown in Figure 2).

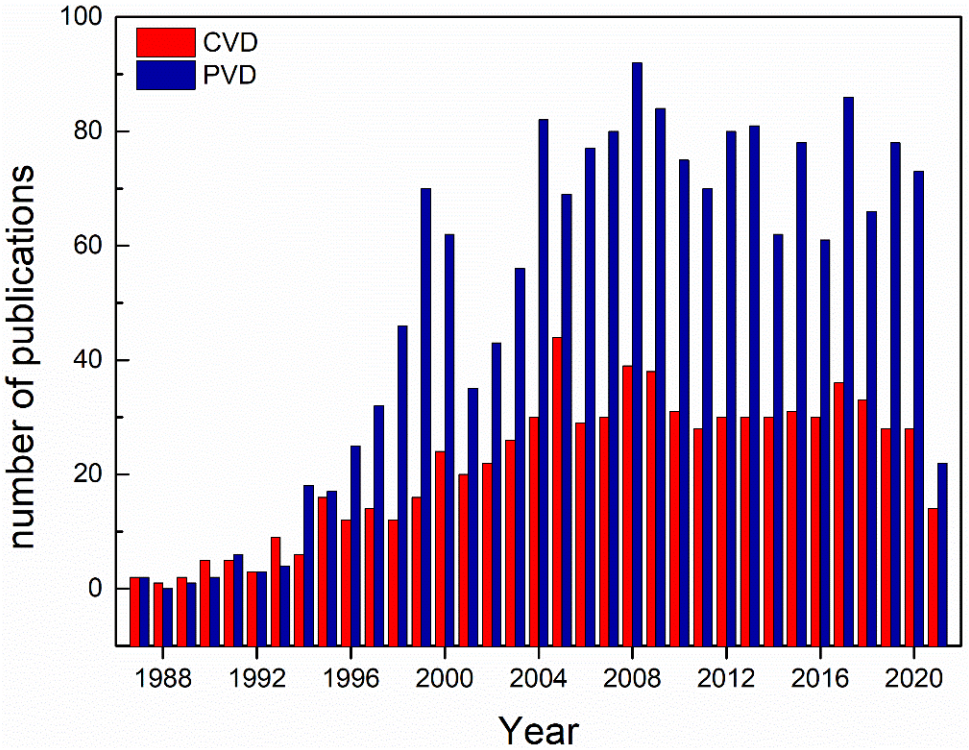

Figure 2. Publication records in "DLC" coatings: Number of articles published yearly concerning "CVD" and "PVD". Data obtained from Scopus on 27 May 2021.

As the structures of DLC materials are affected by the deposition techniques, the properties of DLC are similarly changed [87]. As simple examples, the hardness increases with the sp^3 bonds and when the electrical conductivity is enhanced with the presence of higher amounts of sp^2 bonds (similarly to graphite, which is a good electrical conductor) [88,89].

On the other hand, the presence of hydrogen in the DLC materials, in some situations, can present disadvantages and restrict their use due to their poor electrical properties, such as low electrical conductivity.

There are numerous DLC deposition methods. Two main CVD techniques include plasma-enhanced chemical vapor deposition (PECVD) and electron cyclotron resonance plasma chemical vapor deposition (ECR-CVD). DLC produced by CVD techniques usually is produced in the form of a hydrogenated DLC film, which, depending on the application required, can be a weakness [90].

The PVD processes for DLC deposition usually involve methods such as sputtering [91], direct ion beam [92], pulsed laser deposition [93], and cathodic vacuum arc techniques [94]. PVD deposition mechanisms generally involve the bombardment of carbon atoms onto a substrate; however, the amounts of energy per unit ion are different when producing films with different characteristics [95]. The most used deposition techniques for DLC production are disclosed in Table 2.

Table 2. Deposition techniques reported in literature for DLC coatings.

