

# Analyses of Biofilm on Implant Abutment Surfaces Coating with Diamond-Like Carbon and Biocompatibility

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The aim of this study was to evaluate the surface free energy (SFE), wetting and surface properties as well as antimicrobial, adhesion and biocompatibility properties of diamond-like carbon (DLC)-coated surfaces. In addition, the leakage of *Escherichia coli* through the abutment-dental implant interface was also calculated. SFE was calculated from contact angle values;  $R_a$  was measured before and after DLC coating. Antimicrobial and adhesion properties against *E. coli* and cytotoxicity of DLC with human keratinocytes (HaCaT) were evaluated. Further, the ability of DLC-coated surfaces to prevent the migration of *E. coli* into the external hexagonal implant interface was also evaluated. A sterile technique was used for the semi-quantitative polymerase chain reaction (semi-quantitative PCR). The surfaces showed slight decreases in cell viability ( $p < 0.05$ ), while the SFE,  $R_a$ , bacterial adhesion, antimicrobial, and bacterial infiltration tests showed no statistically significant differences ( $p > 0.05$ ). It was concluded that DLC was shown to be a biocompatible material with mild cytotoxicity that did not show changes in  $R_a$ , SFE, bacterial adhesion or antimicrobial properties and did not inhibit the infiltration of *E. coli* into the abutment-dental implant interface.

## Introduction

Diamond-like carbon (DLC) is the basis of many studies due to its mechanical, physical and chemical properties, as well as its wide range of applications in electronics and in chemical-, mechanical- and bio-engineering. Thin DLC-coated films have been shown to be biocompatible and to exhibit antimicrobial activity (1,2); they may eliminate *Escherichia coli* deposited on stainless steel and decrease the biofilm formation (3). The primary killing mechanism was proposed as describing the physical interaction between carbon-based nanomaterials and bacteria, since these nanomaterials can cause irreparable damage to the outer membranes of some Gram-negative bacteria (*E. coli* and *Shewanella oneidensis*) (4,5). The DLC films are also considered good candidates for biomedical applications due to their biocompatibility with human cells, which was widely studied. Further, because of their carbon and hydrogen composition, DLC films present optimal cell behavior and high rates of cell proliferation (3,4,6-9).

Although the use of implant-supported prostheses for the treatment of partially or totally edentulous patients has shown success, complications with single-unit prostheses may result in loosened screws, which can cause mechanical complications and inflammation in peri-implant tissue and eventually the loss of the implant. If exacerbated, it promotes the infiltration of bacterial flow,

regardless the size of the interface between abutments and dental implants and the type of prosthetic connection (10). Gram-negative bacteria such as *E. coli*, used in this study, have a large size, good mobility and survive in adverse environments; they are able to infiltrate the interior of the interface between abutments and dental implants (24). A protocol to control bacteria and strategies to reduce bacterial adhesion and biofilm formation on the surfaces of fasteners during the treatment and maintenance of implants are needed to improve the long-term survival and the soft tissue health in the treatment of peri-implantitis (11). Even if the relationship between the characteristics of the surfaces and the behavior of biofilm formation has not been well defined, the surface free energy and roughness of surfaces to prevent the accumulation of biofilm are worth mentioning. Previous reports have shown that protein adsorption and bacterial adhesion *in vivo* may result by a threshold surface roughness of 0.2  $\mu\text{m}$  (12).

Thus, if DLC is applied to components of dental implants, it may prevent the migration of bacteria to the interface between the abutment and the dental implant; it may be biocompatible and could contribute to the longevity and success of rehabilitation treatment. So, the objective of this study was to evaluate the preventive action of DLC on the migration of bacteria into the interface between abutments and dental implants. Properties including

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roughness, surface free energy, antimicrobial activity, bacterial adhesion and biocompatibility of DLC coating on titanium surfaces were also investigated and the results were correlated with abutment-implant interface properties.

## Material and Methods

### Bacterial Strains and Growth Media

*Escherichia coli* ATCC 8739 was grown in brain-heart infusion (BHI) culture medium at 37 °C for 3 h. Turbidity was measured in a spectrophotometer (Spectrum, SP-2000 UV) at 600 nm (OD600) and was found to be 0.3, corresponding to  $1.3 \times 10^8$  CFU/mL.

