IDUNAS | NATURAL & APPLIED SCIENCES JOURNAL | 2021 Volume:3 Special Issue, No:12

The Effect of Plasma Treatment Parameters on Antibacterial and Antifungal Activity of Plasma Polymerized Diethyl Phosphite Thin Films

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Abstract

In this study, plasma polymerization technique for the production of antimicrobial surfaces was studied to inhibit the formation of biofilm of *Staphylococcus aureus* (*S. aureus*) and *Candida albicans* (*C. albicans*) for foreign materials in biomedical application. Low pressure RF-plasma system was used to coat Ti surfaces. Ti surfaces were exposed to diethyl phosphite (DEP) plasma generated with different discharge power varying from 25-90 W for 1-10 min of exposure times at a constant pressure of 0.15 mbar. Surface hydrophobicity and surface energies of unmodified and DEP modified Ti surfaces were used to enlighten surface wettability by the sessile drop method using contact angle analyser. All DEP coatings produced with different plasma conditions increased both the surface hydrophilicity from 100° to 30-48° and surface energies of Ti surfaces from 33mJ/m2 to 61-71mj/m2. Aging of the DEP coatings on Ti surfaces was analyzed in terms of change in surface energies by time within 30 days. Even though the stability of phosphorus containing thin films has been problematic due to the post-plasma oxidation, thin films produced with 25 W-5 min, 50 W-5 min, 75 W-10 min and 90 W-1 min were found more stable compared to the others. The antibacterial and antifungal activity of unmodified and DEP modified Ti surfaces was studied against *S. aureus* and *C. albicans*, respectively. While the adhesion and growth of both bacteria and fungi was observed on unmodified Ti surfaces, antimicrobial activity was observed after surface

modification with DEP plasma with different plasma conditions. The highest efficiency for anti-fungal coating was obtained with 50 W-5 min, 75 W-10 min and 90 W-10 min and the highest antibacterial activity was achieved with 25 W- 1min, 50W-5 min, 50 W-10 min and 75 W-10 min. Additionally, surface modification with DEP plasma increased L929 fibroblast cell viability of Ti surfaces. The chosen precursor, DEP, solves problems in reducing the risk of infection associated with Ti implants with plasma polymerization technique.

Keywords: Plasma polymerization; Amphoteric polymer; Titanium; Antimicrobial coating; Fungicidal activity; Antibacterial activity.

1. INTRODUCTION

Increasing lifetimes significantly increase the demand for biomedical devices. Advances in nanotechnology and surface modification in recent years allow the design and development of biomedical devices with improved function or longer life span [1]. Although many products are commercially available, most of them have suffered from polymicrobial infections after implantation due to the adherence of bacteria and fungi and the multiplication of these pathogens on the surfaces. This could cause serious problems in implant surgery as well as short-term implanted biomedical devices. They often require replacing the infected device or implant, and could result in significant costs for the healthcare system [2-4].

Surface coating is one of the most effective ways to develop anti-infective biomaterial/medical devices. The fabrication of various controlled surface structuring with the use of antimicrobial peptides, enzymes, nanoparticles, quaternary ammonium compounds, anti-adhesive polymers, super hydrophobic coatings and chitosan based strategies have been used to obtain antimicrobial surfaces [5]. In addition to the aforementioned methods, various antibiotics can be immobilized to prevent the formation of biofilms on the surface, but the bioactivity and biocompatibility of most of them with host tissues is problematic. Plasma technology is a highly effective method for surface structuring with their shorter reaction times, single-step process, environmental safety and only changing the surface properties of the material without affecting the bulk properties. With proper discharge power and the chemical structure of precursors used for modification, the production of antimicrobial surfaces could be achieved [6]. Phosphorus based nanomaterials have been used as drug nanocarriers, tumor theranostics, biosensors, and bone formation in biomedical application [7]. Diethyl phosphite as a precursor during plasma polymerization has been studied by our group and others with the aim of modifying the physical, chemical, electrical, or biological properties of the silicon, titanium and polystyrene for mainly biomedical applications [8-10]. Depending upon the plasma parameters applied during plasma processing, the surface energy and/or chemical structure could be changed. Therefore, it is important to find optimum conditions which inhibit both the growth candida and bacteria while keeping their biocompatibility.

