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Diamond-like carbon films over reconstructive TMJ prosthetic materials: Effects in the cytotoxicity, chemical and mechanical properties

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ABSTRACT

Increasingly more young patients have been submitted to reconstruction of the Temporomandibular Joint (TMJ), so, the prostheses must to present more functional longevity.

Objective: To evaluate the effect of diamond-like carbon film (DLC) over titanium alloy (Ti6Al4V) and polyethylene (UHWPE) samples, their mechanical and chemical properties and cellular cytotoxicity.

Methods: Titanium and UHWPE specimens, with 2.5 cm in diameter and 2 mm thickness were coated through plasma enhanced chemical vapor deposition (PECVD) with DLC or DLC doped with silver (DLC-Ag). Scanning electron microscopy (SEM) morphological analysis, Energy-dispersive spectroscopy (EDS) chemical analysis, scratching test, mechanical fatigue test, surface roughness analysis, and cellular cytotoxicity were performed. Data were statistically analyzed using one-way ANOVA ($p < 0.05$) or two-way ANOVA and multiple comparison Tukey test.

Results: In the SEM analysis, morphological differences were observed on substrates after DLC deposition. The film chemically modified the substrate surfaces, according to the EDS analysis. The initial critical load failure occurred at 6.1 N for DLC and 9.7 N for the DLC-Ag film. The DLC film deposition over the polyethylene promoted a decrease in the polymer's damaged area after mechanical fatigue cycling. The cytotoxicity analysis demonstrated less biocompatibility in experimental groups, when compared to control, however, increased biocompatibility was observed, at 10 days, in all groups.

Conclusion: The diamond-like carbon coating enhanced the chemical and mechanical properties from substrates, however modified biological interaction course of the titanium alloy (Ti6Al4V) and polyethylene (UHWPE) samples. Parameters for film deposition remain to be improved in order to obtain best biocompatibility.

1. Introduction

The temporomandibular joint (TMJ) is the main joint of the stomatognathic system and its perfect function interferes in the system's harmony. The temporomandibular joint dysfunction (TMD) comprehends a group of pathologies that intervene into TMJ equilibrium such as: development anomalies, neoplasia and ankylosis in this joint.[1–3] The TMJ ankylosis is a condition in which the mandible is fused to the temporal bone glenoid fossa through fibrous tissue, causing structural deformities and functional alterations such as the limitation of the mouth opening, oral hygiene difficulties, malnutrition, dental-skeletal deformities, problems in the development of routine activities and pain4,5. The most common etiological factor is associated to trauma.[6–9]

Early childhood trauma, such as parturition traumas due to the use of obstetric forceps or accidents (falls) result in condyles fractures; when not identified, cause intra-articular hematomas, followed by fibrosis and consequent bone fusion between the condyles and the cranial fossa.[10,11]

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The reconstruction of the ankylosed TMJ using a total prosthesis may be a good alternative for the treatment of this infirmity.[12,13] Currently, many artificial TMJ prostheses are available in the market, which may be stock or customized items. Customized prostheses are manufactured using a prototype obtained with the aid of a Computed Tomography of the patient.[14] These prostheses are composed of metal alloys such as Ti6Al4V and CoCrMo, which are biocompatible, corrosion-resistant, ductile and rigid according Guarda-Nardini, Manfredini and Ferronato (2008).[13] The glenoid fossa is made of commercially-pure titanium and a second component of polyethylene over the articular surface, known as ultra-high-molecular-weight polyethylene (UHMWPE).[15]

Increasingly more young patients have been submitted to this type of therapy, so prostheses must to present more functional longevity according to Mercuri and Anspach (2003).[12] This is one of the major present concerns in this surgery practice. Hip prostheses have a 15-year median survival, which is not sufficient for a population who may demand 30–40 years of prosthetic parts functionality.[16] This example may be extrapolated for other human body joints.[12] Furthermore, the hypersensitivity to metal alloy components (Ni, Al, V), has been reported in literature. It may cause local inflammation due to foreign body reaction and great bone destruction, leading to prosthetic set loss.[17–19] We may conclude that new alternatives therapies are welcome to improve patient outcome.