### Cell Culture

HaCat cells, a human keratinocyte cell line, were maintained in Dulbecco's modified Eagle medium (DMEM) (Sigma Chemical Co., St. Louis, MO, USA) supplemented with 10% fetal bovine serum (FBS; Invitrogen, Carlsbad, CA, USA), 100 UT/mL penicillin, 100 µg/mL streptomycin and 2 mmol/L glutamine (Gibco, Grand Island, NY, USA) in a humidified atmosphere (5%) of CO<sub>2</sub> at 37 °C. Subsequently, the cells were cultured at a concentration of  $5 \times 10^4$  cells/mL in 75 cm<sup>3</sup> flasks.

### Preparation of Discs

Titanium (Ti) discs (7.5 mm in diameter and 2 mm thick) were fabricated (Dentoflex Comércio e Indústria de Materiais Odontológicos Ltda, São Paulo, SP, Brazil) and subjected to polishing and finishing procedures to achieve a low mean roughness; 0.2 µm was accepted as the threshold for surface screw prosthetic components (12). All discs were ultrasonically cleaned with distilled water and isopropyl alcohol. CVDentus<sup>®</sup>, (São José dos Campos, SP, Brazil), coated the discs with DLC (Ti-DLC). Subsequently, roughness ( $R_a$ ) was also measured by profilometry (SurfTest SV-400, Mitutoyo Corp., Kawasaki, Japan). The discs were sterilized by gamma radiation at 25 kGy at the Radiation Technology Center (International Irradiation Association [IPEN-CNEN/SP], Brazil).

### Surface Free Energy

The surface free energy of the discs was calculated from the contact angle (n=3) for each group tested with the following liquids: water, ethylene glycol, polyethylene glycol, and diiodomethane with polarities of 52.20, 19.00, 13.60, and 2.60, respectively. The sessile drop technique (Young-Laplace) was used at 37 °C in a goniometer (Contact Angle System OCA; DataPhysics Instruments GmbH, Filderstadt, Germany) coupled to a computer with SCA 20 software. For analysis of the physical and chemical characteristics of Ti and Ti-DLC, four liquids of

different polarities were dropped (0.25 µL) onto each material test disc and the contact angle between them was measured. Subsequently, contact angle "θ" values were obtained from the right and left sides of the image of the droplet formed with different solutions and contact angles average were calculated by the SCA 20 software. After the contact angle had been measured, the arithmetic mean and standard deviation were calculated to obtain the surface free energy of each group of samples by use of the SCA 20 software with the OWRK (Owens-Wendt-Rabel-Kaelble) equation.

### Colorimetric Analysis of Methylthiazol Tetrazolium (MTT)

Biocompatibility testing of the DLC was performed by colorimetric analysis of methylthiazol tetrazolium (MTT). The evaluation of the viability and proliferation of HaCaT cells was measured by cytochemical activity of succinic dehydrogenase (SDH), which represents the rate of mitochondrial respiration of viable cells. Ti, Ti-DLC discs (n=24) and glass cover slips (control group) were used in this test. The discs were distributed in 24-well microtiter plates. Then,  $5 \times 10^4$  cells/mL were seeded and maintained in a 5% CO<sub>2</sub> atmosphere at 37 °C for 24 h. After this period, the samples received MTT solution (Sigma Chemical Co, St Louis, MO, USA) and the cells were incubated for 4 h at 37 °C. Cell viability was assessed with an ELISA microplate reader (3550-UV ; BIO-RAD, Hercules, CA, USA) at a 570 nm wavelength.

### Bacterial Adhesion Test

*E. coli* ( $1.3 \times 10^8$  CFU/mL) in the BHI medium was maintained for 1.5 h in an orbital shaker at 75 rpm at 37 °C for microorganisms to adhere to the surface of the Ti and Ti-DLC discs (n=24). After the adhesion period, the discs were rinsed with sterile phosphate buffered saline (PBS) to remove non-adherent microorganisms. Then, new medium was added and the discs were incubated at 37 °C for 24 h at 75 rpm. They were then washed with PBS, placed individually in sterile saline and ultrasonicated for 20 min. The resulting material was diluted and plated on BHI agar for 24 h at 37 °C for further quantitative analysis of CFU/mL.