Technique	Number of Publications	Deposition Rate (nm/s)	Reference
DC/RF sputtering	898	1–10	[29,49,50,58,59,83,96–107]
Plasma enhanced chemical vapor deposition (PECVD)	610	1–10	[88,96,108–114]
Pulsed laser deposition	347	0.1–1	[93,96,115–121]
Filtered cathodic arc (FCA)	141	0.1–1	[94,96,122–126]
Plasma immersion ion implantation	121	>0.28	[127–129]
Direct ion beam (IB)	36	0.1–1	[92,96,130–132]
Electron cyclotron resonance plasma chemical vapor deposition (ECR-CVD)	41	1–10	[96,133,134]

The given deposition technique affects the mechanical, electrical, chemical, tribological, and biological properties of DLC depending on the deposition rates, kinetic energies of carbon species, carbon precursor, or substrate (type, bias, temperature) [29,135]. Another way to tune DLC properties is by elemental doping or by producing composite films. Evaristo et al. [136] produced DLC films with silicon and oxygen. They reported a reduction in friction from 0.17 for the undoped carbon coating to 0.034 for the coating of the system with a-C:Si:O with the highest O content.

Due to the properties of DLC materials, especially including the chemical and tribological properties, these coatings are also widely used in biological applications [137–140].

Most of the researchers in the biology field are focused on the antimicrobial characteristics of DLC films regarding their mechanical, chemical, and tribological properties. To add an antimicrobial effect to DLC materials, different elements can be added (such as Ag, Zn, and Cu) [49,59,83,103,104,141–143].

Peng et al. [142] deposited a DLC coating on zinc substrates by magnetron sputtering using a high-purity C target. They studied the electrochemical properties after immersion in phosphate-buffered saline (PBS) for seven days and found that the corrosion resistance probably decreases due to galvanic oxidation between the DLC film and Zn substrate. Katouno et al. [144] observed a higher calcification of osteoblast cultures using Zn-DLC when compared to a bare DLC coating. On the other hand, Fialho et al. [145] showed a more controlled release of Zn ions when a tantalum oxide-zinc nanoparticle surface was

covered by a carbon thin layer. For the deposition of the DLC layer, they used magnetron sputtering and acetylene (C_2H_2) as the carbon precursor.

More recently, copper has been applied as an alternative element for antimicrobial applications. Jastrzębski et al. [141] deposited Cu-DLC coatings by magnetron sputtering. In this case, they used a graphite target as a carbon source and a Cu target to add the antimicrobial agent; however, the amount of copper required for an antimicrobial effect consequently presents low cell viability and this limits the potential applications of Cu-DLC coatings.

One well known strategy to add an antibacterial effect to a coating is the inclusion of silver. Ag^+ release in Ag/a-C films can be dependent on the aqueous environment [49], i.e., by galvanic couple formation with the a:C matrix [58] and by galvanic couple formation with other metals when bimetallic particles are used [59].

Wang et al. [91] fabricated Ag-DLC films via dual-target high power impulse magnetron sputtering (HiPIMS). The deposited AgNPs have a small size, which promotes the release of silver ions and antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*. Salehizadeh et al. [104] studied the role of Au incorporation in the electrochemical behaviors of Ag/DLC films deposited by a DC magnetron sputtering technique and showed that Ag-Au nanoparticles on a DLC matrix have improved corrosion resistance after the immersion of samples in an artificial urine solution for 14 days, mimicking the electrolyte of the ureter (urine).

One less explored application of DLC materials is biological sensors. Carbon-based coatings are attractive as their chemical properties can be tailored easily and effectively via a covalent functionalization approach [146]. DLC hydrogenated matrixes can be modified to attach antibodies to enable antigen detection [147].

3. Antimicrobial Response

DLC films obtained by sputtering are generally classified as amorphous carbon-based materials and have well known mechanical properties, as mentioned before [77,148,149]. A few studies still show that DLC coatings have antibacterial properties [150,151]; however, a large majority of studies show that these films do not have any antibacterial activity, where it is consequently necessary to add an antibacterial agent to promote this functionality [50,152]. Currently, different antibacterial agents based on organic (e.g., cellulose, chitosan) and inorganic (e.g., Ag, Si, Cu) materials are being studied to improve the performance of devices susceptible to causing infections [50,59,97,153–155].

