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## ABSTRACT

Diamond-like carbon (DLC) coatings have become a prominent strategy to enhance the performance and hemocompatibility of ventricular assist devices (VADs). This review presents a comprehensive overview of the historical development of DLC coatings for VADs, the main DLC deposition techniques, types of blood pumps, substrate materials, and specific applications in VADs. DLC coatings, deposited via methods such as plasma-enhanced chemical vapor deposition, offer high hardness, chemical inertness, excellent wear resistance, and hemocompatibility, making them suitable for blood-contacting components in continuous-flow centrifugal pumps and continuous axial-flow pumps. The ability to apply DLC to various substrates, including metals and polymers, further broadens its biomedical utility. Comparative analysis with other hemocompatible coatings, such as heparin, titanium nitride, and 2-methacryloyloxyethyl phosphorylcholine polymers, demonstrates that DLC provides a unique balance of durability and blood compatibility, although challenges remain regarding long-term adhesion and stability. Advances in doped and nanostructured DLC films continue to improve antimicrobial and antithrombotic properties. Overall, DLC coatings represent a significant advancement in the quest for safer, longer-lasting VADs, but further research is needed to optimize their clinical performance and address remaining limitations.

**KEYWORDS:** Diamond-like carbon, Biomaterials for cardiology, Hemocompatibility, Ventricular assist devices, DLC coatings.

## CARBONO-TIPO DIAMANTE EM DISPOSITIVOS DE ASSISTÊNCIA VENTRICULAR: HISTÓRIA, TÉCNICAS, APLICAÇÕES E HEMOCOMPATIBILIDADE

### RESUMO

Revestimentos de carbono-tipo diamante [*diamond-like carbon* (DLC)] tornaram-se uma estratégia importante para melhorar o desempenho e a hemocompatibilidade de dispositivos de assistência ventricular (DAVs). Esta revisão apresenta uma visão geral abrangente do desenvolvimento histórico de revestimentos de DLC para DAVs, as principais técnicas de deposição de DLC, tipos de bombas de sangue, materiais de substrato e aplicações específicas em DAVs. Os revestimentos de DLC, depositados por métodos como a deposição química de vapor assistida por plasma, oferecem alta dureza, inércia química, excelente resistência ao

desgaste e hemocompatibilidade, tornando-os adequados para componentes em contato com o sangue em bombas centrífugas de fluxo contínuo e bombas de fluxo axial contínuo. A capacidade de aplicar DLC a vários substratos, incluindo metais e polímeros, amplia ainda mais sua utilidade biomédica. A análise comparativa com outros revestimentos hemocompatíveis, como heparina, nitreto de titânio e polímeros de 2-metacriloxietilfosforilcolina, demonstra que o DLC oferece um equilíbrio único entre durabilidade e biocompatibilidade, embora ainda existam desafios em relação à adesão e estabilidade a longo prazo. Os avanços em filmes de DLC dopados e nanoestruturados continuam a aprimorar as propriedades antimicrobianas e antitrombóticas. No geral, os revestimentos de DLC representam um avanço significativo na busca por DAVs mais seguros e duradouros, mas são necessárias mais pesquisas para otimizar seu desempenho clínico e superar as limitações remanescentes.

**PALAVRAS-CHAVE:** Carbono-tipo diamante, Biomateriais para cardiologia, Hemocompatibilidade, Dispositivos de assistência ventricular, Revestimentos de DLC.

## HISTORICAL OVERVIEW OF DIAMOND-LIKE CARBON AND VENTRICULAR ASSIST DEVICES

Heart failure is a cardiovascular disease characterized by dysfunction of the heart muscle; the heart loses its pumping function and compromises the maintenance of organs and tissues. In severe cases, heart transplantation and mechanical circulatory support through the implantation of a ventricular assist device (VAD) that supplies cardiac flow in parallel with the patient's heart are indicated<sup>1</sup>. Hemolysis and thrombogenic response are the two main challenges in the development of a fully implantable target VAD, and the influencing factors are directly related to the interaction phenomena that occur during the performance of its function<sup>2</sup>.

The International Organization for Standardization (ISO) 10993 standard: Biological evaluation of medical devices specifies that constituents of an implantable medical device that remain in contact with circulating blood for an indefinite period must remain stable for at least 5 years. In this type of application, the biological medium must not compromise the biofunctionality of the implant by corrosive action, for example; likewise, the biomaterial cannot generate negative biological stimuli to any system of the organism, such as hematological and/or immunological reactions<sup>3</sup>.