This full text explains the optimization of plasma parameters to inhibit both candida, *Candida albicans (C. albicans)*, and bacteria, *Staphylococcus aureus (S. aureus)* biofilm formation using plasma polymerized thin-film coating on Ti surfaces and to evaluate the influence of different thin films produced with varying plasma power and exposure time onto the attachment and growth of both microorganisms. Ti surfaces were chosen as a substrate due to their frequent usage in orthopedic and dental implant applications [11-12]. Furthermore, the biological response of the L929 fibroblast cells incubated with plasma modified Ti surfaces with different plasma conditions was monitored and compared with unmodified Ti.

2. METHOD

A. Coating Process

During research, commercially available Ti coated glass slide surfaces (Ti surfaces) were used as a substrate (Oncel Advanced Materials & Surface technologies, Istanbul, Turkey) and surface coating process of Ti surfaces was achieved with low pressure plasma produced with radio frequency of 13.56 MHz in a stainless-steel plasma chamber of 150 mm radius/320 mm length (Pico, Diener Electronic GmbH, Germany). A RF (13.56 MHz) generator was chosen to keep glow discharge in the plasma chamber. Before the process, Ti surfaces were placed in the plasma chamber. Before the plasma generation, air in the chamber was evacuated until 0.15 mbar with the vacuum pump (Trivac 2.5E, Leybold Vacuum GmbH, Germany). Then, precursor, DEP was degassed with the freeze-pump-thaw method. After the degassing process, the atmosphere of the plasma chamber was changed from air to DEP for 15 min. Ti surfaces were exposed to DEP plasma generated with different discharge power varying from 25-90 W for 1-10 min of exposure times at a constant pressure of 0.15 mbar. Discharge power losses during the plasma process were kept minimum by the help of a matching network. After the surface modification process, plasma modified Ti surfaces were left in the vacuum medium for 15 min to passivate the active radicals.

B. Surface Characterizations

The contact angle (CA) of water and diiodomethane with the unmodified and DEP modified Ti surfaces was measured by monitoring the drop using a contact angle analyser (KSV Instruments Ltd., Finland). Five measurements were recorded to calculate the average contact angle and standard deviation (±SD). The solid surface free energies (SFE) of the unmodified and DEP modified Ti surfaces were calculated using the Young-Dupré's equation. Hydrophobic recovery of the DEP thin films was analyzed in terms of change in surface energies by time within 30 days to monitor air stability (aging).

C. Microbiological Testing

A modified version of the Japanese Industrial Standard Z 2801 was used to measure both the antibacterial activity of plasma polymerized films against the prokaryotic bacterium *Staphylococcus aureus* (*S. aureus*) (29213 ATCC) and the antifungal activity against the eukaryotic fungus *Candida albicans* (*C. albicans*) (90028 ATCC) [13]. The details about both antifungal and antibacterial assay were given in previous study [9].

D. Cytotoxicity of Plasma Modified Ti Surfaces

Cytotoxicity of Ti surfaces after modification with DEP plasma with different plasma powers for 10 min exposure times at a constant pressure of 0.15 mbar against mouse L929 fibroblasts (ATCC, CCL-1, Rockwille, USA) were analyzed with a modified version of 3-(4,5-dimethylthiazol- 2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay [14].