Silver has gained considerable attention since it exhibits a strong antibacterial activity for a wide range of microorganisms, low susceptibility to bacterial resistance, and the ability to inhibit polymicrobial colonization. Although the mechanism of antibacterial action for silver is not yet well established, high antibacterial efficacy has been demonstrated by released Ag ions, the direct interaction of ions and/or nanoparticles with the cell membrane, and the generation of reactive oxygen species (ROS) produced by Ag^+ and/or Ag nanoparticles. Ag^+ is known to interact with cell thiol groups, binding to the main functional groups in some components, such as enzymes, and avoiding bacterial division, damaging the cell envelope [156] and the nanoparticles disrupt the cell membrane, thus killing the bacterium [157]. The induction of oxidative stress caused by ROS production includes peroxides, superoxides, and hydroxyl radicals by reaction with Ag^+ and/or Ag nanoparticles causing mitochondrial damage [158,159].

Several studies have shown that the main bactericidal effect of silver is obtained through the release of Ag^+ ions through an oxidative reaction in aqueous solutions or biological medium [160]. The kinetics of this dissolution process, which are related to the duration of the antibacterial effect, can be exponentially increased if silver is used in the form of nanoparticles due to the greater proportion between the surface and volume. Another feature of DLC coatings is the possible formation of a galvanic pair between the carbon matrix and silver, in which carbon behaves as the cathode and silver as the anode, thus promoting the ionization of silver [50,58].

Numerous studies with silver-doped DLC coatings have demonstrated their toxic effect against various microorganisms. Chekan et al. demonstrated that DLC films doped with Ag up to 6.5 at % can inhibit *Staphylococcus aureus (S. aureus)* [161]. Baba et al. found antibacterial activity against *S. aureus* in Ag-DLC films with Ag contents of about 4 at % [162]. Although these studies refer to the different mechanisms of antibacterial activity of silver, none of them suggest a possible mechanism. Lan et al. showed that composite films of silver clusters in hydrogenated carbon matrix exhibited an anti-bacterium rate of over 93%, attributing this effect to the release of Ag ions that destroy the cell walls and cell membranes of *Escherichia coli* (*E. coli*), which was the bacteria under study [163]. Almaguer et al. also verified the inhibition of the adhesion of several oral strains to a-C:Ag films. This study suggests that the antibacterial effect is due to Ag ions or radicals that are produced by erosion/corrosion processes [164]. Mihailescu et al. also demonstrated that silver-doped carbon films with up to 7 at % Ag featured antimicrobial effects. They tested Gram positive and Gram negative samples and a yeast and showed that this antimicrobial activity was not always proportional to the amount of Ag in the coatings. The lack of microbial adhesion was due to physical properties, such as the roughness and hydrophobicity of the surfaces of the coatings under study [165]. Carvalho et al. obtained antibacterial Ag- and AgAu-coated amorphous carbon films against *Escherichia coli* (*E. coli*) with a small amount of Ag (between 3 to 5 at %). This study shows that, in addition to the release of Ag ions, the direct interaction of the nanoparticles with the bacteria also promotes the death of the bacteria [104]. Also, Wang et al. have shown long-lasting antibacterial activity from similar coatings which was essentially due to the segregation of Ag [152]. Few studies have also shown antibacterial activity with DLC coatings doped with other elements as N, Si, and Cu. Liu et al. demonstrated the antibacterial performance of Si- and N-doped DLC coatings, where they related the antibacterial effect to the decrease in roughness caused by the doping of Si and N [166]. Chan et al. showed that the incorporation of copper into a hydrogenated DLC matrix (Cu / a-C: H) promoted antibacterial activity against *E. coli* [155].

For orthopedic, cardiological, ureteral stents, or dental implant applications, there must be a compromise between the amount of a bactericidal agent added and the possible toxicity that these agents may impart. In addition, biocompatibility is a requirement of these materials that must also be evaluated, an issue that will be addressed in more detail in the next section, in the case of DLC as coatings for biological applications.