### Antimicrobial Test

Sterile Ti and Ti-DLC discs (n=24) were distributed in microtiter plates (24 wells) containing BHI and *E. coli* ( $1.3 \times 10^7$  CFU/mL) and maintained at 37 °C for 3 h and 24 h. Gentamycin at 100 g/mL (Sigma Chemical Co.) was used as positive control. After the incubation period, aliquots were removed from each well and read by an ELISA reader at 570 nm (Ascent 354 Multiskan; Labsystems CE,

Les Ulis, France). For the CFU/mL counting, aliquots from each period were removed, plated on BHI agar medium and maintained at 37 °C for 24 h.

### *Microbiological Analysis: Bacterial Leakage at Implants with External Hexagon-Abutment Connections*

In the present study, external hexagon implants 4.0 mm in diameter and 13 mm long (n=28) with their respective anti-rotational abutment were used (Dentoflex). Fourteen screws and surfaces of the implant and the abutments in contact with them were coated with DLC film. During the experimental stages, biosafety standards were adopted.

The implants were fixed in a "bench vise" and the abutments were carefully connected to the implant with a digital torque meter to 32 Ncm (TQ-680; Instrutherm, São Paulo, SP, Brazil), according to the manufacturer's protocol. The assemblies were suspended and stabilized in a glass beaker by a special device manually made from orthodontic chromium nickel (CrNi) wire and wire mesh, so that only the region of the interface between the abutment and dental implant had contact with the culture medium, whether or not it was contaminated with *E. coli*.

All groups were submerged in sterile BHI broth, and the test groups were inoculated with *E. coli* ( $1.3 \times 10^7$  UFC/mL) and maintained at 37 °C for 24 h. Subsequently, the joint surfaces were removed and washed twice with sterile saline solution and cleaned with sterile gauze to remove the biofilm from the external surfaces. Each set was disconnected and the components (implant, screw and abutment) were placed individually in sterile saline and maintained in ultrasonic bath for 10 min. Subsequently, aliquots of the samples were removed by serial dilution, plated on BHI agar medium and maintained for 24 h at 37 °C; then, they were quantified in CFU/mL.

### *Microbiological Analysis: Preparation of Collected Samples for DNA Extraction*

To check for contamination by *E. coli* within the implant-abutment joint, PCR was used. Bacterial samples from the implant-abutment joint were collected, centrifuged, re-suspended in sterile PBS and frozen at -20 °C. Later, DNA extraction was performed by heating the samples at 97° C for 10 min (Thermomixer; Biorresearch, São Paulo, SP, Brazil).

Aliquots of the samples were placed in phenol/chloroform/isoamyl alcohol (25:24:1) and centrifuged. Next, DNA precipitation was performed at -20 °C, with the addition of 0.25 volumes of NaCl (5 M) and 2.5 volumes of cold absolute ethanol. After this period, the samples were centrifuged and the DNA was washed with 75% ethanol. The final pellet was re-suspended in TE (10 mM Tris-HCl,

pH 8.0, 1 mM EDTA, pH 8.0) and frozen at -20 °C until later use. The same procedure was used for purifying DNA from the *E. coli* bacterial strain (ATCC 8739), which was used as a positive control.

### *Semi-quantitative Polymerase Chain Reaction - PCR*

The sequence of primers used for *E. coli* 16S14F was as follows: 5' CTTGTACACACCGCCCGTC 3' and 23S1R 5' GGGTTTCCCATTCGGAAATC 3' (Life Technologies of Brazil, Ltda). The oligonucleotides were dissolved in sterile solution containing Tris-HCl (10 mM, pH 7.6) and EDTA (1 mM, pH 8.0).

PCR was performed in a final volume of 25 µL, containing approximately 1 µL of each oligonucleotide (Invitrogen Tech-Line<sup>SM</sup>), 10.5 µL dNTPs (Invitrogen Tech-Line<sup>SM</sup>), from 1 to 2.5 µL of Mg<sup>2+</sup> and 0.2 µL of Taq DNA polymerase (Invitrogen Tech-Line<sup>SM</sup>). All reactions were performed in the presence of a positive control containing specific genomic DNA of this bacteria and a negative control without a DNA template. Thermocycling regimen was 94 °C for 3 min, 35 cycles at 94 °C for 45 s, 55 °C for 30 s and 72 °C for 1.5 min. The presence of the amplified DNA was observed via electrophoresis using 1.5% agarose gel (Invitrogen Tech-Line<sup>SM</sup>), along with Sybr Safe DNA gel stain 10000X (Invitrogen Tech-Line<sup>SM</sup>). The 100-bp marker (GibcoBRL) was used as a molecular weight standard. The bands on the gel were observed in the UV light transilluminator and the images were scanned using Vision Works LS Version 5.5.4 software.

