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Biological evaluation of modified DLC coatings – a review

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ABSTRACT

Recently, due to their high biocompatibility, DLC coatings have been extensively researched in the context of biomedical applications. It was proven that the biological properties of DLC coatings can be tailored by the incorporation of different dopants into the carbon matrix. Depending on the element used, different biofunctionality may be achieved. The dopants preventing the bacteria adhesion include certain toxic elements, such as Ag or Cu, and allow to limit the risk of post-implantation infections. Antithrombogenic agents like Si, Ca, or P not only increase the hemocomaptibility, but also the proliferation of endothelial cells and thus, may be applied for cardiovascular implants. In the case of orthopaedic implants, it is recommended to use dopants which enhance the osseointegration process, for example Ti or Si. Summing up, the literature shows that the modification of DLC coatings may allow to obtain highly functional coatings for different medical implants.

***Keywords*:** DLC coatings, dopants, biofunctionality, biomaterials

1. INTRODUCTION

The main goal of the implantation of a biomaterial into the human body is to restore or augment certain mechanical or biological functions in order to improve the quality of life. Since biomaterials remain in constant contact with living tissues and body fluids, they have to

fulfill strict requirements concerning their biocompatibility. Biocompatibility can be understood as a number of features, including non-toxicity and no immunological response, that allow a material to be safely inserted into the living organism. Interaction of a biomaterial with the surrounding tissues and body fluids is mainly dependent on its surface properties, which have a direct role in different post-implantation biological events, including protein adsorption, cell proliferation and bone-tissue deposition [28,35].

Despite the excellent mechanical properties, commonly used metallic biomaterials possess relatively poor surface properties. This is connected with the highly aggressive environment of the human body and the fact that metals do not exhibit total chemical stability [18,25,42]. Hence, even the corrosion resistant materials can cause negative biological response via release of the degradation products [42]. The degradation of the biomaterials can occur not only as a result of the corrosion process, but also due to friction or intrinsic dissolution.

The clinical consequence of at least partially degrading implants is the release of variable compounds, such as metal ions, that may cause adverse reactions of the organism. One of the most common post-implantation complications include allergies, inflammations, hypersensitivity reactions and tissue irritation [18,25,42,55]. The average percentage of metal sensitivity concerning Co, Cr, and Ni, which are well-known allergens, is approximately 10% for the general population, 22% for patients with well-functioning implants, and 60% for patients with faulty implants [25]. Another major problem concerning the use of metallic implants is their thrombogenicity. Apart from that, the contact of the biomaterial with the biological environment may lead to the development of post-implantation infection caused by bacteria [6].

As a result, the growing interest is given to the surface modification of metallic implants that will enhance the biocompatibility and biological response of the biomaterial. One of the possible solutions that is widely discussed in the literature is the application of diamond-like carbon (DLC) coatings, which exhibit a combination of highly desirable properties in the context of biomedical applications [19,29,38]. The DLC layers are not only characterized by very good physiochemical and mechanical properties, but also by the excellent biocompatibility confirmed in numerous in vitro and in vivo studies [2,9,10,12,37,40]. Consequently, DLC coatings are nowadays extensively studied in terms of their possible biomedical applications [19,29,39]. The most promising applications include the blood contacting implants, such as heart valves and stents as well as bone implants [1,11].

The cell behaviour and body reaction towards the DLC coatings can be altered through the incorporation of different elements into the carbon matrix [28]. Owing to that, biological properties of the DLC films may be tailored in order to achieve certain features. Depending on the dopant used, different parameters of DLC coatings can be improved. Numerous studies have proven that the incorporation of variety of different elements, such as Ag, Si, Ti, Cu, F, P or Ca, allows the enhancement of either biocompatibility, hemocompatibility, antibacterial properties or the osseointegration process.

As a consequence, the development of highly biocompatible modified DLC coatings with particular functionality and superior biological properties for strictly defined biomedical application is possible.

2. MODIFIED DLC COATINGS WITH SUPERIOR ANTIMICROBIAL PROPERTIES

The inhibition of bacteria adhesion on the surface of the biomaterial is essential since the biofilm formation may lead to the development of serious infections [6]. The improvement of antibacterial properties of DLC coatings can be achieved through the addition of heavy elements such as Ag [3,5,14,22,34,39] or Cu [13] that exhibit toxicity towards the bacterial cells. During the exposure of such a film to the biological fluids, the metallic ions are successively released causing the adverse reactions in the bacteria attached to the surface [28]. As a result, the concentration of the toxic element near the surface of the implant is high enough to prevent the biofilm formation and sufficiently low not to cause side effects [28].