4. DLC Biocompatibility

Together with the advent of new biomaterials, the definition of biocompatibility has been evolving over the last few decades and is assumed as "the ability of a material to perform with an appropriate host response in a specific application" [167]. The vast worldwide implantation of medical devices represents a crucial concern for medical and scientific communities, as well as for the global population, who benefits from its utilization. The implantation of a prosthetic element can cause damage to host tissues that will initiate a nonspecific host immune response (the "foreign body reaction"), ultimately leading to infection, chronic inflammation, and poor implant outcomes, or even implant failure and body rejection [168,169]. Thus, in order to limit and avoid these types of reactions, a complete study of the desired implant or coating materials should be performed and approved before use, meeting the requirements of the International Organization for Standardization (ISO) defined experiments for biocompatibility (ISO10993). These tests, intended to access materials extractable components and their effects on in vitro and in vivo research models, will dictate if the scaffold and coating materials can be classified as biocompatible [168]. Due to their properties, carbon-based structures have stood out as efficient compatible biomaterials for future review.

Thin carbon-based films, such as amorphous carbon (a-C) and amorphous hydrogenated DLC (a-C:H) are widely used in implant device coatings. In a comparative study, the hemocompatibility of carbon-based thin films, developed by magnetron sputtering under various deposition conditions, was focused on the surface adsorption of two im-

portant human plasma proteins, namely, human serum albumin (HSA) and fibrinogen (Fib). The sp^3 content was found to control the hemocompatibility of the a-C films, with HSA/Fib ratio increasing with the sp^3 fraction [170]. The interface between a-C:H films and different human cell types was studied extensively. Martino and colleagues showed that human bone marrow-derived mesenchymal stem cells (hBM-MSCs) responded to different nanopattern designs with specific changes of microtubule organization. During the 21 days of testing, the hBM-MSCs preserved the growth rate, morphology, and viability, suggesting that the films were biocompatible and suitable for stem cell cultures. The a-C:H coating did not alter cell adhesion and the hBM-MSCs cultured on a-C:H films differentiated toward adipose or bone tissue at a comparable rate to stem cells cultured on control cell substrates [171]. In another study, different 2D and 3D scaffolds with several hole sizes and surface topographies have been manufactured and functionalized for in vitro study with human mesenchymal stem cells (hMSCs). Cell growth and aggregation depend on the DLC surface texture, which will lead to niche structures enabling tissue formation and organ repair [172]. DLC layers applied onto silicone scaffolds have been used as substrates for human endothelial (ECV304), smooth muscle (SMCs) and human umbilical vein endothelial cell (HUVEC) studies. Hydrophobicity, rough pre-deposition substrate, low surface free energy, low hydrogen content, and large residual stress are adverse for cell viability, with the ejection of cellular lamellipodia and controlled escalation of the cells depending on substrate topography [173–175]. Higher hydrogen precursor contents (>30%) give rise to smoother surfaces, lower contact angles, and higher surface free energy, which seem to be favorable to cell cultures, presenting greater cell growth [176]. In human fibroblast primary cells (AG 01522D), DLC coatings deposited by magnetron sputtering show the best biocompatibility results, demonstrating that the electron-beam modification of DLC coatings is a suitable process for controlling and guiding cell adhesion on implant surfaces [177]. Also, in DLC silicon coatings, the presence of silicon enhanced the adsorption of HAS onto the surface of a DLC, reducing the adsorption of Fib and indicating a protein conformation change from α-helix to β-sheet [178].

The incorporation of different amounts of titanium (Ti) into a-C:H films has also been widely explored. Cell cultures of primary adult rat bone marrow cells (BMC) and osteoblasts and in vivo Wistar rat experiments with a-C:H/Ti have shown increased bone cell proliferation while reducing osteoclast (bone resorbing cells)-like cell activation (in particular at 7.2 at % Ti in the carbon matrix) [179,180]. Human fibroblast MG-63 and red blood cells (RBC) enhanced the a-C:H/Ti films cell adhesion and proliferation, as well as hemocompatibility [181]. The incorporation of Ti revealed non-toxic effects and induced surface absorption of proteins, improving the initial hemocompatibility and decreasing the platelet activation in human osteosarcoma Saos-2 cells and whole blood samples [182,183].

DLC coatings deposited on an equiatomic nickel-titanium (NiTi) alloy inhibited the release of Ni ions from the NiTi substrate, enhancing its biosafety. The adhesion, morphology, and viability of endothelial cells (ECs) showed to be better on DLC coatings than on bare NiTi alloy, leading to faster endothelialization after surgical implantation [184]. Mononuclear macrophages (THP-1 monocytes) cell proliferation revealed no significant promotion between TiN coated and uncoated stainless steel; however, pro-inflammatory TNF-α levels were found to be higher, indicating better biocompatibility [185].

