Potential tribological and antibacterial benefits of pulsed laser-deposited zirconia thin film on Ti6Al4V bio-alloy

S. Kedia1,3 · A. Das2 · B. S. Patro2 · J. P. Nilaya1,3

Received: 19 April 2022 / Accepted: 29 June 2022 / Published online: 13 July 2022
© The Author(s), under exclusive licence to Springer-Verlag GmbH, DE part of Springer Nature 2022

Abstract
The experimental results depicting the advantages of pulsed laser deposition of zirconia thin film on Ti6Al4V bio-alloy at room temperature and at 200 °C substrate temperature in improving its biofunctionality are presented. A comparison of the change in surface roughness, wettability, surface free energy, tribological and antibacterial properties of uncoated and zirconia-coated Ti6Al4V samples clearly revealed the advantage, especially when deposition was carried out at higher substrate temperature. The coated samples exhibited lower coefficient of friction and a notable reduction in the wear rate. The in vitro bacterial retention test showed a clear inhibition in growth of Staphylococcus aureus and Klebsiella pneumonia bacteria on the surface of the coated samples indicating the possibility of prevention of biofilm formation. Superior antibacterial properties of the coated surface were also revealed by fluorescence microscopy where an increased percentage of bacteria was found to be decimated. Thus pulsed laser deposition of zirconia thin film coating on Ti6Al4V improves both, its tribological and antibacterial properties simultaneously. This, in return, is expected to increase the durability of artificial implant in the human body.

Keywords Ti6Al4V · Zirconia · Pulsed Laser Deposition · Tribology · Antibacterial effect · Biomaterial · Wear resistance

1 Introduction
As the demand for artificial implants in orthopedic fixation, dentistry, stents, and tissue engineering is increasing, research activity towards improving their quality and longevity has garnered attention [1, 2]. Based on important properties such as biocompatibility, mechanical stability, and non-toxicity some materials, e.g., titanium alloys, stainless steel, cobalt alloys, aluminum oxide, zirconia, calcium phosphates, and poly(methyl)methacrylate have been recognized as suitable biomaterials to construct artificial body implants [3]. However, the issue of early and late implant failures in patients caused by poor osseointegration, corrosion, wear, or bacterial infections remains a cause of concern [4]. A satisfactory osseointegration of the implant in the body ensures its primary stability and eliminates the risk of fibrous tissue formation at the bone-implant interface, both these factors being responsible, more often, for an implant failure. An implant faces unavoidable wear and corrosion in the harsh body environment. A biomaterial with poor corrosion resistance can degrade and release toxic ions in the body leading to complications. Moreover, an implant with a high wear rate can generate wear debris which can expedite dimensional instabilities [5]. This debris, in the form of free metallic ions, colloidal complexes, metal oxide, or metal particles, under certain circumstances, can induce inflammatory reactions and allergies in the patient. The complex process of wear is largely dependent upon the contact area between the two surfaces and the sliding distance, both of which increase gradually with physical activity and weight gain of the patient [6]. Another major issue that is accountable for implant failure is bacterial infection and biofilm formation on the implant surface [7]. Bacterial adhesion can, in some cases, lead to bacterial colonization and biofilm formation as well. The general sources of bacteria include the environment of the operation room/hospital, surgical equipment, and the bacteria already present on the patient’s...
skin or body. Bacterial infection during or after implantation can lead to early complications or permanent contamination and remedial action in these situations accounts for an enormous medical cost, the surgical removal of the infected implant becoming the sole solution in some cases [8]. All these factors lead to a decrease in the service life of an artificial implant and therefore the need for the development of biomaterials with superior properties.