The diamond-like carbon (DLC) film is a hard lining with diamond-like characteristics, biocompatible to human cells,[20,21] presenting high wear resistance, low coefficient of friction, high chemical inertia, semiconductor gaps and bactericidal properties[22] and this last property may be increased with the silver nanoparticles becoming also fungicide.[23] The DLC has been used in implants as a surface coating for prosthetic screws[24] and prosthetic pillars[25] aiming to torque maintenance. The DLC doped with silver nanoparticles has been reported effective in the reduction of bacterial adhesion in acrylic resin26 and bacterial infiltration in the implant/abutment interface.[27]

Several studies seek, with variation in the deposition, composition, and application parameters, to obtain more stable conditions of this coating over the desired surface.[28–35] However, the deposition parameters for this coating are not established in Dentistry literature. We suppose that a good-quality film with high adherence, presenting adequate tribological properties and high resistance to corrosion may be used as coating material of TMJ prosthesis. This study aimed to evaluate the effect of DLC and silver-doped DLC (DLC-Ag) films over titanium alloy and polyethylene UHMWPE; their cellular cytotoxicity, chemical and mechanical properties.

2. Material and methods

2.1. Preparation of specimens

Thirty discs of ultra-high-molecular-weight polyethylene (UHMWPE/ASTM F648) and forty discs of titanium alloy (Ti-6Al-4V) were produced with dimensions of 2.5 cm in diameter and 2 mm thickness. All specimens were polished with water sandpaper of 600–1200 granulation using an automatic polisher (Automet 250, Buehler Ltd., IL, USA) and cleaned with ultrasound with isopropyl alcohol at 10% during 4min.

2.2. Plasma deposition over metallic alloy samples

Half of the titanium specimens received DLC coating and the other half, DLC-Ag. The deposition was performed in a plasma reactor (ITA, São José dos Campos, Brazil). The deposition technique was the Plasma-enhanced chemical vapor deposition (PECVD). Previous to deposition, an argon plasma application was conducted on specimens during 30 min, at 3×10^{-1} Torr, tension of −900 V; aiming to reduce impurity of specimens. An interface containing silicon (SiOxCy) was deposited over specimens at 1×10^{-2} Torr for 30 min, with the application of liquid hexamethyldisiloxane ($C_6H_{18}OSi_2$) (Sigma Chemistry-Aldrich, Munich, Germany) and oxygen. Then, the methane plasma (C_6H_{14}) was applied followed by argon (Ar). Finally the DLC deposition occurred at 1.1×10^{-1} Torr during 90s. The deposited film thicknesses were standardized using a silicon blade placed inside the reactor's chamber, along with the samples receiving deposition. Part of this silicon blade is coated with another silicon blade, forming a platform between the covered (no film) and uncovered (with film) blade. This platform was measured using optical profilometry (INPE/São José dos Campos - Brazil) and the film thickness was of 1.58 µm.

The DLC doped with silver (DLC-Ag) films were deposited under the same DLC film plasma deposition conditions. The only different parameter was the gaseous precursor which, in this case, was the hexane (C_6H_{14}) containing silver nanoparticles in the order of 30–50 nm suspension at 0.5 g/L. The average DLC-Ag film thickness obtained was 2.91 µm, measured using an optical profilometer. In both deposition processes, the gas flow was controlled by a flow meter and the deposition power was kept around 200 w. The cathodic tension and the electric current were kept constant during the entire deposition process and samples temperature was kept at 500 °C by the plasma itself. After deposition, the source of electric current was shut down, and the gas flow was interrupted, and the vacuum system was kept on (approximately 1 h) until samples temperature dropped and reached 40 °C so that they could be removed from the reactor.