One of the most frequently used antibacterial dopants is silver [3,5,14,22,34,39]. Antibacterial properties of silver-doped diamond-like carbon layers have been confirmed in numerous studies against a variety of different bacterial strains, including E. coli, S. aureus, A. israelii, S. sanguinis, F. nucleatum, C. rectus, E. corrodens, P. micra, P. intermedia and A. actinomycetemcomitans [3,5,14,22,34,39]. In general, the higher is the silver concentration, the higher antibacterial activity of the Ag-DLC films. Marciano et al. demonstrated that the silver nanoparticles solution embedded within the DLC matrix can lower the E.coli adhesion to the surface even by 68% within 3h, and by 32% within 24h [39]. Even more impressive results were obtained by Kwok et al. who showed that Ag-DLC layers can exhibit the antibacterial activity higher than 98% [34]. Furthermore, Baba et al. indicated that even small content of silver, i.e. 3.8 at.%, can result in high antibacterial activity at the level of around 80% [5]. The antibacterial properties of Ag-DLC films may be associated with the decreased wettability caused by the incorporation of silver, as it was suggested by Endrino et al. [22]. It was also proven that Ag-DLC layers do not only prevent the formation of the biofilm on the biomaterial's surface, but also exhibit the bactericidal activity [22]. This was confirmed by Endrino et al., who demonstrated that the silver contained in the DLC film can cause the death of S. aureus bacteria adhered to the surface [22].

The antibacterial properties of Cu-doped DLC coatings were proven by Chan et al. [13]. As it was presented in [13], for the coatings with the Cu content exceeding 58.76 at.%, the antibacterial activity against gram-negative bacteria E.coli reaches the level of 99.9% [13]. This means that for a DLC coating with such high concentration of copper, the attachment of the bacteria to the surface is almost completely eliminated. Moreover, it was also suggested that the copper can act as a fungistatic agent suppressing the growth of the various fungi species on a DLC surface [30]. According to Ivanov-Omskii et al. the Cu-DLC coatings with the Cu content equal to 9 at.% can withstand the fungal attack of such species as A. niger, Ch. globosum, C. cladosporioides, E. nigrum and P. heteromorpha [30]. The authors suggested that the inhibition of fungal growth is achieved through the release of copper ions under the action of organic acids produced by fungi [30]. Hence, the Cu-doped DLC layers may also act as protective coatings against the biodeterioration caused by microorganisms.

Another approach towards the improvement of antibacterial properties of DLC coatings involves the incorporation of the elements which reduce the surface energy of the DLC coatings and thus, prevent the bacterial adhesion [50]. The examples of such elements are Si and F [50]. Ren et al. showed that with the increasing concentration of either Si or F, the surface energy of carbon layers decreases, and as a consequence, the bacteria adhesion to the surface drops [50].

3. HIGHLY HEMOCOMPATIBLE MODIFIED DLC COATINGS FOR CARDIOVASCULAR IMPLANTS

In the case of cardiovascular implants it is essential to prevent the clot formation and hence, it is required from the biomaterial to be non-thrombogenic [28]. In order to inhibit the thrombus formation, it is necessary to limit the platelet adhesion, activation and aggregation [28]. The adhesion and activation of circulating platelets is mainly governed by the surface-absorbed proteins, mainly fibrinogen and albumin [16].

Fibrinogen is well-known to prompt the platelet aggregation and activation, while albumin prevents the adhesion of the platelets [16]. Thus, the high albumin/fibrinogen ratio is treated as the good indicator of antithrombogenic properties [16]. Many studies proved that the incorporation of certain elements into the DLC films, such as F, Ag, N, Si or others, can enhance their hemocompatibility [16,17,26,27,31-34,45-47,51,53].

One of the examples of non-thrombogenic coatings is fluorinated DLC film [26, 27, 51]. Saito et al. showed that the incorporation of F into the DLC layers can significantly suppress the adhesion of platelets and their activation [51]. It was suggested that the lower platelet adhesion is associated with the increased hydrophobicity of the surface of F-DLC coatings [51]. Similar results were obtained by Hasebe et al. who also showed that fluorinated diamond-like carbon coatings exhibit lower wettability, what may be beneficial in terms of their hemocompatibility [26,27]. Those results indicate that the F-DLC layers can be a promising candidates for use as a coating material for blood-contacting devices [26,27].