The properties of the diamond-like carbon (DLC) coating provide high mechanical strength, high hardness, low coefficient of friction, chemical inertness, electrical insulator characteristics, and biocompatibility to the substrate surface, among other characteristics<sup>4</sup>.

DLC coatings were first explored in the 1970s for their exceptional hardness and chemical inertness. Their biomedical use accelerated in the 1990s, with applications in orthopedic and cardiovascular devices due to their biocompatibility and wear resistance<sup>5,6,7</sup>. The first DLC coatings for blood-contacting devices, such as stents and heart valves, demonstrated reduced thrombogenicity and improved durability<sup>6,8</sup>.

In VADs, DLC coatings were introduced in the late 1990s and early 2000s, notably in implantable centrifugal pumps and rotary blood pumps, where they significantly improved hemocompatibility and device longevity [9]. VADs themselves have evolved from large external devices in the 1960s to compact, implantable rotary pumps, now essential for advanced heart failure management.

Comparative analysis between DLC and other hemocompatible coatings shows that various coatings are used to improve the hemocompatibility of VADs, including titanium nitride (TiN), heparin, 2-methacryloyloxyethyl phosphorylcholine (MPC) polymers, textured surfaces, and endothelial cell coatings<sup>9,10</sup>.

DLC and heparin coatings are the most established for commercial VADs due to their excellent hemocompatibility and durability. TiN offers good wear resistance but is generally outperformed by DLC in hemocompatibility<sup>9,10</sup>.

MPC polymers provide superior hemocompatibility but are less durable and not suitable for long-term implants. Textured and endothelialized surfaces can promote natural antithrombotic properties but are challenging to produce reliably and may lack long-term stability<sup>9</sup>.

DLC stands out for its balance of mechanical strength, chemical inertness, and proven hemocompatibility, although challenges remain regarding adhesion and long-term performance in dynamic physiological environments<sup>5,8,9</sup>.

The well-adhered DLC coating on textured pure titanium has been shown to inhibit the adhesion and protection of biological material in cardiovascular applications, where it remains in contact with arterial blood, in addition to improving the mechanical properties of the surface, increasing the fracture toughness, and improving hemocompatibility in long-term applications<sup>11</sup>. This experimental study will be further discussed in Applications of coatings DLC in VADs.

Table 1 shows some types of surface modifications applied to improve the performance and durability of VADs and compares the effects obtained with DLC coatings and other types of coatings.

**Table 1:** Comparison of DLC and other VAD coatings.

Coating type	Hemocompatibility	Durability	Clinical use	Limitations	Citations
DLC	High	High	Yes	Adhesion, long-term data	[10; 5; 8; 9]
Heparin	High	Moderate	Yes	Degradation, leaching	[10; 9]
TiN	Moderate	High	Yes	Lower hemocompatibility	[9]
MPC polymer	Very high	Low	Limited	Poor long-term stability	[10; 9]
Textured/endothelial	Potentially high	Variable	Experimental	Production, reliability	[9]
DLC in TiO <sub>2</sub> texturized	Very high	Moderate	Experimental	Reproducibility	[11]

Source: Elaborated by the authors.

## DLC DEPOSITION TECHNIQUES

As a coating process, plasma-enhanced chemical vapor deposition (PECVD) process is used to deposit elemental materials (Al, a-C, c-Si), nitrides (AlN, Si<sub>3</sub>N<sub>4</sub>), and carbides (BCN, SiC) on solid surfaces, and can be carried out in different ways, depending on the energy source used to activate the gaseous species: at atmospheric pressure, low pressure, laser-assisted, or plasma-assisted. The PECVD method is the most suitable for obtaining uniform, homogeneous coatings of regular thickness on samples with complex geometries, since the vapor reaches all areas without the need to manipulate their positioning during the process<sup>12</sup>.

The dynamics of the process are multifactorial and require control of several parameters during its execution to result in a coating with the desired characteristics. The reactions occur through a luminous plasma discharge, sustained by an electrical energy source, whose voltage can vary from a few hundred volts to tens of kilovolts, inside the deposition chamber<sup>13</sup>.