2.3. Plasma deposition over polyethylene samples

The DLC films over polyethylene were deposited using a process chamber made of stainless steel in a cylindrical shape with internal diameter of 550 mm and 300 mm in length. It had two water-refrigerated magnetron cathodes made by Edwards Vacuum (Albany, NY, USA). A carbon target was used (99.99% purity). The vacuum system was composed of one mechanical pump (Edwards, Model E2M − 18, Albany, Ny, United States) used for pre-vacuum, and a turbo-molecular pump (Edwards, Model EXT351), serially-connected. The process chamber work pressure was controlled with the aid of a gate-type valve and Pirani-type pressure measurer (Edwards, Albany, NY, USA) for primary vacuum (up to 10^{-3} Torr), and by Cold-cathode gauge type (APGX – Edwards, Albany, Ny, United States), for secondary vacuum (10^{-3} a 10^{-6} Torr).

The plasma was generated with Advanced Energy DC power (MDX-1K) the power of 300 W. The work pressure was kept at 3 mTorr. The argon flow was of 10 sccm and the deposition time was 30 min. Under the described conditions, the deposition resulted in 10 nm thick DLC films.

2.4. Samples characterization

The surface topography of specimens was visualized using Scanning Electron Microscopy (SEM Inspect S50, FEI Company, Orlando, FL, USA) (n = 2). The chemical elements identification was carried out using the Electron Dispersive Spectroscopy method (EDS). The Bruker's EDS method, with the Espiret 1.9 software, used an EDS detector attached to SEM using the samples above.

The critical load (scratch test) for DLC and DLC-Ag film failure analysis was evaluated using a UMT-CERT tribometer (Bruker, Campbell, USA). During the scratch test, a Rockwell C diamond tip was used. The applied load ranged from 0.20 to 20 N, at 0.1 mm/s, applied along the entire specimen (n = 1).

Twenty polyethylene specimens (UHMWPE) were submitted to mechanical fatigue. They were previously embedded onto a polyurethane base (Araldite/Bras cola/Brazil), with 2.5 cm in diameter and 2.0 mm height. Ten specimens received DLC film and the other ten were not coated. Finally, 300,000 cycles of fatigue were carried out with a spherical stainless steel tip (r = 3.5 mm), maximum load of 200 N, 3 Hz