### *Statistical Analysis*

A statistical analysis was performed for the data of quantitative variables by means of both nonparametric (Mann-Whitney U) and parametric tests (Student's *t* test) for analysis of the results. Data were expressed as mean ± standard deviation. All statistical tests were considered at a significance level of 5% ( $p < 0.05$ ).

## **Results**

### *Surface Roughness, Contact Angle Measurements and Surface Free Energy*

The results of surface roughness ( $R_a$ ), contact angle of the tested liquids (water, ethylene glycol, polyethylene glycol, and diiodomethane), and surface free energy (SFE) on the surfaces of Ti and Ti-DLC discs are reported in Table 1. The  $R_a$  values were 0.15 and 0.14 µm for Ti and Ti-DLC surfaces, respectively, and were not significantly different ( $p > 0.05$ ). The surfaces presented the highest contact angle values with water and the lowest ones with hydrophobic wet agents and there were no statistically significant differences ( $p > 0.05$ ) between groups. The SFE for each tested surface was calculated based on the polarity of the

liquids tested by the OWRK (Owens-Wendt-Rabel-Kaeble) equation. The obtained values of 5.4 and 5.6 mN/m for Ti and Ti-DLC, respectively, corresponded to the evaluated capacities of the polar surfaces.

### Biocompatibility

The viability of human keratinocytes (HaCat) grown on discs of Ti, Ti-DLC and control discs, was assessed by the MTT assay. The surfaces of Ti and Ti-DLC discs showed respective decreases in cell viability of 58.99% and 66.07%, and presented a statistically significant difference compared with the control (Table 2). Thus, both tested surfaces showed mild toxicity. Statistical analysis revealed no significant differences between the two experimental groups ( $p > 0.05$ ).

### Antimicrobial and Anti-Adhesive Properties

In the antimicrobial test, there was no inhibition of *E. coli* in the tested groups during the review periods of

3 h and 24 h (Fig. 1). The positive and negative control groups corresponded to the gentamycin and BHI cultures, respectively.

The adhesion of *E. coli* did not decrease on the surfaces of Ti-DLC discs compared with the clean surface of Ti discs and the values were approximately between 7 and 7.2 log (CFU/mL) (Fig. 2).

### Bacterial Infiltration

After 24 h of incubation in a medium containing *E. coli*, all dental implants with their respective abutments were disconnected and all sets showed bacterial infiltration. The average values of viable *E. coli* present in the interface between abutment and dental implant are shown in Fig. 3. The analysis showed no statistically significant difference between the tested groups ( $p > 0.05$ ).

Microbiological analysis by semi-quantitative PCR with the specific primer for *E. coli* showed presence of positive DNA in 100% of the tested samples of both groups, confirming the contamination at the interface between the abutment and dental implant (Fig. 4).

Table 1. Means and standard deviation (SD) of surface roughness ( $\mu\text{m}$ ) and contact angles (degree) of Ti and Ti-DLC groups

Groups	Ti	Ti-DLC
Surface roughness ( $\mu\text{m}$ )	0.15 $\pm$ 0.03	0.14 $\pm$ 0.02
Contact Angle (degree)		
Water	74.8 $\pm$ 4.0	69.9 $\pm$ 4.1
Ethylene glycol	25.8 $\pm$ 5.9	24.1 $\pm$ 2.7
Polyethylene glycol	27.2 $\pm$ 7.1	21.9 $\pm$ 3.5
Diiodomethane	23.8 $\pm$ 2.5	19.6 $\pm$ 1.4
Surface free energy (mN/m)	5.4	5.6

\*Note: The surface free energy (mN/m) is shown as the value of its polar component (*t* test,  $p > 0.05$ ).

## Discussion

Table 2. Cell viability (%) as determined by the MTT assay

Treatment	Cell viability (%)
Glass cover slip	99.26 (85.99-114.07) A*
Ti	58.99 (56.28-72.99) B
Ti - DLC	66.07 (58.51-70.08) B

\*Note: Different capital letters represent statistically significant differences between the cell biomasses in the tested groups (Mann-Whitney,  $p > 0.05$ ).