Four stages occur in a PECVD deposition process, and they are listed below:

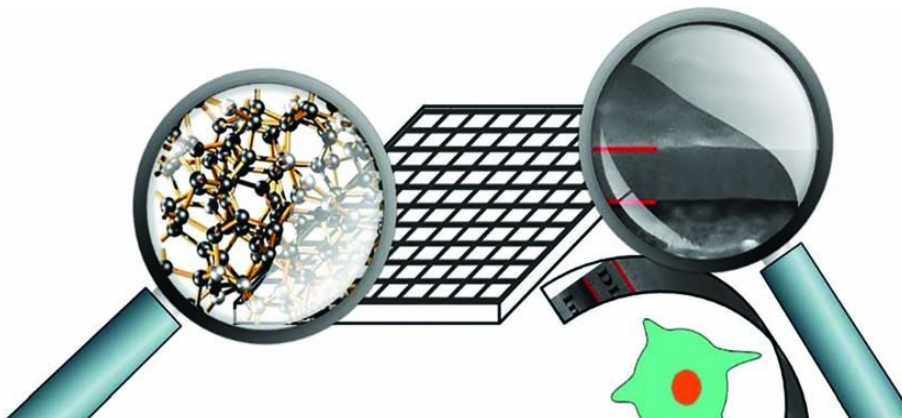
- Primary reactions between electrons and other reacting species in the plasma. Ions and free radicals of the reactive species may be formed.
- The reactive species formed in stage I are transported to the substrate surface, where additional elastic and inelastic reactions may also occur, such as ion-radical and photon-molecule interactions, etc.
- Incorporation of ions into the substrate.
- Anchoring of new species to the surface of the film that is already adhered to the substrate surface. There may be re-emission of non-adherent species from the surface to the gaseous phase.

The first DLCs were prepared as thin films by Heisenberg and Chabot in 1971 through ion beam deposition<sup>14</sup>. There are several deposition methods, which can be improved according to the objectives and needs for laboratory studies or industrial production. Regardless of the method, the DLC film is condensed from a beam of carbon or

hydrocarbon ions of medium energy (~ 100 eV). It is the physical impact of these ions on the growing film that induces  $sp^3$  bonding; at the same time, the chemical process stabilizes this  $sp^3$  bonding<sup>15</sup>.

DLC films are typically deposited using physical vapor deposition (PVD) and chemical vapor deposition (CVD), including PECVD. These methods allow precise control over film thickness,  $sp^3/sp^2$  carbon ratio, and hydrogenation, which influence mechanical and biological properties.<sup>5,8,16</sup>

DLC can be deposited on a wide range of substrates, including titanium, stainless steel, ceramics, and polymers like polyether ether ketone (PEEK)<sup>17,11,8</sup>. The choice of substrate depends on the mechanical requirements and location within the VAD. Surface preparation and the use of interlayers (e.g., CrN) are essential for ensuring strong adhesion and long-term stability of the DLC coating<sup>11,8</sup>. In Fig. 1, DLC thin films on the surface of metallic implants protect the biomaterial.



**Figure 1:** DLC thin films on the surface of metallic implants protect the biomaterial.

Source: Adapted from Derakhshandeh, et al.<sup>16</sup>

Low-temperature processes, such as periodic PECVD, are preferred for heat-sensitive substrates and complex geometries found in VADs<sup>8,17</sup>. Doping DLC with elements like silver, copper, or fluorine can further enhance antimicrobial and antithrombotic properties<sup>18,19</sup>.

In Brazil, the construction of a large-scale high-vacuum system made it possible to generate relatively high gas flows capable of accelerating ions with energies up to 170 eV and obtaining adherent DLC films on different types of substrates. The growth parameters of thin DLC films have been improved over the years to impart novel properties and for specific applications, such as functionalized mechanical and tribological properties<sup>13,20</sup>.

The DLC film growth system via the PECVD technique has also been widely studied due to the ease of deposition in a single step, allowing the production of commercially better films with more possibilities to expand applications due to the greater number of properties of industrial interest.

Of the related national patents, the PECVD technique with significant modifications has made it possible to obtain new pulse forms and amplitudes through the introduction of an additional cathode with an active screen, where a more uniform and more energetic discharge can be obtained at much lower pressures, increasing the conditions for better adhesion on different types of substrates; this is also known as the DLC coating process via partial confinement of electrons and ions<sup>21</sup>.

This technique also permits reaching low low-pressure vacuum of  $10^{-4}$  Torr, which means that the particles inside the reactor are in a low-pressure region; the collisional effect in terms of energy produces an effect on the particles, increasing the probability of collisions with the substrate. These issues are represented in high adherence and a homogeneous film, increasing the hardness and the film performance due to the large mean free path present in the additional cathode device.

It is important to mention that the additional cathode helps deposition on the substrate when it is necessary to grow the film on irregular geometries. Thin films can be deposited over the 3D sample geometries when the additional cathode is used in the PECVD system<sup>22</sup>.