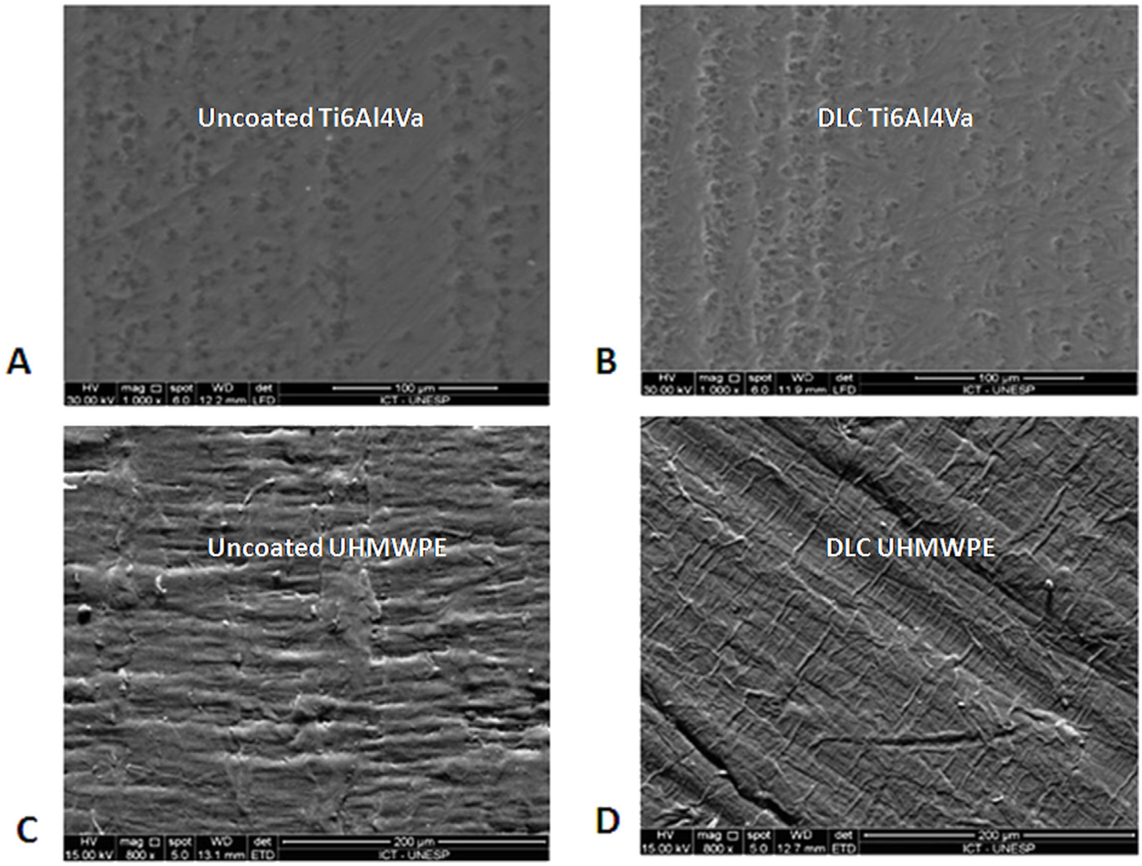

Fig. 1. SEM images of substrates: A- Uncoated titanium (1000× magnification). B- DLC-coated titanium (1000× magnification). C- Uncoated polyethylene (800× magnification). D- DLC-coated polyethylene (900× magnification).

frequency on embedded specimens, positioned in a chewing cycle simulator (Biocycle, Biopdi, São Paulo, Brazil) under liquid environment. Each cycle consisted on the tip being in constant contact with the sample, making constant sliding movements of 5 mm (2.5 mm to the right and 2.5 mm to the left side of the specimen). After the fatigue cycles, the specimens were analyzes using a stereomicroscope (Discovery.V12, Carl Zeiss, LLC, USA).

The surface roughness was measured on the coated (two different films) and uncoated titanium alloy specimens and coated UHMWPE before and after mechanical fatigue test, according to the ISO 4287 regulation, using a profilometer (Mitutoyo SJ-400, Suzano, Brazil) under the roughness average pattern (Ra - Roughness Average) (n = 3).

2.5. Biological assay

All animal procedures were performed in accordance with guidelines of the Research Ethics Committee of the School of Dentistry (UNESP) in São José dos Campos (027/2008-PA/CEP). Cells from newborn (2–4 days) Wistar rat (Rattus norvegicus) calvaria were harvested using the enzymatic digestion previously described.[36] The animals were born and purchased from the University of Taubaté (UNITAU, SP, Brazil). Rats weighing 4 g, approximately, were allocated with mother and brood in solid-floor plastic cages coated with wood shavings. The environment was carefully monitored to maintain the temperature in approximately 20 °C and the humidity was maintained at 55%, with daily cycles alternating 12 h corresponding to the light period and night time. Cells were isolated from six newborn rats calvarial bone through enzimatic digestion immediately after euthanasia. The number of rats is based in previous experiments in which the approximate number of cells obtained was determined. Briefly, rats was euthanized by guillotine and calvaria were removed and incubated in α-MEM (Gibco-Life Technologies, NY, USA), supplemented with 10% fetal bovine serum (Gibco), 50 mg/mL gentamicin (Gibco) and trypsin/collagenase. All medium was collect, separated, cell counting was performed thought Newbawer chamber and 2×10^4 viable cells were plated in each well of the 24 well plate containing samples (Nunc, Denmark) for evaluation of cell viability. The cells were cultivated over the samples during 3 and 10 days (n = 5). Cytotoxicity test was performed through MTT (3–4,5-dimethylthiazol-2yl)-2,5-diphenyltetrazolium bromide (Sigma-Aldrich, St. Louis, USA) assay.