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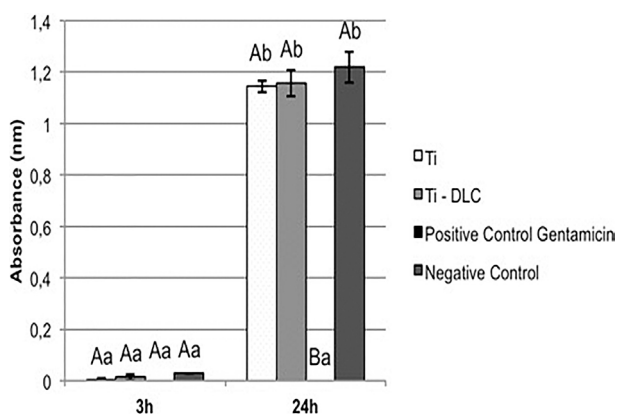


Figure 1. Inhibition of bacterial growth after 3 h and 24 h of incubation for Ti and Ti-DLC groups. Columns represent means and error bars represent standard deviations (Mann-Whitney, *t* test,  $p > 0.05$ ).

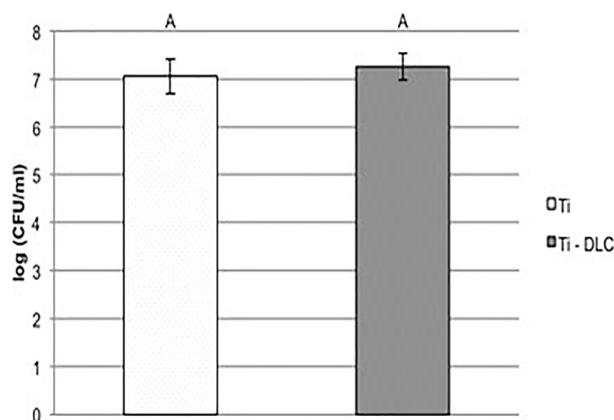


Figure 2. Microorganisms adhering (CFU) to the disc surfaces coated with Ti and Ti-DLC. Columns represent means and error bars represent standard deviations (*t* test,  $p > 0.05$ ).

In this study, surfaces coated with diamond-like carbon (DLC) were evaluated because the technique has recently attracted attention due to its unique physical, chemical and mechanical properties and it may be applied in many different areas of health care, including dentistry. According to the method of preparation and deposition, the DLC may have different characteristics and properties. Contradictory results have been found regarding the effect of the DLC coating (7). In this study, the samples were coated with DLC by CVDentus® by means of technical chemical vapor deposition (CVD).

Antimicrobial tests on DLC-coated surfaces showed antibacterial properties when tested with *E. coli* (5). The total increase in biomass growing in a liquid medium bacterial culture can be monitored by the measurement of optical density (absorbance). Figure 1 shows that none of the tested groups was able to inhibit bacterial growth. Different authors (5,13) have reported that DLC films exhibit antibacterial activity of 33% in a 3 h incubation, which differs from the results of this study. The methodology applied in the present study (in terms of bacterial strain, culture medium and the microorganism concentration) was the same as that used in the studies mentioned above.

Bacterial adhesion on a given surface depends on many factors, including the specific characteristics of the bacterial strains as well as the type of biomaterial. The ability of the biomaterial to resist bacterial adherence is one of the factors that can influence colonization or bacterial infection (14). The free surface energy and roughness of surfaces are important aspects in preventing the adhesion of biofilm and were shown as having direct impact on the adhesion phase in bacteria. A relationship between the types of substrates and the bacterial species has been studied (15) and surface roughness above a threshold of 0.2  $\mu\text{m}$  can influence the initial phase of bacterial adherence; hence, in this study,

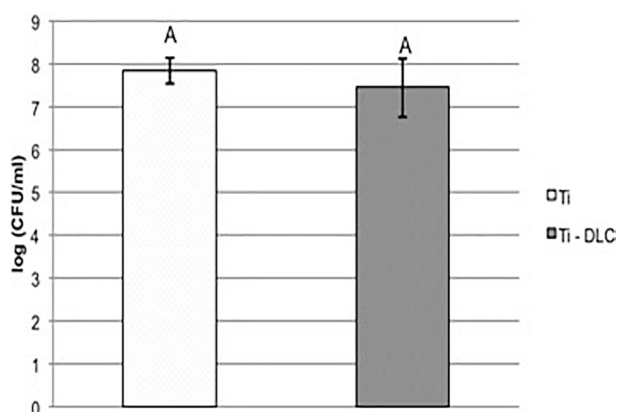


Figure 3. Columns represent means and error bars represent standard deviations for the presence of *E. coli* (log CFU/mL) after 24 h of incubation in the Ti-DLC and Ti groups (*t* test,  $p > 0.05$ ).