3. RESULTS

Since the surface wettability was expected to increase after plasma coating due to polar groups found in the chemical structure of DEP, the measurement of water and diiodomethane contact angles is a great way to monitor the surface modification of highly hydrophobic Ti. The water and diiodomethane contact angle results of unmodified Ti and *pp*(DEP)-Ti surfaces produced by different plasma parameters were given in Table 1. Water contact angles of unmodified and plasma modified Ti surfaces with plasma power 25W to 90 W for 10 min exposure time were found 100±7°, 33±1°, 33±3°, 30±2° and 40±13°, respectively. A sharp decrement right after DEP plasma coating was seen compared to unmodified Ti surfaces and no significant relation between the increment of plasma power applied during plasma polymerization and water contact angle was observed. The influence of exposure time on water contact angle was also investigated to observe which exposure time provides sufficient polymerization time. Although all of the coatings gave more hydrophilic results in contact angle measurements compared to unmodified one, more hydrophilic surfaces were obtained with the increment of exposure time from 1 or 5 min to 10 min as expected. Moreover, the increment of the exposure time from 1 to 5 min at all plasma power except 90 W did not change the contact angle.

Surface energies (SE) of unmodified Ti and *pp*(DEP)-Ti surfaces produced by different plasma parameters were also given in Table 1. The SE value of unmodified Ti surfaces were calculated as 33.4 mJm^2. After DEP plasma modification with plasma power 25W to 90 W for 10 min exposure time, two times higher surface energies were seen around 70mJ/m^2 compared to unmodified one except 90 W-10 min. The most striking increase in surface energies can be observed in surfaces which were modified with 25 W-10 min, 75 W-10 min and 90 W-5 min plasma parameters.

Table 1. Comparison of contact angles results of unmodified Ti and *pp*(DEP)-Ti surfaces with different discharge power varying from 25-90 W for 1-10 min of exposure times at a constant pressure of 0.15 mbar.

Plasma Conditions		Contact Angle (°)		
Plasma Power (W)	Exposure Time (min)	Water	Diiodomethane	Surface Energy (mJ/m^2)
base	**-**	***100±7***	***52±2***	***33.4***
25	1	48±1	33±8	61.0
	5	46±5	38±9	60.9
	10	33±1	29±3	69.9
50	1	40±8	39±4	67.5
	5	40±4	48±3	61.8
	10	33±3	32±5	69.0
75	1	40±7	34±2	68.5
	5	40±2	43±1	62.9
	10	30±2	31±5	70.7
90	1	41±3	35±3	64.4
	5	30±2	31±4	70.8
	10	40±13	33±9	65.6

One of the main problems that affect the adaptation of plasma polymerized thin film in surface modification of implants have been the aging in ambient air. After surface modification with plasma, surfaces face with the hydrophobic recovery over time and this leads to simultaneous decrement in the surface energies [15-19]. It has been reported that re-orientation of polar groups located on the surface and/or post-oxidation of functional groups on the surface could be causing the decrease in hydrophilicity over time. Therefore, the surface energies (SE) between 1 and 30 days were also studied to understand the shelf-life of DEP plasma modified Ti surfaces with different plasma parameters and given in Figure 1. The clear change in surface energies was seen in the first 5 day due to the post-plasma oxidation except those produced with 25 W-5 min, 50 W-5 min, 75 W-10 min and 90 W-1 min [20]. The thin films produced with these parameters were found more stable compared to the others.