Among mentioned biomaterials, grade-5 titanium alloy (Ti6Al4V) has been considered as the most promising material for orthopaedic due to its inherent properties of low density, high tensile strength, excellent biocompatibility, good corrosion resistance [9] and the near similar value of elastic modulus of Ti6Al4V (55–110 GPa) and natural bone (10–40 GPa) [10]. However, Ti6Al4V has a high coefficient of friction (CoF) and low wear resistance which restricts its uses in load-bearing applications [5, 11]. Alteration in the surface properties either by topographical modification [12] or by suitable surface coating [13] is an alternative route to achieve desired functionalities in a bio-material. Various materials, such as diamond-like carbon, graphite-like carbon, tantalum and titanium nitride, hydroxyapatite, zirconia, alumina, have been used as coating materials for biomaterials [14–16] by sol–gel [17], pulsed laser deposition (PLD) [18], chemical vapor deposition [19], or RF magnetron sputtering [20] techniques. PLD, a technique capable of providing a precise control over thickness of deposition, maintaining stoichiometry of the target material, freedom of using versatile targets, and option of increasing substrate temperature during deposition, is a sought after method to modify the surface of a biomaterial [13]. A surface coating that can simultaneously improve wear resistance and inhibit bacterial adhesion on Ti6Al4V surface without losing its bulk mechanical properties will ensure a better tissue integration and also stretch the service life of an implant. A promising candidate meeting these requirements is zirconia (ZrO2), a crystalline dioxide of zirconium that is widely accepted biomaterial in dental and orthopedic because of its elevated hardness, good biocompatibility, and antibacterial properties [21–23]. The direct use of ZrO2 as an implant can be prohibitively expensive. However, its characteristics can be imparted to the implant by coating it as a thin film on Ti6Al4V.

This work is aimed to assess the potential tribological and antibacterial benefits of ZrO2 thin film coating by PLD on Ti6Al4V bio-alloy. The PLD was performed at two substrate temperatures, viz., room temperature and 200 °C. A Nd:YAG laser (Model # N311, Ekspla make) capable of delivering a max of ~ 150 mJ/pulse at 355 nm, pulsed duration of 6 ns and repetition rate of 10 Hz was employed as the ablation source. The surface morphology, roughness, adhesion, wettability, and surface free energy of the coated samples were recorded using a Scanning Electron Microscope (SEM), Atomic Force Microscope (AFM), scratch test, static sessile drop technique, and geometric-mean methods, respectively. The tribological analysis was carried out in ambient using standard ball-on-disc method at 3 different loads of 2 N, 5 N and 7 N. A clear reduction in CoF of ~23% at 2 N and wear rate up to 49% at 5 N were observed for sample coated at 200 °C substrate temperature. This is the first report, to the best of our knowledge, where such an improvement in the tribological behaviour of PLD coated ZrO2 on Ti6Al4V has been reported. The antibacterial properties of samples were tested against two bacteria: gram positive Staphylococcus aureus (S. aureus) and gram negative Klebsiella pneumoniae (K. Pneumoniae). Adhesion and growth of both the bacteria were found to be reduced on coated samples as studied by total viable count. A qualitative confirmation was obtained by confocal microscopy where reduction in number of bacterial colonies, biofilm formation and increase in the percentage of decimated bacteria was found on the coated samples. A method of improving both the wear resistance and antimicrobial properties of Ti6Al4V by PLD of ZrO2 is demonstrated here.

2 Materials and methods

2.1 Materials

As-received ZrO2 pellet (M/s. Merck, KGaA) of thickness 2 mm and diameter 10 mm was used as PLD targets. Ti6Al4V substrate of dimensions (20 mm × 20 mm × 1 mm) was surface polished using SiC papers of grit size 400, 800, and 1200. The polished samples were cleaned with acetone, ethanol, and water for 10 min each followed by ultrasonic cleaning and stored in a desiccator till further use.