Briefly, MTT aliquots of 5 mg/mL in phosphate buffered saline solution (PBS) were prepared, then mixed to osteogenic culture media at 10%. Following, the incubation of cultures was performed during 1 h at 37 °C, under humid atmosphere containing 5% CO_2 and 95% atmospheric air. After this period, the cultures were washed in PBS. Then, 1 mL of dimethylsulfoxide (DMSO-Sigma) solution was added in each well and agitation was performed during 20 min, for complete solubilization of the precipitate salt. The MTT salt is reduced by the mitochondrial proteases, active only in viable cells, producing a soluble product in the culture environment. The spectrophotometer EL 808 (BioTek Instruments, Winooski, USA) was used and colorimetric analysis was performed at 570 nm. Absorbance is directly proportional to the number of living cells in the culture. The surface unit of a 24-well culture plate with cellular growth and with no samples was adopted as control in test.

2.6. Data analysis

The surface roughness results were submitted to ANOVA one-way and the cell cytotoxicity was evaluated with ANOVA-two-way, followed by the post hoc Tukey test. Significance adopted level was considered at $p < 0.05$ in all tests.

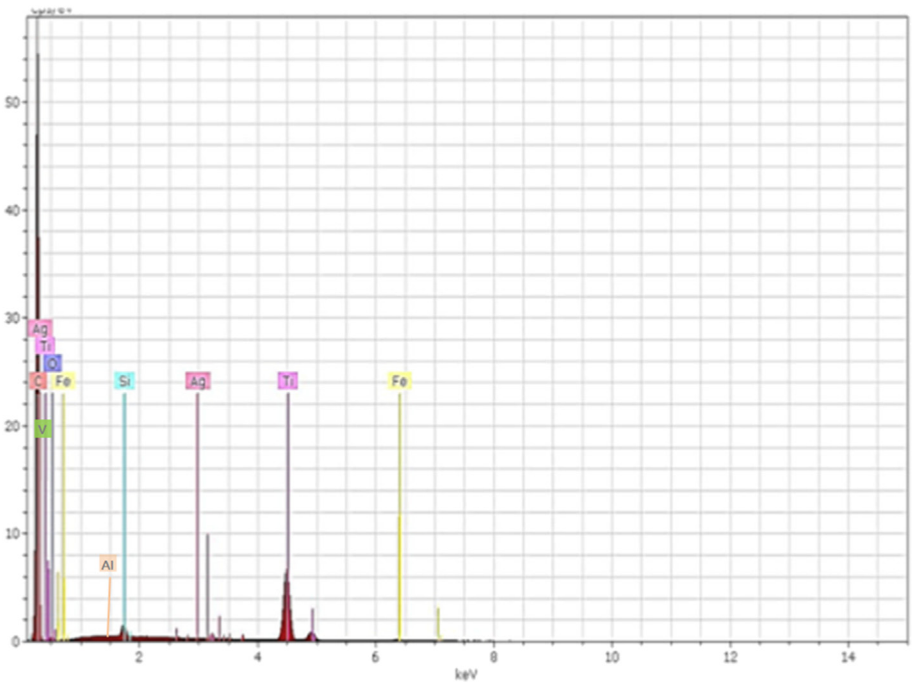

Fig. 2. EDS chemical analysis, DLC-Ag film coating titanium.

3. Results

3.1. Samples characterization

Morphological differences were observed on both substrates after DLC deposition through SEM analysis (Fig. 1). Ti6Al4V coated surface appears to be less rough than uncoated surface. The superficial cavities were partially covered by film and were discretely reduced. UHMWPE surface topography changed after DLC film deposition, that provides a surface full of demarcated parallel lines (Fig. 1).

The PECVD successfully provide a film presenting carbon and silver as demonstrated by means of EDS of a coated metallic sample that also presented many elements after chemical analysis (Fig. 2). Comparing uncoated and coated UHMWPE, the plasma deposition promoted the addition of new elements over film-modified substrate, not only carbon, as seen in Fig. 3.