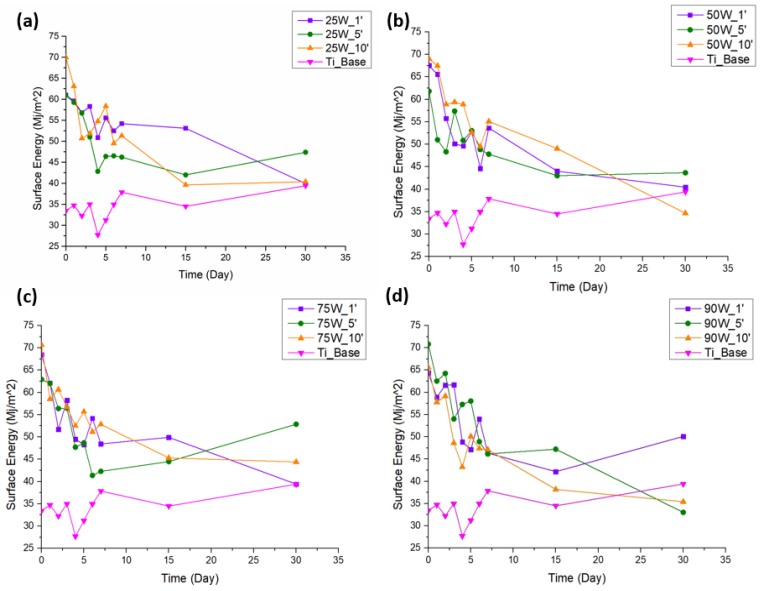

Figure 1. Aging of unmodified Ti and *pp*(DEP)-Ti surfaces with different discharge power varying from 25-90 W for 1-10 min of exposure times at a constant pressure of 0.15 mbar.

The effect of different plasma parameters onto the growth of *S. aureus* on unmodified and *pp*(DEP)-Ti surfaces were studied and given in Figure 2. At the end of the 24h incubation time, surface modification with DEP plasma decreased the adhesion and growth on Ti surfaces compared to control except surfaces modified by 75 W-5 min and 25 W-10 min. The highest antibacterial activity was seen on Ti surfaces modified with 25 W-1 min, 90 W-1 min, 50 W-5 min plasma conditions. Because of the fact that incubation time could affect the antibacterial activity, bacterial biofilm formation was also evaluated for 24, 48 and 72 hrs. When DEP plasma polymer films produced with 25, 50, and 75 W of plasma power and 1, 5, and 10 min of exposure time were analyzed, it was seen that the inhibition was increased with the increment in incubation time. However, the plasma power of 90 W behaved differently with the increment of exposure time.

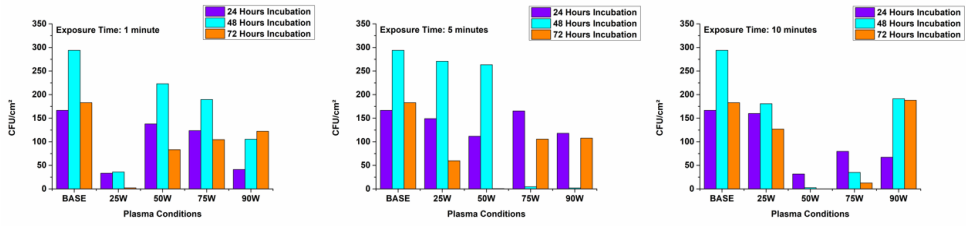

Figure 2. Growth inhibition of *pp*(DEP)-Ti surfaces with different discharge power varying from 25-90 W for 1-10 min of exposure times at a constant pressure of 0.15 mbar against *S. aureus* after culturing for 24 h, 48 h and 72 h. The unmodified Ti surface was used as a control material. The results are averaged from three replicates [9].

Ti surfaces that modified with 1 min exposure times were found insufficient to gain anti-fungal properties in all plasma powers (Figure 3). Ti surfaces modified by 25 W-1 min plasma parameters showed similar trend in terms of the formation of candida colonies. When plasma power increased to 50, 75 and 90 W, higher candida biofilm formation was observed compared to unmodified Ti at their 72-hour incubation. Even though DEP plasma coating was successfully achieved as seen in surface energy results in all parameters, some conditions stimulated the formation of candida colonies such as 50 W- 1 min, 50 W-10 min, 75 W-1 and 75W-5 min. Also, some of them totally behaved like antifungal surfaces like 50W-5 min, 75W-10 min and 90W-10 min. Such a sharp diversity caused by the nature of plasma polymerization technique. According to overall results, while the highest efficiency for anti-fungal coating was obtained with 50 W-5 min, 75 W-10 min and 90 W-10 min, the antibacterial surface was produced by 25 W- 1min, 50 W-5 min, 50 W-10 min and 75 W-10 min.