2.2 PLD of ZrO2 thin film

PLD is a physical process in which a focused pulsed laser beam ablates the desired target material, and a thin film deposition occurs on an appropriately positioned substrate in a high vacuum background (~2 × 10⁻⁵ mbar). Figure 1 illustrates the experimental setup of PLD chamber used for deposition of ZrO2 on Ti6Al4V. The laser beam (λ = 355 nm, pulse duration 6 ns, repetition rate 10 Hz) was focused using a lens (f = 50 cm) onto a ZrO2 pellet placed at a distance of 40 mm from the substrate. A deposition time of 1 h was found to be optimum to obtain a uniform layer when the laser fluence was maintained at ~20 J/cm². Provision was made to change the temperature of the substrate using a heater and film deposition was carried out at two substrate temperatures, viz. room temperature and 200 °C and the corresponding samples are referred to as S-RT and S-200, respectively. The nomenclature of the samples and their experimental condition are listed in Table 1. The results
of the ZrO₂-coated samples were compared with the results of
the pristine sample termed as S–P. Since deposition occurs in a
clean environment, the transfer of target to the substrate is stoi-
chiometric, and the thickness of the film can be controlled by
the number of pulses and duration of the deposition process.

2.3 Surface characterization

2.3.1 Topography, film thickness, and surface roughness

Topography and surface roughness of samples before and after
ZrO₂ coating were analyzed from SEM and AFM images,
respectively. Adhesion of ZrO₂ thin film on Ti6Al4V sur-
faced was analyzed by Rockwell scratch test using a diamond
indenter of radius 100 μm (M/s. Anton Paar). Progressive load
starting from 0.03 N to 5 N with the rate of 4.97 N/min was
applied during the measurement and speed was maintained at
0.5 mm/min for a length of 0.5 mm.

2.3.2 Wettability and Surface free energy

The interaction between the surface of a biomaterial and its
environment, e.g. bacteria in an aqueous solution, depends
upon the chemistry of both and can be estimated from their
interfacial energy that is a direct measure of the intermolecu-
lar attractive forces. The adhesion of bacteria on the sam-
ple surface is governed both, by dispersive as well as polar
interactions and hence the interfacial energy comprises of the
Lifshitz-van der Waals attractive interaction component, the
electrostatic double-layer repulsive component, the Lewis
acid–base component, and the Brownian motion component.
Microbial adherence to the sample surfaces occurs when the
total interaction energy is negative [24]. Direct measurement
of interfacial surface energy is difficult; however, it can be
estimated from a series of contact angle (CA) measurements
of polar and non-polar liquids of known surface tension on the
sample surface using standard software (M/s. VCA optima).

In the present work, two polar solvents, water and for-
mamide and a non-polar solvent, xylene have been used.
The change in surface energy of uncoated and ZrO₂-coated
Ti6Al4V samples was directly obtained from the software
by feeding contact angle data of all three liquids. The CA
was evaluated using static sessile drop technique with VCA
optima analyser. A software controlled micro-syringe was
used to drop liquid of volume ~ 1 μL on the sample, and
photo of the drop was recorded immediately using an inbuilt
CCD camera. The drop image was processed using image
processing software to record advancing (θₐ) and receding
(θᵣ) CA from the shape of the drop. Three measurements
with each liquid at three different locations on the sample
surface were performed to determine average CA of all
samples.

2.3.3 Tribological tests

The dry sliding wear tests were carried out by a ball-on-
disc tribometer (M/s. Anton Paar). A standard stainless steel
(SS) ball of diameter about 5 mm was used as counterpart.

| Table 1 | Nomenclature and description of different samples used in this work |
|---------|---------------------------------------------------------------|
| Nomenclature | S-P | S-RT | S-200 |
| Sample description | Pristine Ti6Al4V substrate | ZrO₂ coating on Ti6Al4V substrate at room temperature | ZrO₂ coating on Ti6Al4V substrate heated to 200 °C |
Sliding friction and wear tests were measured at constant normal load of 2 N, 5 N and 7 N at room temperature in air. During the wear tests, the sample was fixed and SS ball was rotated against the stationary sample at liner velocity of 300 mm/min for 2000 numbers of cycles and variation in CoF was directly recorded during the rotation. The wear area on the sample was estimated using a stylus profilometer and wear area on counterpart was obtained from optical microscope image. The wear rate was directly calculated by the software. The wear mechanism was studied by topographical analysis of wear traces in SEM.