## TYPES OF BLOOD PUMPS – VADS

VADs primarily use centrifugal and axial-flow pumps. DLC coatings are applied to blood-contacting surfaces such as rotors, bearings, and housings to reduce friction, wear, and platelet adhesion<sup>9,11,17</sup>. These modifications are critical for minimizing hemolysis and thrombosis, thereby extending device life and improving patient outcomes.

This section was subdivided into two parts. Initially, commercially available VADs with significant relevance in the medical device market are presented, with the analysis restricted to a class of long-term devices driven by rotary actuators. Subsequently, adverse events (AEs) inherent to the use of these devices are discussed, namely undesirable events caused by the direct interaction between the pump and the cardiovascular system. Emphasis is placed on AEs triggered by disturbances in blood coagulation and on the importance of applying DLC coating techniques to minimize these complications.

### Commercial medical devices

The information in this section is limited to presenting a class of VADs commercialized in recent years. Three devices are presented as follows: the HeartMate 2 (HM2) (Abbott Laboratories, California, USA), HeartMate 3 (HM3) (Abbott Laboratories, California, USA), and the HeartWare Ventricular Assist Device (HVAD) (Medtronic, Florida, USA)<sup>38</sup>. Among the devices presented, the HVAD is no longer implanted in new patients, as the Medtronic Company decided to discontinue new implants due to the increased risk of death, AEs, and the potential to cause patient harm resulting from VAD failures<sup>23</sup>.

Information regarding each of these devices is presented below, including structural characteristics, operational details, and approval status from the U.S. Food and Drug Administration (FDA).

The HM2 was approved by the FDA for use as destination therapy on January 20, 2010. It is an axial-flow VAD that propels blood in the direction of the rotor's axis, which rotates at high speeds (a minimum of 9,200 rpm and a maximum of 11,500 rpm). Its controller and batteries are placed in wearable external supports outside the body, and a driveline in the abdomen connects the batteries to the pump for electrical power and data transmission. Two additional cables connect the batteries to the controller<sup>24</sup>.

The HM3 was approved by the FDA for use as destination therapy on October 18, 2018. It is a VAD that incorporates MagLev technology, with magnetic levitation of the rotor, and propels blood using centrifugal force at lower rotational speeds than the HM2. The controller, batteries, and pump are connected by cables like those of the HM2, and the wearable support for the batteries and controller is also comparable<sup>23</sup>.

The HVAD was approved by the FDA for use as destination therapy on September 27, 2017. It combines hydrodynamic and magnetic forces to support and rotate the rotor around its axis, propelling blood by centrifugal force at speeds between 1,800 and 4,000 rpm. Its controller and batteries are placed in wearable external supports outside the body, and a driveline at the abdominal level, connected to the batteries and the pump through a percutaneous incision, provides electrical power and data transmission. Two additional cables connect the batteries to the controller<sup>25</sup>.

### Adverse events

For a historical period, recurrent cases of thrombus formation in VADs were reported, resulting in a significant increase in hospital readmission rates due to blood coagulation disorders. Reports indicate that some patients required surgical extraction or replacement of the pump, as well as cases of death associated with thrombus-related complications in the device<sup>26,27,28</sup>.

Blood coagulation occurs through the action of a sophisticated control mechanism of the coagulation cascade in response to tissue injury<sup>40</sup>. In the context of VAD use, clot formation may develop as a result of direct interactions with the interior of the pump, leading to the development of a thrombus that is an AE. AEs remain the main cause

of hospital readmission and regarding coagulation disorders, since the combination of these kinds of events may lead to additional harm to patients, such as stroke and pulmonary embolism<sup>26,29,30</sup>.

An additional relevant AE is hemolysis, which results from high levels of shear stress applied to red blood cell (RBC) membranes, leading to their mechanical rupture<sup>31</sup>. While hemolysis is a natural physiological process caused by various factors, blood passing through circulatory devices (as in a VAD) is subjected to markedly higher stress, often due to frictional forces influenced by the device's material properties. This mechanism underscores the critical importance of rigorous biocompatibility studies.