titanium discs with mean roughness values of  $0.15 \pm 0.03 \mu\text{m}$  (Table 1) were used. The results showed that after the DLC coating, these films showed no significant changes in values of roughness values, indicating that the surface properties of the DLC coating did not have a significant influence on bacterial adherence. These findings contradict those of other studies (14,16) that evaluated DLC surfaces and showed that they exhibited high resistance to bacterial adherence of *S. aureus* and *S. epidermidis*. Conversely, multispecies anaerobic microorganisms in biofilms on DLC surfaces have demonstrated that the surfaces were unable to reduce bacterial adherence (17). The determination of the antibacterial DLC coating surfaces with only single species testing cannot be considered a basis for *in vivo* response, since the oral microbiota includes a variety of microorganisms.

Different intermolecular forces can define how bacteria interact with a specific type of surface. Consequently, the interactions can be stronger or weaker, depending on the characteristics of these forces. In this study of *E. coli*, Gram-negative bacteria were used. These species possess a thinner peptidoglycan layer and an outer membrane that consists of proteins, phospholipids and lipopolysaccharides (LPS) (18), which have a negative net charge that contributes to hydrophilic characteristics (19). According to the results of this study, the average contact angle (Table 1) showed that Ti and Ti-DLC surfaces have characteristically high hydrophobic and lipophilic values that could have influenced the ability of *E. coli* to adhere to these materials. These results corroborate those of another study (20) showing similar values for the contact angle with water in DLC-coated surfaces, with values of  $69.9 \pm 4.1$ .

The OWRK equation is most commonly used in the literature for the determination of the surface free energy (21), suggesting that the polarities of the surfaces are an

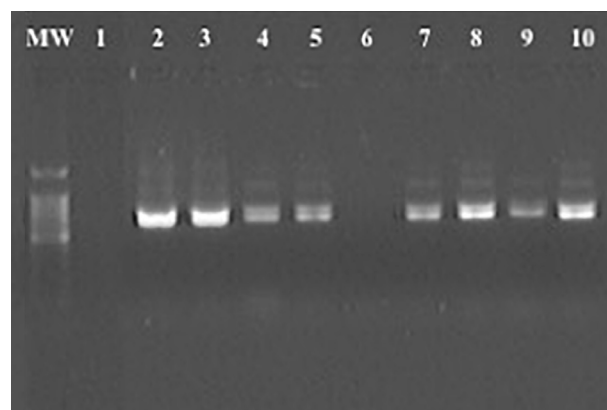


Figure 4. Semi-quantitative PCR for the detection of *E. coli* in the dental implant-abutment joint. MW: molecular-weight standard (100-bp ladder); lines 2–5, Ti-DLC testing group; lines 7–10, Ti control group.

important part of solid-liquid interactions. Thus, the polar components of the tested samples (Table 1) showed values of 5.4 and 5.6 mN/m for Ti and Ti-DLC, respectively, without any significant difference. Diab Al Radha et al. (22) found similar values (5.19 mN/m) for the polar component in titanium samples with average roughness values of less than 0.2  $\mu\text{m}$ ; this was the same pattern and results seen in this study. Moreover, Podgornika et al. (21) assessed steel surfaces coated with DLC by the CVD technique, and found a value of 10 mN/m for the polar component. As shown, DLC coating on titanium samples does not modify the surface properties of the material, considering the surface free energy.

*In vitro* studies evaluated the biocompatibility of the materials by cell viability tests with the MTT assay. Marciano et al. (1) and Wachesk et al. (2) noticed that the first time period (24 h) is essential for cell adhesion due to the migration and proliferation of cells on the surface. The results of the viability of human keratinocytes (HaCat) evaluated on Ti and Ti-DLC discs indicated that the DLC coating does not affect cell response when compared with titanium discs. According to the ISO 10993-5 2009 Standard: Biological Evaluation of Medical Devices: *In vitro* Methods, the reference value of 99.26% was used in the present study for cell viability (control); thus, it can be stated that both surfaces tested showed mild toxicity. These results agree with those of previous studies, which also found that for *in vitro* studies, the biocompatibility of DLC films is well-tolerated and does not cause adverse effects (8,14). However, information about the effect of DLC on cellular metabolism is scarce, as are *in vivo* studies of DLC. Additional testing is required to confirm cellular integrity, such as tests that prove the structural and functional integrity of the biological material.