### 2.3.4 Bacterial adhesion tests

*S. aureus* MTCC 7373 and *K. pneumoniae* MTCC 109 were isolated in Nutrient Agar and grown in Nutrient broth overnight followed by centrifuging. The supernatants were discarded and pellets were re-suspended in phosphate-buffered saline (PBS), followed by a second centrifuging and resuspension in PBS. The OD value of the cell suspension was measured using a spectrophotometer at an excitation wavelength of 600 nm, and the value was adjusted to 0.1 by gradually adding PBS [25]. The samples were placed separately in 60 mm × 15 mm sterilized Petri dishes followed by the addition of 500 µL of the bacterial suspension. The samples were incubated at room temperature with intermittent mixing by pipette for 4 h. At the end of the incubation period, the suspension was gently pipetted from the surface and the plates were then put into a new tube containing 5 ml of PBS, and vigorously vortexed for 30 s to remove the adhering micro-organisms. The pipetted culture was mixed with the vortexed culture, centrifuged, and re-suspended in PBS. Viable organisms were quantified by plating serial dilutions on Nutrient agar plates. Nutrient agar plates were incubated for 18 h at 37 °C, and the colony-forming units (CFU) were counted visually.

For the staining procedure, two fluorescent dyes were used in combination: SYTO9 (Invitrogen AG, Basel, Switzerland), and PI (Invitrogen). Stock solutions of the dyes were prepared as follows: PI and SYTO9 were used from the LIVE/DEAD BacLight system as proposed by the manufacturer. All stock solutions were stored at 20 °C. All the samples, S-P, S-RT and S-200 were cultured (10⁶ cells/ml), immediately stained with a mixture of SYTO9 (5 µM final concentration) and PI (30 µM) [26]. Samples were incubated in the dark at room temperature for 15 min before analysis. The samples were washed once in PBS and 2 µL was spotted on 1% agarose-coated slides. Fluorescence microscopy analysis of the slides was performed using an LSM 780 Meta laser scanning confocal microscope (Carl Zeiss, Oberkochen, Germany) with a 60 × 1.4 NA Plan-Apochromat oil immersion objective [27].

### 3 Results

#### 3.1 Surface analysis

Figure 2 depicts SEM images, AFM images, scratch test results, and water contact angle of S-P, S-RT, and S-200 samples. Change in the morphology of the surface after deposition of ZrO₂ thin film was observed in the SEM images. The density of ZrO₂ globules was more on S-RT while the average size was larger on S-200. AFM was used to measure the change in average surface roughness of the sample. A reduction in surface roughness from 218 nm of S-P to 104 nm and 82 nm for S-RT and S-200, respectively, was observed. The diagram of friction force, coefficient of friction, normal force and optical images of scratch lines is shown in Fig. 2. It can be clearly seen that the frictional force exhibited different behaviour for S-RT and S-200. For S-RT sample the frictional force raised proportionally with increased load up to a point when it sharply increased, indicated ZrO₂ coating failure. However, such abrupt change of the frictional force was not observed for S-200 sample. The scratch morphology images are shown as insert of each graph in which an increase in scratch width with the progressive load was observed on S-P. Complete delamination of ZrO₂ film was observed on S-RT at 0.4 mm distance (marked with a circle); however, the film was almost intact in S-200 at the same distance. This indicates superior mechanical property and better adhesion of ZrO₂ film in the case of S-200. The photograph of the water drop on S-P revealed its hydrophilic nature with a water CA of 78°. The wettability of the sample decreased and the sample became hydrophobic with CA of 100° (S-RT) and 103° (S-200) after ZrO₂ coating. To know the change in surface energy of Ti6Al4V after ZrO₂ coating, the advancing and receding CA of polar solvents water, formamide, and non-polar solvent xylene were measured on the left and right sides of a 2D image of 3 drops at three different locations. Measured values of the CA are listed in Table 2. While the CA for water and formamide increased on coated samples, it showed a reverse trend for xylene for which the CA decreased as a result of coating. The resulting surface free energy of all samples, calculated using these CA values, is listed in Table 2. The surface free energy of Ti6Al4V can be seen to reduce from 18.5 dyne/cm to 17.5 dyne/cm for S-RT. This value further reduced to 14.9 dyne/cm for S-200.