Evaluating the importance of silver on film composition through scratch test, it was observed that the initial critical load failure occurred at 6.1 N in the group coated with DLC and 9.7 N in the group coated with a DLC-Ag film.

Comparing coated UHMWPE and uncoated UHMWPE surfaces, damage area after the mechanical fatigue test was lower in cover samples (Fig. 4).

The surface roughness did not present statistical difference among the titanium specimens (Ti6Al4V) with or without coating ($p = 0.426$); and the polyethylene became rougher after mechanical fatigue (Table 1).

3.2. Biological assay

The cell viability increased with time, presenting significant difference between the two experimental periods ($p = 0.001$). Coated surfaces presented significant less viability when compared to uncoated Ti6Al4V surface. The group DLC-Ag film did not differ from DLC, so silver did not altered cell viability. Smaller viability values were demonstrated in UHMWPE at 3 and 10 days (Table 2).

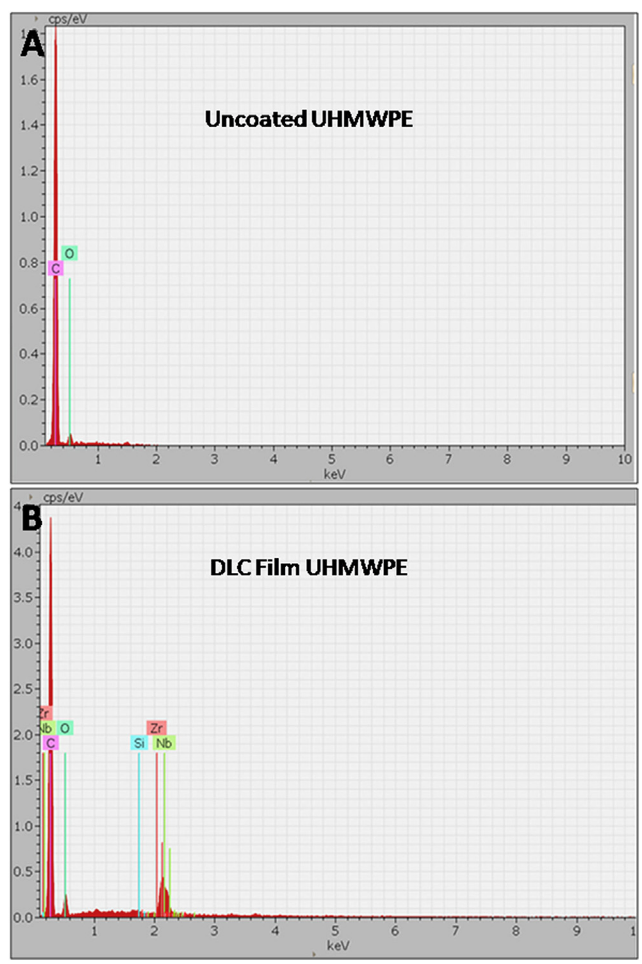

Fig. 3. EDS UHMWPE samples chemical analysis. A- Uncoated polyethylene, the dominant presence of carbon, and oxygen is observed. B- DLC-coated polyethylene, the dominant presence of carbon, silicon, zirconia, niobium, and oxygen is observed.