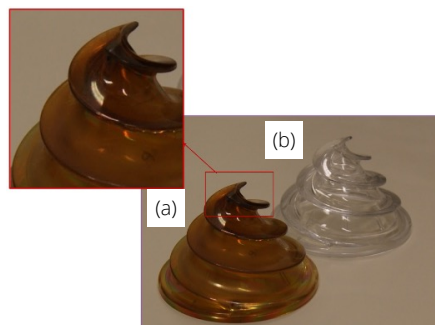
Considering the context presented, the improvement in patient survival rates in recent years<sup>32,33</sup> and the results demonstrated with the use of DLC coating techniques in VADs<sup>9,11</sup> indicate significant advances in reducing AEs and the above-mentioned complications. AEs result from behavioral deviations in the patient's organism associated with the direct interaction between the VAD and the cardiovascular system<sup>34</sup>. Therefore, undesirable events such as hemolysis and coagulation caused by interactions with the pump's interior are minimized through the application of DLC coatings, since this technique offers improvements in hemocompatibility.

## APPLICATIONS OF COATINGS DLC IN VADS

The main application of DLC in VADs is to enhance hemocompatibility and wear resistance of blood-contacting components. DLC coatings reduce platelet activation, prolong clotting time, and minimize protein and cell adhesion, which are crucial for preventing thrombosis and device failure<sup>8,9,17</sup>.

Doped DLC variants (e.g., fluorinated or silver-doped) offer additional antimicrobial and antithrombotic benefits<sup>18,19</sup>. *In vivo* and *in vitro* studies confirm the biocompatibility and durability of DLC-coated VAD components<sup>9,17,35</sup>.

The CVD technique was used to coat the Spiral Pump® VAD rotor with a DLC film at the Associated Materials Laboratory of the National Institute for Space Research (INPE) in Brazil (Fig. 2). The reagent, composed of atomic hydrogen and methane, was introduced into a pulsed DC PECVD reactor, as shown in Fig. 3. A metal cage trapped the ionized gas and enabled the deposition of the DLC coating in hard-to-reach regions, such as negative angles present in the polymeric rotor<sup>36</sup>.



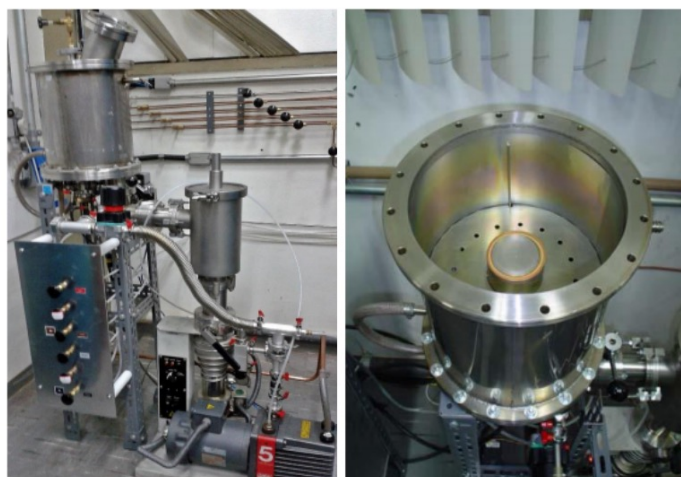
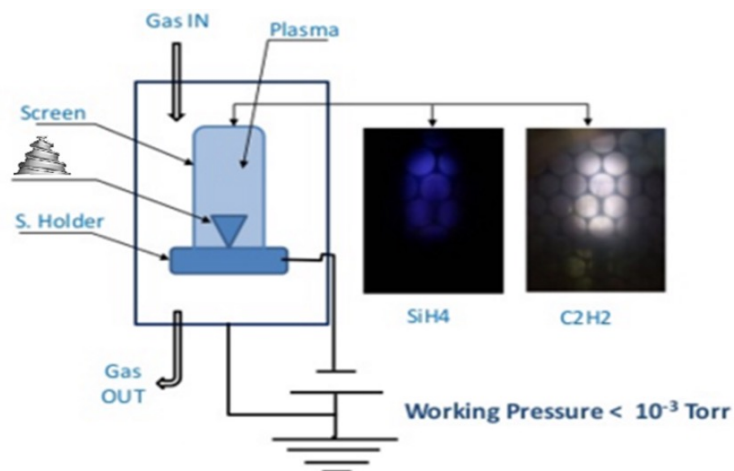
**Figure 2:** Rotor Spiral Pump® VAD: (a) polycarbonate with DLC coating and (b) pure polycarbonate.