The antithrombogenic character of DLC surfaces can be also prompted by the incorporation of silicon [45-47]. In general, the higher content of silicon is associated with the lower number of adhering platelets, what was proved by Ong et al. [47]. The decreased adhesion of platelets on the Si-doped DLC surfaces may be caused either by low surface energy or by the increase in its polar component, which diminishes the affinity of platelets and proteins to the surface [47]. Also Okpalugo et al. showed the decrease in the number of platelets aggregates on the Si-doped DLC coatings [45,46]. Moreover, Okaplugo et al. demonstrated that the incorporation of silicon can lower the surface energy and wettability of DLC coatings and hence, decrease their thrombogenicity [45].

Apart from the already mentioned elements, also nitrogen can suppress the thrombus formation and thus, improve the hemocompatibility of DLC coatings [17,33,53]. It was demonstrated that the addition of nitrogen leads to lower platelet adhesion and activation [17,33,53]. Srinivasan et al. pointed out that this effect can be caused by the increased contact angle and surface energy of the N-doped DLC coatings [53]. However, Kwok et al. showed that despite the possible beneficial effect of nitrogen on hemocompatibility of DLC coatings, too high concentration of this element can lead to the deterioration of this property [33].

Another dopant increasing the hemocompatibility of the DLC coatings is silver [16, 34]. It was proven that with the increase of silver concentration in the DLC layer, the ratio of albumin/fibrinogen absorption also increases [16]. As a consequence, the thrombogenicity of the DLC surface is successfully reduced. Choi et al. also suggested that the enhancement of the hemocompatibility of Ag-DLC coatings may be connected with more hydrophobic character of the surface and its lower energy [16]. Lower surface energy and hence, the increased hemocompatibility of silver-doped DLC films was also demonstarted by Kwok et al., who showed preferential absorption of albumin as well as weak adsorption and less conformation of fibrinogen on Ag-DLC surface [34].

Hemocompatibility of DLC coatings can be also improved by the addition of Ca and P [17,31,32]. Kwok et al. demonstrated that strong adhesion of albumin and low conformational change of fibrinogen on Ca-DLC and P-DLC surfaces may be achieved [31]. The beneficial effect of P on the hemocompatibility of DLC coatings was also indicated by Chu [17]. However, it was suggested that the increased hemocompatibility of Ca- and P-doped DLC layers results from their high wettability [31]. This stays in a contradiction to the previously mentioned studies that point out the increased hydrophobicity of the surface as the reason behind the decreased thrombogenicity. Similarly, also the effect of the surface energy on the hemocompatibility of the DLC surface is not clear and requires deeper understanding.

4. MODIFIED DLC COATINGS ENHANCING BONE TISSUE DEPOSITION FOR BONE IMPLANTS

As far as bone implants are concerned, the most important biological property is the interaction of the biomaterial's surface with the osteoblasts, i.e. bone-forming cells. The proper bone-healing is strongly dependent on the osseointegration process and thus, the promotion of the bone-tissue deposition at the bone-implant interface is desirable. Numerous different studies showed that this can be achieved through the use of modified DLC coatings [4,8,15,24,49,52,54].

Among various different elements, titanium is one of the most commonly used as an osseoinductive agent [4,8,15,24,49,52,54]. It was demonstrated that DLC coatings containing either pure titanium or titanium oxide exhibit excellent biocompatibility towards the osteoblasts [4,8,15,24,49,52,54]. Shroeder et al. demonstrated that the addition of Ti into the DLC matrix promotes the bone marrow cells proliferation, and simultaneously reduces the activity of the osteoclast-like cells, which are responsible for bone resorption [52]. Similar results were obtained by Francz et al., who indicated the usefulness of Ti-DLC coatings for bone implants [24]. Cheng et al. suggested that the good biocompatibility of the Ti-DLC coatings may be associated with the larger contact area and more negative electrostatic state of the surface, leading to better adsorption of calcium ions [15]. The beneficial effect of TiOx incorporation into the DLC films was confirmed by Thorwarth et al. [54]. The research presented in [54] showed that the addition of TiOx favours the proliferation of osteoblasts on DLC coatings [54]. Likewise, also Amin et al. proved that TiO_2-DLC coatings are a very promising material for bone implants [4]. However, Randeniya et al. proved that the lower concentration of Ti, equal to about 3 at.%, is more favourable for the adhesion and proliferation of osteoblasts than high content of this element [49]. Furthermore, Bharathy et al. showed that the biocompatibility of the Ti-DLC towards osteoblasts is even better when the Ti concentration does not exceed 1.1 at.% [8]. This was explained by the fact that higher concentration of Ti may lead to the release of titanium ions having negative impact on the proliferation of osteoblasts [8].