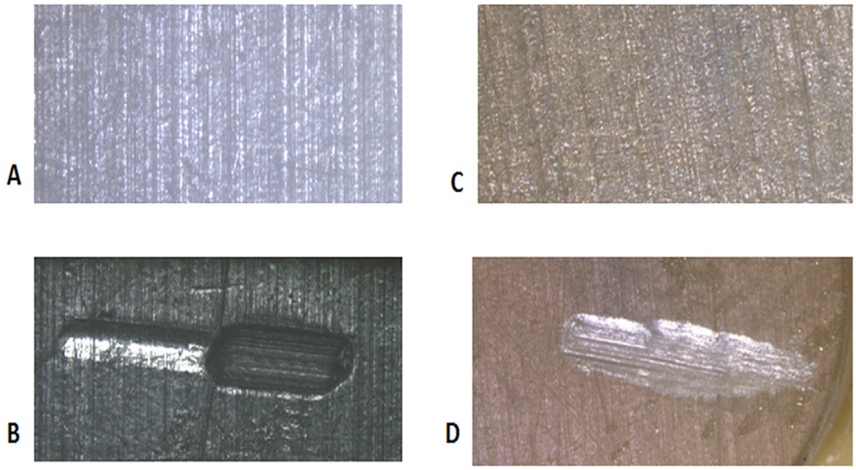

Fig. 4. Stereo microscope images after mechanical fatigue. A- Initial uncoated specimen. B- Uncoated specimen after mechanical fatigue. C- Initial DLC-coated specimen. D- DLC-coated specimen after mechanical fatigue. (10× magnification).

Table 1
Substrates, Groups, Mean (μm) and standard deviation of the surface roughness (Ra).

Substrate	Groups	Mean ± Standard deviation
Ti-6Al-4V	Ti-6Al-4V	245.0 ± 19.8
	Ti-6Al-4V + DLC	241.4 ± 33.5
	Ti-6Al-4V + DLC-Ag	229.3 ± 41.8
UHWPE + DLC	Cycled polymer	3.243 ± 1.54[a]
	Non-cycled polymer	1.237 ± 0,42[b]

*Values that do not share same superscript letter are significant different from each other.

Table 2
Groups (substrates and days), Colorimetric averages (nm) obtained with the spectrophotometer from MTT assay and Tukey Test results.

Groups		Mean (nm)	Grouping			
Substrate	Time (days)					
Ti-6Al-4V	3	0.0802	a			
	10	0.3564		A		
Ti-6Al-4V + DLC	3	0.0898	a			
	10	0.3442			B	
Ti-6Al-4V + DLC-Ag	3	0.0866	a			
	10	0.3418			B	
UHMWPE	3	0.0818	a			
	10	0.3004				C

4. Discussion

Herein we describe some chemical, mechanical and biological behavior of DLC and DLC-Ag films, coating two main substrates used in total TMJ replacement therapy. DLC coatings present outstanding properties, such as high hardness and low friction.[37] Both are important characteristics in biomaterials.

Since design of prosthesis involves a Ti alloy core covered with a polyethylene surface, the deposition of DLC film intend different results when covering metal and composite. In polyethylene, the film must act reducing wear, since the surface is challenged constantly by joint movement. In metal, DLC film must be biocompatible, act as an isolator to corrosion elements that are related to complication in many patients, as well as decrease risk of infection, through doped Ag.

Starting to characterize the obtained film, roughness was accessed qualitatively by means of SEM and quantitatively using profilometry. Despite the data from roughness analysis did not display significant differences, DLC film tends to reduce roughness, as seen in SEM images. In literature, DLC coating leads to a broadening of the sharp edges of the faceted crystals, increasing the roughness. This behavior is reported in small DLC film thickness. However, the roughness decreases in thicker coatings according Salvadori et al.[38] Our film presented 1580 nm thickness and Salvadori et al. thicker film exhibited 200 nm thickness. Corroborating with our results, they demonstrated that roughness decreases in thicker coatings.

Many techniques are described in literature successfully depositing DLC film.[37] Herein, we choose PECVD and we also reached success covering Ti6Al4V with a DLC-Ag film, using gas hexane containing silver nanoparticles (30–50 nm) in suspension. EDS spectra confirm Ag peak in 2.985 keV.[39]

Many functions are attributed to Ag when it is incorporated into DLC films.[28] We demonstrated a higher force, measured in N, in critical load scratch test in DLC-Ag films. Our results corroborate with previous study, in which an increase of the adhesion tested through scratch test was observed in DLC-Ag films presenting many different atomic concentrations of silver.[40] Similarly, the progressive-load scratch test performed in Muguruma et al. study's revealed that DLC-coated samples displayed significantly higher hardness due to the diamond-rich structure and significant lower frictional forces as well as lower wear rate friction than the non-coated disk specimens.[41]