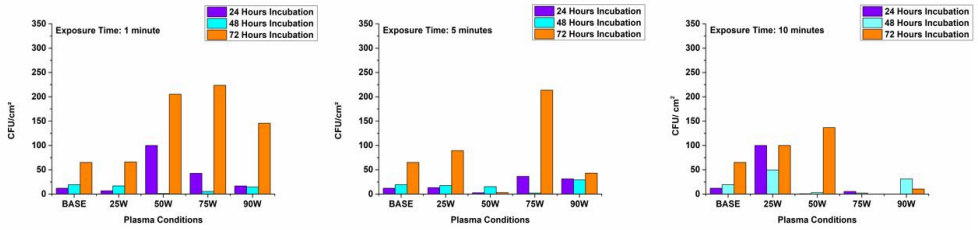

Figure 3. Growth inhibition of *pp*(DEP)-Ti surfaces with different discharge power varying from 25-90 W for 1-10 min of exposure times at a constant pressure of 0.15 mbar against *C. albicans* after culturing for 24 h, 48 h and 72 h. The unmodified Ti surface was used as a control material. The results are averaged from three replicates [9].

The proliferation of L929 fibroblast cells on the Ti surfaces modified with different plasma parameters after 24, 48 and 72 hrs of culture were shown in Figure 4. The cell viability results were shown that unmodified Ti surfaces had the least biocompatibility in all days compared to the plasma modified Ti surfaces. The proliferation rate of cells on the DEP plasma modified Ti surfaces with different plasma power compared with each other was not significantly different at the end of 24 and 48 hrs. As seen in the figure, only surfaces modified with 90 W of plasma power lost their biocompatibility at the end of 72 hrs. The DEP plasma modified Ti surfaces could provide a proper surface for the viability of L929 fibroblast cells.

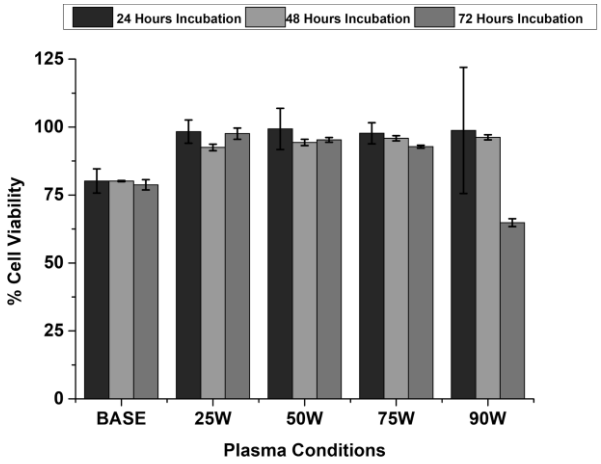

Figure 4. The biocompatibility of *pp*(DEP)-Ti surfaces with different discharge power varying from 25-90 W for 1-10 min of exposure times at a constant pressure of 0.15 mbar after culturing for 24 h, 48 h and 72 h. The unmodified Ti surface was used as a control material. The results are averaged from three replicates [9].

5. CONCLUSION

In this study, Ti surfaces were modified with DEP plasma under different plasma power for different exposure times at a 0.15 mbar pressure and the best conditions to inhibit microbial adhesion on Ti surfaces during plasma polymerization was optimized during this study. DEP plasma modified Ti surfaces were found to be hydrophilic and have higher surface energy compared to unmodified surfaces regardless of plasma power and exposure time. Antibacterial and antifungal tests indicated that dramatic decrement in the number of viable pathogen cells was seen at the Ti surfaces produced with 50 W-5 min and 75 W-10 min compared to unmodified Ti surfaces. The cell viabilities of L929 fibroblast cells on DEP plasma modified Ti with 50 W-5 min and 75 W-10 min was also increased compared to unmodified Ti regardless of plasma power.

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