The presence of microbial colonization in the dental implant-abutment interface contributes to periimplant inflammation, which may result in serious clinical complications. Leonhardt et al. (23), reported that microorganisms not associated with periodontitis caused more than 50% of peri-implant lesions; enteric bacteria such as *E. coli* and *E. cloacae* were shown to exist in the lesions. *E. coli*, used in this study, is motile and its physiological characteristics associated with survival in adverse environments guarantee the possibility of infiltration inside the dental implant-abutment interface, which ranges from 1 mm to 10 mm (24). In bacterial infiltration tests, the method of infiltration from the external to the internal environment was established by simulation of the *in vivo* process. After 24 h of contact with the bacterial suspension, the external surfaces of the sets were manually decontaminated, because the

objective was to evaluate only the dental implant-abutment interface; however, this procedure could have influenced the results, due to different forces applied by the operator. The maladaptation of dental implant-abutment joints may be caused by loosening of the screws and thus can cause mechanical or inflammatory disease complications. This is related to the amount of bacterial leakage where the torque is directly applied (10). However, the amount of torque used in this study was set according to the manufacturer's instructions; thus, all sets would have received the same applied force. In Figure 3, both groups show bacterial infiltration; the coating of the screw as well as the contact areas coated with DLC had no antibacterial characteristics and DLC did not prevent bacterial infiltration into the interface. These results corroborate the findings of Neves et al. (24), who evaluated microleakage in external hexagonal dental implants using screws with and without DLC coating and found no significant differences. Thus, it seems that coating surfaces with DLC have no bactericidal effect on the evaluated groups. The whole procedure was developed according to established biosafety standards to avoid external contamination; however, no external contamination was observed and none was excluded. This result was also supported by a microbiological analysis with semi-quantitative PCR in which the results showed that all samples were contaminated with *E. coli* and not by other microorganisms from the outside environment.

There are few studies in the literature focusing on the effect of this type of film applied on dental implants and abutments. The results of this study show divergences of the physical and chemical properties of DLC compared with those reported in the existing literature. In contrast, there are several techniques used to produce DLC, which may result in different characteristics and properties. Even so, further studies are required to clarify important characteristics of the DLC, including delamination and various films thicknesses or application of this material to abutments and dental implants of different materials. Thus, this study provides additional information about DLC and its application to material surfaces in health care and dentistry settings.

The results showed that diamond-like carbon (DLC) does not modify roughness and surface free energy applied to titanium surfaces. Also, this material has shown to be biocompatible, with mild cytotoxicity. DLC has no antimicrobial properties and does not interfere with bacterial adhesion when tested against *Escherichia coli*. Similarly, the DLC coating on the bolt and areas of contact (dental implant-abutment joint) does not exhibit antimicrobial properties and does not inhibit bacterial leakage in this interface.

## Resumo

O objetivo deste trabalho foi avaliar a energia livre de superfície (ELS), molhabilidade e propriedades de superfície assim como propriedades antimicrobianas, de adesão e biocompatibilidade de superfícies recobertas com Diamond-Like Carbon (DLC). Além disso, investigou-se a infiltração de *Escherichia coli* por meio da interface abutment-implante dentário. ELS foi calculada a partir dos valores de ângulo de contato;  $R_0$  foi medida antes e depois do revestimento com DLC. Foram avaliadas propriedades antimicrobianas e de adesão contra *E. coli* e citotoxicidade do DLC utilizando queratinócitos humanos (HaCaT). Além disso, também avaliamos a capacidade para impedir a migração de *E. coli* na interface do implante hexágono externo. Uma técnica estéril foi utilizada para a reação em cadeia da polimerase semi-quantitativa (PCR semi-quantitativo). As superfícies mostraram uma ligeira diminuição da viabilidade celular ( $p < 0,05$ ), enquanto a ELS,  $R_0$ , adesão bacteriana, testes antimicrobianos e de infiltração não apresentaram diferenças estatisticamente significativas ( $p > 0,05$ ). Concluiu-se que o DLC demonstrou ser um material biocompatível levemente citotóxico que não mostra alterações na  $R_0$ , ELS, adesão bacteriana ou propriedades antimicrobianas e não inibiu a infiltração de *E. coli* na interface abutment-implante dentário.

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