Some researchers also suggest that the osseointegration process can be enhanced through the addition of silicon into the DLC matrix [54]. Thorwath et al. presented that the slight enhancement of osteoblast adhesion and proliferation can be achieved for Si-DLC coatings [54]. On the other hand, Bendavid et al. and Randeniya et al. did not show any significant influence of either Si or SiOx on the osseointegration process [7,48].

5. BIOCOMPATIBILITY OF MODIFIED DLC COATINGS

The incorporation of different elements into the DLC coatings can significantly improve their biological properties and allow the development of DLC films with certain functionality. However, it has to be remembered that each dopant may influence also the biocompatibility of the DLC layers. Therefore, it is always necessary to consider not only the desired bioactivity of the DLC coatings, but also their biocompatibility.

One of the elements raising doubts about the biocompatibility of modified DLC coatings due to its toxic effect on the bacterial cells is silver [28]. It was reported that addition of silver to the DLC coatings may have cytotoxic effect on bone marrow cells [28]. In [28], it was indicated that with the increasing concentration of silver, the number of cells on the surface decreases. At the same time, the tendency of the bone marrow cells to differentiate into the osteoclasts increases [28]. Those results implies that the addition of silver may inhibit the deposition of bone tissue and promote its resorption [28]. However, different studies showed that incorporation of silver does not cause the deterioration in the biocompatibility of DLC films [14,23]. Endrino et al. demonstrated that despite the initial decrease in the number of mouse osteoblastic cells on the surface of Ag-DLC films, after one week the number of the cells present on the surface increases [23]. This may be an indicator of good osseointegration properties of Ag-DLC coatings. Similar results were obtained in [14] for human osteoblasts. Moreover, it was proven that the addition of silver may inhibit the mitotic activity of rat glioma cells [3]. This means that the Ag-DLC coatings can prevent the formation of certain tumours.

Apart from silver, also other dopants may have significant influence on the biocompatibility of DLC films. As it was already described in the previous section, elements such as Ti or Si may have positive effect on the biocompatibility of DLC coatings towards the osteoblastic cells. Additionally, the good biocompatibility of Si-DLC films was also confirmed towards other cell lines [41,43,44]. Ogwu et al. demonstrated that the increasing concentration of Si results in a proper surface energy that favours the attachment of human endothelial cells [41]. Moreover, it was proven that the Si-DLC coatings do not exhibit cytotoxicity towards those cells [41]. This was confirmed by the experiments carried out by Okpalugo et al. [44,43]. As well as that, it was demonstrated that the silicon contained in DLC coatings does not affect the viability of the bovine retinal pericytes [43].

Another dopant that may enhance the biocompatibility of the DLC coatings is nitrogen [36,44,56]. Liao et al. stated that the incorporation of nitrogen into DLC films may result in improved biocompatibility and bioinertness [36]. This was proven by the increased attachment and proliferation of mouse fibroblasts on the N-DLC surface [36]. Moreover, Yang et al. obtained results indicating the proper growth of human microvascular endothelial cells on N-doped DLC coatings [56].

The beneficial effect of nitrogen on the cell growth on DLC coatings was confirmed by the increased MTT activity reflecting the proper cell metabolism [56]. Also Okpalugo et al. proved that the attachment of human microvascular endothelial cell on the N-doped coatings is better than for undoped DLC coatings [44].

The biocompatibility of DLC coatings can be also affected by the incorporation of calcium [20,21]. Dorner-Reisel et al. showed that DLC coatings with Ca-O promote the adhesion and proliferation of mouse fibroblasts [20,21]. Moreover, the cell morphology investigations indicated that the viability of the cells on the Ca-O modified DLC surfaces is

higher than in the case of unmodified DLC coatings [20,21]. The authors explained that this effect may be caused by the incorporation of $CaCO_3$ into the DLC coatings that can act as bioactive agent that increases the biocompatibility [20].