Source: Sá, et al.<sup>36</sup>

The plasma phenomenon was obtained between the electrodes under low-pressure conditions ( $8 \times 10^{-4}$  Torr) by means of electrical discharge voltages between the cathode and the anode, varying from 600 to 1,000 volts, with a pulse frequency of approximately 20 kHz. In this way, in 60 minutes it was possible to deposit approximately 1 micron of DLC coating on the polycarbonate rotor surface at a maximum temperature of 80 °C, a temperature considered suitable so as not to compromise its structural integrity, since polycarbonate is a thermoplastic with a glass transition temperature of approximately 147 °C<sup>36</sup>.

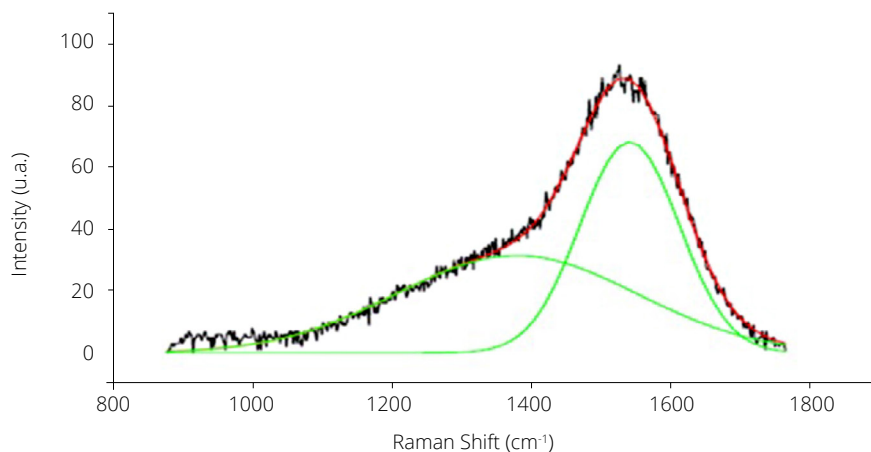
Raman spectroscopy is a high-resolution photonic technique commonly used to identify, in seconds, the chemical and structural composition of organic or inorganic compounds. It was used to record the characteristic bands of

polycarbonate coated with DLC; the amorphous carbon could be observed in two broad bands, G ( $1,560\text{ cm}^{-1}$ ) and D ( $1,350\text{ cm}^{-1}$ ), where the largest fraction of the scattered light upon reaching the material had the same frequency as the incident light and the remainder, inelastically, as shown in Fig. 4.



**Figure 3:** Small reactor, 120 liters in volume, for the growth of DLC at INPE Brazil.

Source: Sá, et al.<sup>36</sup>



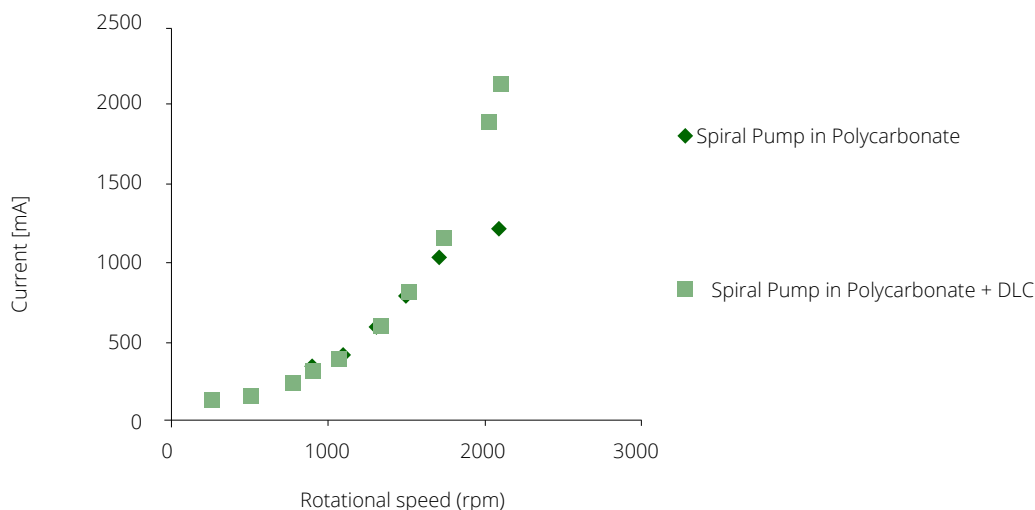
**Figure 4:** Raman spectroscopy characterization of the C-H bonding on the polycarbonate surface.

Source: Sá, et al., 2016<sup>36</sup>.