Cycled UHWPE + DLC presented higher surface roughness and less damage area after mechanical fatigue test, as seen in Fig. 4. So, the present result demonstrated that DLC protected polyethylene from wear induced through friction of spherical stainless steel tip. This behavior is explained by the reduction of the friction coefficient, noticeably with the presence of the DLC coating, since a transfer layer with graphitic characteristics is formed. This layer works as a solid lubricant between the coating and the counterpart reducing the friction coefficient.[42]

Fretting is a contact degradation process that occurs due to reciprocal relative displacement between surfaces, exactly what may happen with polyethylene in TJM prosthesis. As fretting wear can lead to loosening of joints, resulting in increased vibration and consequent acceleration of damage in contact components, the durability of a coating should be assured.[43] For this motive, we choose to perform fatigue test in uncoated and coated UHWPE. Wear behavior of DLC coatings deposited on steels or metallic substrates has been studied in different sliding situations but not much in fretting.[43] To the best of our knowledge, it is the first description of this kind of test over DLC film deposited on polyethylene. Our result highlights a very interesting property in case of use of coated UHWPE in TMJ replacement therapy. After fatigue cycles the roughness was increased in the DLC coated UHWPE. Possible due to the attrition in the damaged are, that leads to loosening of nano and microfragments from the DLC film.

The biological characteristics of DLC films, such as biocompatibility, are important due to their application as coating of new instruments and implants in the Biomedical Engineering,[21,44] so we decide to test it using calvarial bone osteoblasts. Using rat bone marrow cells, Schroeder et al.[45] perform the analysis of a DLC film over titanium, deposited using a combined radio frequency plasma and magnetron sputtering deposition process in an all stainless-steel high vacuum system. The authors demonstrated that the relative cell number was significantly increased in titanium samples covered by DLC (amorphous hydrogenated carbon) differently from the results presented herein. Our cells were differentiated osteoblasts, a primary culture experiment. Schroeder et al.[45] used MSCs chemically induced to differentiate in osteoblasts and then plated the subculture cells over the samples. As less manipulation in the cells phenotype was applied herein, we strongly agree with the hypothesis that response is closer than Schroeder et al.[45] study, to *in vivo* situation.

Previous studies demonstrated that the incorporation of nanoparticles such as V, Ti, H, F, Ag in the microstructure has the purpose of promoting bactericidal capacity to the coating.[30,31] But it is important that these nanoparticles do not interfere in the cellular response against the coating. Our cytotoxicity results showed the DLC-Ag film decrease the biocompatibility. However the cellular viability values increased with time, suggesting an acceptable interaction between the cells and the substrates. In fact, at 10 days, normalizing cell viability percentage considering Ti6Al4V as 100% demonstrated that 84% of cells were viable in contact with UHWPE and 96% of cells were viable when contact with two types of DLC film covering titanium alloy.

Vinculin adhesion was significantly reduced in fibroblasts cultivated over UHMWPE when compared with other materials. Those cells also synthesized significantly less DNA when in contact with polyethylene.[46] We believe that reduced ability to attachment and to replicate DNA justify the reduced viability reported herein.

The slight lower cell viability demonstrated in the experimental groups also may be explained by the hydrophobic behavior of DLC film. The relative amounts of the sp2 and sp3 bonded carbon present in the network dictate the physical properties. The sp3 bonding determines the mechanical properties and hydrophobicity while electrical properties are dependent on the sp2.[47] Surface energy and protein adsorption analysis show that a lower surface energy in DLC-Ag film, a hydrophobic film, is related to the increase of sp2/sp3 content in the amorphous carbon matrix in the film.[48]

5. Conclusion

The diamond-like carbon coating enhanced the chemical and mechanical properties from substrates, however modified biological interaction course of the titanium alloy (Ti6Al4V) and polyethylene (UHWPE) samples. Parameters for film deposition remain to be improved in order to obtain best biocompatibility.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jobcr.2019.04.003.

Disclosure statement

No potential conflict of interest was reported by the authors.

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