6. CONCLUSIONS

The incorporation of various elements into the DLC matrix can cause the significant enhancement of their biological properties. The modification of DLC layers can influence not only their biocompatibility, but also their hemocompatibility, antibacterial properties as well as osseointegration properties. Therefore, the elements used as dopants for DLC coatings can be divided into four main categories (see Figure 1):

1. Improving the general biocompatibility.
2. Enhancing the hemocompatibility and suppressing the thrombogenicity.
3. Promoting the osseointegration process.
4. Preventing the bacteria adhesion.

The categories listed above are strictly connected with different fields of biomedical applications of DLC coatings modified by the incorporation of various elements.

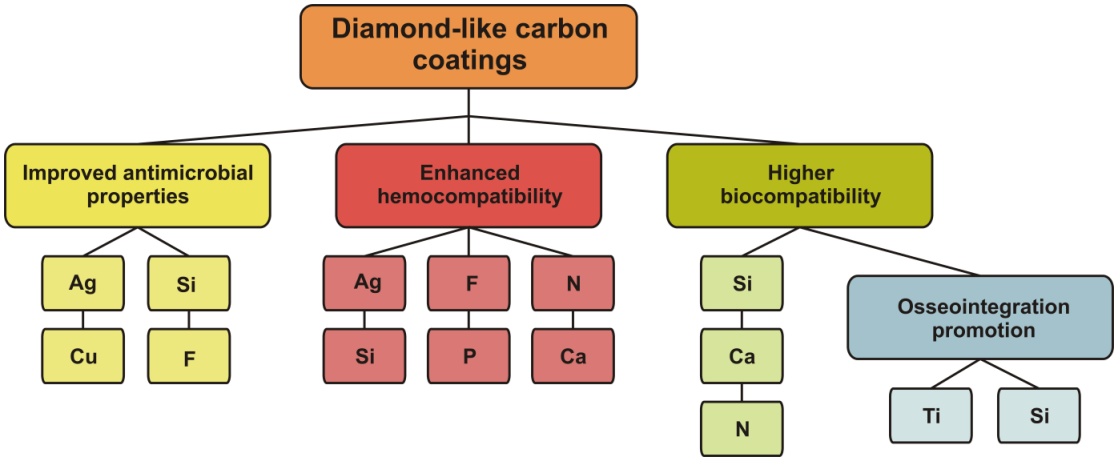

Figure 1. Scheme showing the influence of different dopants on the biofunctionality of DLC films based on the literature review.

The modified DLC coatings with improved biocompatibility and antibacterial properties may be useful for all kinds of implants. Due to the better biocompatibility, the risk of adverse tissue reaction after the implantation, such as inflammation, allergy and irritation, is lowered. At the same time, modified DLC coatings with increased antibacterial properties prevent the formation of the biofilm on the surface of the implant and hence, the danger of the development of post-implantation infection is eliminated. The dopants with the positive effect on the biocompatibility include Ti, Si, Ca, and N, while Ag, Cu, Si and F are the elements capable of enhancing the antibacterial properties.

Another group of modified DLC coatings is comprised of films with the addition of the antithrombogenic agents. Such coatings can be used for cardiovascular devices, such as peacemakers, heart valves or stents, that are in constant contact with blood and thus, cannot cause the formation of thrombus in order to be safely implanted. The examples of the elements that may enhance the hemocompatibility of DLC coatings include Ag, Si, Ca, P, F and N.

Apart from the improvement of biocompatibility, certain elements may specifically improve the biological response of the DLC coatings towards the bone-forming cells. DLC coatings modified with those dopants are able to promote the process of bone-healing through the enhancement of the osseointegration process. The most known additive causing the improved adhesion of osteoblasts is Ti. However, also the addition of Si may result in a slight increase in the bone-tissue deposition at the implant site. Such coatings can be applied for any orthopaedic implants that substitute bones.

To conclude, by the addition of particular dopants, distinct biofunctionality can be achieved. Thus, it is possible to tailor biological properties of DLC films by alloying with different elements in order to obtain features necessary for strictly defined biomedical applications. Nevertheless, it has to be remembered that each dopant influence not only the bioactivity of DLC coatings, but also its biocompatibility and other physiochemical and mechanical properties. Therefore, during the development of a new biomaterial it is always necessary to conduct a detailed and complex investigation not only in terms of the desired biofunctionality, but also concerning the general biocompatibility and physiochemical characteristic. This is of the utmost importance since the biological and physiochemical properties of the biomaterials are mutually dependent on each other, and are both required for the biomaterial to function properly and fulfill its role.

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