Raman spectroscopy characterized the C-H bonding on the polycarbonate surface, with carbon atoms bonded primarily in  $sp^3$  and  $sp^2$  hybridizations. The hydrogenated form of DLC, with 50% hydrogen, is (a-C:H). Typical a-C:H spectra indicate increased polarization, as increased  $sp^3$  is correlated with increased hydrogen. The conversion from  $sp^2$  to  $sp^3$  occurs through saturation of the C=C bond by H.

At the Dante Pazzanese Institute of Cardiology in São Paulo, Brazil, the rheological influence of viscous friction on the spiral pump rotor in pure polycarbonate and coated with DLC was compared in an *in vitro* assay through the analysis of electrical current consumption; the circuit consisted of a DRV8x Evaluation Kit development tool (Texas Instruments, Texas, USA), digital multimeter, motor, reservoir, solution with viscosity analogous to that of blood (glycerin + 1/3 water + 70% ethanol water), and two spiral pump rotors: one in pure polycarbonate and the other coated with DLC<sup>36</sup>.

With a fixed electrical voltage of 20 volts, rotation intervals of 250, 500, 750, 1,000, 1,250, 1,500, 1,750, and 2,000 rpm were assigned, and the current was recorded at each rotation (Fig. 5). The *in vitro* current consumption indicated that polycarbonate imparts lower yield stress at high rotations, due to the slightly superior hydrophobic character of DLC compared to polycarbonate.

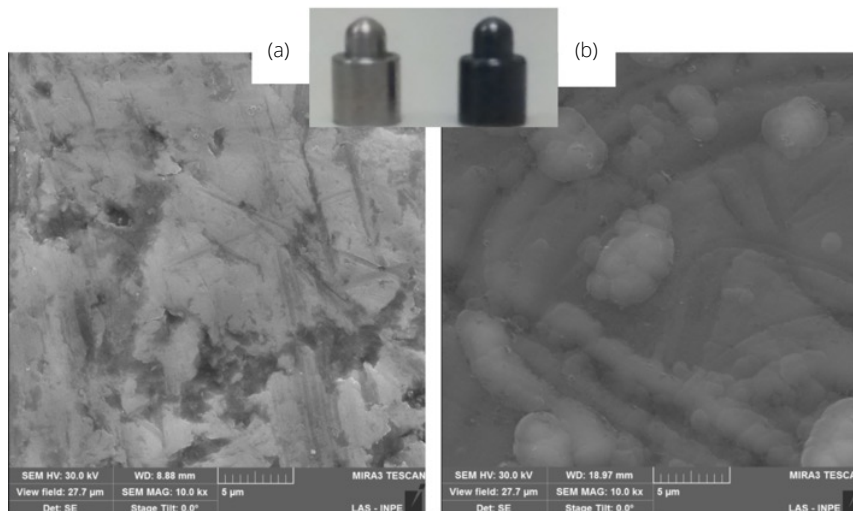


**Figure 5:** *In vitro* comparison of electrical current at different rotation speeds with spiral pump rotors with and without DLC coating.

Source: Sá, et al., 2016.<sup>36</sup>

A VAD must be made of a biomaterial that can withstand highly corrosive environments without triggering a negative response in the body for at least 5 years; a critical condition for VADs that have a pivoting rotor support system, which must be biocompatible and have high durability to resist abrasion wear resulting from the support point. For a decade, the centrifugal VAD pivoting support system has been composed of PEEK or ultra-high molecular weight polyethylene for bearings and titanium or ceramic for pivots with DLC application [37] (Fig. 6).

*In vitro* friction and durability tests simulated the conditions of a VAD to evaluate the pivoting support system capable of imparting less wear using a test bench composed of a digital scale, a milling machine (LFG, X63250), a bearing, a pivot, a support, and a blood analog solution. One pivot in pure titanium and another with DLC coating were subjected to 1,500 rpm with a uniaxial load of 2.5 kg for 6 continuous hours, compatible with the axial magnetic load imposed by the magnets of a centrifugal rotor during magnetic coupling.



**Figure 6:** Pivots, bearings, and micrographs of titanium before and after DLC coating.

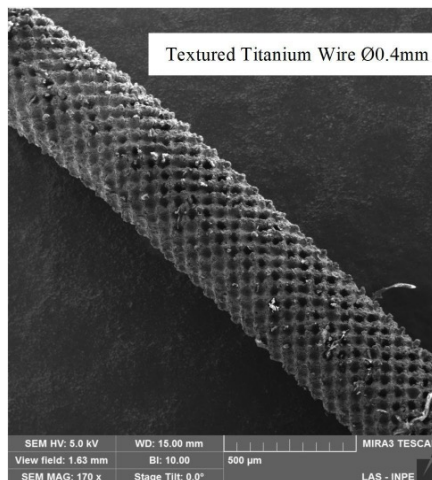
Source: Sá, et al., 2019.<sup>37</sup>

The abrasive wear rate was controlled by plastic deformation and represented by the adapted equation of Archard; this study proved the underlying assumptions concerning abrasive wear, such as:

- The pivot, abrasive, penetrates the weathered surface at a depth proportional to the ratio of the hardness of the material and the applied force.
- The formed groove volume is completely removed and released into the circuit.
- The angle of attack is formed by the geometry of the abrasive.