L-929 mouse fibroblast cells were used in tests with a-C NM film on Ti6Al4V alloy with no cytotoxic effects. Also, in vivo mice and rabbit experiments with a-C NM film for 28 days revealed no visible signs of inflammation or bone graft rejection, and reorganization of bone tissue around the implantation site [186]. The use of films based on DLC accelerated the formation of mature trabeculae at the interface between the bone and implant [187]. The same L929 cell line was used to access fluorinated DLC (F-DLC) films in terms of biocompatibility. F-DLC films exhibited higher albumin and lower fibrinogen adsorption than uncoated specimens, preventing thrombus formation, together with no loss of cell integrity and non-toxic effects [188]. In a comparative study of different a-C:H materials (pure and N, Si, and Ti-doped a-C:H), human MRC-5 and murine L929 fibroblasts presented

no alterations in cell viability upon contact with the test samples [189]. Fibroblast cell adhesion on uncoated stainless steel, a-C, a-CN, and Ti-coated steel samples was similar for all tested samples; however, osteoblast adhesion for a-C films and cellular proliferation for a-C and Ti were higher when compared to uncoated steel [190].

Regarding chromium-modified DLC (Cr-DLC) films, the biological response of human microvascular endothelial cells (HMV-EC) has been studied and cell adhesion and growth have been found to be affected by the Cr content, with disordered graphitic phases in the DLC film leading to enhancement in the seeding of endothelial cells [191]. Subperiosteal implants made of Co/Cr, Ti, and carbon nanocomposite-coated (CNC) Co/Cr alloys were implanted in rabbits for 12 weeks, with coated specimens presenting higher biocompatibility when compared to the metal alloy specimens. CNC deposited on a Co/Cr alloy significantly improved the implant durability, acclimation rate, and formation of new tissues. Average characteristics were shown by the Ti implant and negative results were found with the uncoated Co/Cr alloy implant [192]. A DLC-coated Co-Cr-Mo alloy with the use of titanium gradient interlayer (Ti/Ti-C:H) implant was simulated in human U-2 OS osteoblast-like cells and no cytotoxic wear debris was formed [193]. Cr-doped DLC layers of different Cr concentrations have been used to evaluate osteoinductivity with human bone marrow mesenchymal stromal cells (hMSCs), along with the immune activation potential with RAW 264.7 macrophage-like cells and their effect on apoptosis in Saos-2 human osteoblast-like cells and neonatal human dermal fibroblasts (NHDFs). Cr-doped DLC layers with an optimized Cr content (0.9 at %) have improved osseointegration, long-term biocompatibility, and the functionality of metallic prostheses for use in humans [194].

Silver nanoparticles embedded within DLC matrix have been used in an exploratory study comparing nanocomposite a-C:H–Ag and a-C–Ag coatings, with no cytotoxicity with mouse MC3T3 osteoblastic cells in all deposited amorphous carbon films [195]. The hemocompatibility of Ag-incorporated DLC films was investigated and, with higher Ag incorporation, a higher HSA/Fib adsorption ratio was found when compared with DLC and less incorporated DLC films, which is an indicator of the good compatibility of an increase in Ag content [196]. The Ag-DLC coating of PET surfaces also has revealed a decrease in thrombus formation and blood protein adsorption [197]; however, the biocompatibility of carbon layers on PET, using a 3T3 mouse fibroblasts model, revealed increased 3T3 cell adhesion on carbon films deposited for up to 30 min. Cell adhesion declined with longer deposition times, which was probably due to surface morphology and roughness changes [198]. In a study using L929 fibroblasts, primary human osteoblasts, and HUVEC, clear cytotoxicity at high silver concentrations was measured; however, at lower nontoxic Ag concentrations, a proangiogenic cell phenotype was observed [199].