In 2024, the two hypotheses considered capable of minimizing the negative responses of interaction in VADs and dependence on anticoagulant drugs were tested *in vivo*. Nanosecond laser processing technology imparted highly uniform, micrometric-sized “pores” homogeneously distributed within the implants, capable of trapping sufficient air to promote the Cassie-Baxter effect, as demonstrated in preliminary wettability tests with water and blood. This is because the microstructure also had nanometric roughness inside. This same micro- and nanometric surface structure promoted the inverse, plasmaphilic effect when coated with thicker DLC<sup>11</sup>.

The method developed to biofunctionalize surfaces dedicated to the blood of constituents of VADs proved reproducibility even in pieces with reduced dimensions, such as 0.4 mm titanium wire implants (Fig. 7).



**Figure 7:** Implants in titanium wire textured and coated with DLC.

Source: Sá, et al., 2024.<sup>11</sup>

Both proposed hypotheses proved capable of minimizing undesirable interactions with blood. The analytical results obtained with the implants after *in vivo* evaluation demonstrated that the DLC coating inhibits the adhesion and proliferation of biological material, even those with a hydrophilic profile, due to its higher concentration of carbon.

The titanium surface was potentially improved with the introduction of microstructures, but mainly by the DLC coating, which contributed to a low coefficient of friction, increased wear resistance, and, consequently, high durability of the biofunctional effect of the surfaces. The high adhesion of the DLC film to the microtextured titanium improves the mechanical properties of the surface, increasing the crack threshold and of the entire mechanical circulatory system, allowing flow for a long time without alterations in hemorheological properties and hemodynamic performance<sup>11</sup>.

## CONCLUSIONS

DLC coatings have become a benchmark for improving the hemocompatibility and durability of VADs, outperforming most alternatives in clinical and experimental settings. Ongoing research focuses on optimizing adhesion, long-term stability, and multifunctionality of different materials as substrates, enhancing VAD performance and patient safety.

Even with the advancement of control systems for magnetic levitation of rotors, DLC coating in pivoting systems and components of blood-contact VADs must be improved, because the release of particles can compromise system performance and human organs.

Regarding hemocompatibility studies, according to the dynamic action of each component of a VAD, the ideal type of biofunctionalization for the surface is adopted, for example: for the rotor, a plasmaphobic surface is indicated because this component remains in constant and high rotation, approximately two thousand rotations per minute; while for the internal region of the blood pump, a plasmaphilic surface is indicated so that the endothelialization process occurs and, consequently, there is a gradual reduction in the administration of heparin as an anticoagulant.

It is worth noting that this product definition must have the support of the surgical medical team so that the circulatory assistance technique is improved by the biofunctional function of the surfaces in contact with the blood.

## CONFLICT OF INTEREST

Nothing to declare.

## AUTHOR CONTRIBUTIONS

**Conceptualization:** Sá RCL, Leão T, Drigo E, Barbosa G, Nishida B, Sobrinho JRS, Ramirez M, Moro JR, Corat E, Trava-Airoldi V, Bock E; **Methodology:** Sá RCL, Leão T, Drigo E, Barbosa G, Nishida B, Sobrinho JRS, Ramirez M, Moro JR, Corat E, Trava-Airoldi V, Bock E; **Research:** Sá RCL, Leão T, Drigo E, Barbosa G, Nishida B, Sobrinho JRS, Ramirez M, Moro JR, Corat E, Trava-Airoldi V, Bock E; **Writing - First draft:** Sá RCL, Leão T, Drigo E, Barbosa G, Nishida B, Sobrinho JRS, Ramirez M, Moro JR, Corat E, Trava-Airoldi V, Bock E; **Final approval:** Sá RCL.

## DECLARATION OF USE OF INTELLIGENCE ARTIFICIAL TOOLS

No artificial intelligence tools were used.

## AVAILABILITY OF DATA AND MATERIALS

Not applicable.

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