Tantalum carbide (TaC) and TaC/amorphous carbon (a-C) coatings, with various carbon contents, have been assessed for cell viability with human osteosarcoma cell line MG-63, showing good biocompatibility for this cell line. Optical and fluorescent confocal imaging have also confirmed the cell attachment and MG-63 distribution, suggesting the use of TaC/a-C as a coating with the highest biocompatibility for cell adhesion, distribution, and growth [200]. Human fetal skin fibroblasts (WS1, derived from the soft tissue) were also tested with Ta, TaC, TaC/a-C, and Ta-containing amorphous hydrogenated carbon films (Ta-C:H), with Ta-C:H coatings exhibiting the highest biocompatibility, suggesting a TaC/a-C and Ta-C:H cell-dependent biocompatibility [201].

Yate and colleagues compared Nb−C and a-C films biocompatibility using an osteoblast precursor cell line (MC3T3-E1). Nb incorporation changed the surface chemistry of the a-C films, and led to a refinement of film nanotopography, contributing to the cell adhesion [202]. To study the biological response of deposited DLC and silicon Si-DLC coatings, Bociaga and colleagues demonstrated the hemocompatibility of examined coatings in whole blood samples. Increases in Si concentration affected the hydrophobicity of the surface, suppressing platelet adhesion and decreasing platelets activation. Si-DLC coatings demonstrated to be non-cytotoxic, regardless of the Si amount. In endothelial

cell line EA.hy 926, the addition of Si to the DLC coating deposited on a Ti6Al7Nb alloy also showed positive results for the proliferation and viability of endothelial cells [100,203]. Using hBMSCs, host tissue response and angiogenesis activity were studied along 21 days, comparing uncoated and Cu/a-C:H-coated implants after subcutaneous implantation in rabbits. The Cu/a-C:H coatings showed higher angiogenesis and osteogenesis activities, with a higher development of blood vessels [204]. The development of DLC films with the ability to release Zn (Zn-DLC) revealed a potential to affect osteoblasts. In a mouse osteoblastic cell line (MC3T3-E1), the benefits of DLC biocompatibility were combined with those of Zn, enhancing osteogenesis and allowing a controlled Zn released by altering the manufacturing process [144].

DLC biocompatibility, in its multiple compositions, has been largely proved over the last few years; however, the constant improvements in the development and production of new implant materials and techniques should be accompanied with close attention to the biological properties while always aiming for a perfect host fit.

5. Conclusions and Perspectives

Carbon-based materials are inherently biocompatible due to their chemical compositions. This is the case for DLC, which has other outstanding properties, such as its hardness, corrosion resistance, and self-lubricity due to its sp^2/sp^3 bond structure. Given these properties, DLC materials have been gaining a strong position in biomedical applications.

The validation of coatings that can be used in host environments requires a whole process of in vitro analysis regarding their behavior with cells that will serve as a basis for further studies with in vivo models. From these analyses, it is essential to derive the biological responses of coatings such as DLC, and in particular the antibacterial and biocompatibility features.

In line with this, such results require careful analysis, since the environments and cell types, as well as the given microorganism, influence the results obtained, with no consensus on a generic validation of the use of DLC in various biomedical applications, especially those that directly contact blood. The dangerous components released from coatings that may lead to thrombus formation are a concern of the medical community, requiring a more thorough evaluation than in cases where the coatings are not in direct contact with the bloodstream; however, both in blood-associated devices and orthopedic prostheses, component release and induction of acute and chronic inflammatory processes are two main issues that must always be kept under surveillance.

With this review, it is possible to move towards clarification of all the antibacterial and biocompatible behaviors of DLC in the most diverse environments and with different cells/bacteria, where the main objective is to show its diversity. Also, the chemical composition of doped DLC has been addressed here, contributing to highlighting its potential as a biomedical material.

In addition, regarding the healthcare field, which is not restricted to biomedical implant materials, doped DLC coatings can also stand as an important research area for the progress of antiviral surfaces. Currently, with the COVID-19 pandemic, the importance of disinfected surfaces has been emphasized, and some metal nanoparticles, such as Ag, Au, and Cr nanoparticles, have already been shown to possess antiviral characteristics. So far, there are no studies demonstrating this antiviral effect in DLC coatings, which means that the antibacterial coatings in use today could also be analyzed and explored regarding this property.

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