The roughness of Ti6Al4V surface on which coating was done was ~0.2 µm and hence the thin film deposited is masked by the inherent surface roughness inhibiting its independent evaluation. However, we estimate that the thickness would be ~200 nm as measured from deposition under identical conditions on a mirror finish sample.
3.2 Tribological behaviour

Figure 3a–c shows variation in CoF of S-P, S-RT, and S-200 samples as a function of sliding cycles at different loads of 2 N, 5 N, and 7 N, respectively. The CoF for S-P exhibited a sharp rise to a value of 0.63 during the initial few cycles (15 cycles) and with an oscillating behaviour reached 0.92 at the end of 2000 cycles. In case of S-RT, the minimum value of CoF at 0.28 sustained till ~100 cycles after which it suddenly rose to 0.59. This implies that after
100 cycles the counterpart pierced the ZrO₂ coating and touched the Ti₆Al₄V surface. A significant reduction in CoF was observed for S-200 where the initial CoF was only 0.19, ~23% less than S-P that sustained up to ~1500 cycles, before rising to a maximum value of 0.63. Similar trend of improvement in CoF in case of S-200 as compared to S-RT and S-P was observed at higher loads too although the sharp rise in the CoF occurred for lower number of cycles. For a load of 5 N, the minimum CoF for S-RT and S-200 remained intact (about 30% lower value for both) up to ~143 and ~772 cycles, respectively, after which both samples showed a sharp rise in CoF and reached the same value as S-P. At a higher load of 7 N, S-P showed almost constant CoF from starting while S-RT and S-200 had lower CoF up to ~90 and ~327 cycles, respectively, which increased at the end and was highest for S-RT. The minimum and maximum values of CoF of all samples under all load conditions are listed in Table 3 for comparison.

To calculate the wear rate, the trace area was estimated from the resultant wear track on the sample using a stylus profilometer and the depth profile of all samples is shown in Fig. 4a where the wear depth indicates the amount of material removed from the sample, and trace width represents the contact area between the sample and the counterpart at the end of wear cycles. In general, S-P sample exhibited a deeper wear groove compared to S-RT and S-200 samples. The wear depth increased, understandable, in all cases with increase load. A significant reduction in the wear rates of 24%, 49% and 46% were observed at 2 N, 5 N and 7 N, respectively for S-200 sample in comparison with S-P, as shown in Fig. 4b. Here, the wear rate is an accumulative effect of wear of ZrO₂ thin film and subsequent wear of the

### Table 2

| Sample/solution | WATER          | FORMAMIDE      | XYLENE         | Surface energy (dyne/cm) |
|-----------------|----------------|----------------|----------------|--------------------------|
|                 | θₐ  | θᵣ  | θₐ  | θᵣ  | θₐ  | θᵣ  | θₐ  | θᵣ  | θₐ  | θᵣ  |
| S-P             | 75.60 | 74.60 | 53.30 | 57.90 | 16.40 | 20.70 | 18.5 |
| S-RT            | 95.40 | 90.60 | 84.80 | 85.20 | 12.30 | 13.00 | 17.5 |
| S-200           | 101.70 | 103.20 | 99.50 | 89.10 | 99.00 | 91.10 | 14.9 |

### Table 3

| Samples/CoF | 2 N | Min | Max | 5 N | Min | Max | 7 N | Min | Max |
|-------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| S-P         | 0.63 | 0.92 | 0.45 | 0.56 | 0.42 | 0.45 |
| S-RT        | 0.27 | 0.92 | 0.18 | 0.60 | 0.16 | 0.56 |
| S-200       | 0.19 | 0.63 | 0.13 | 0.60 | 0.13 | 0.43 |

Fig. 3 CoF of S-P, S-RT and S-200 samples at (a) 2 N, (b) 5 N and (c) 7 N
substrate (Ti6Al4V) and therefore it is difficult to isolate the two effects to explain the observed overall behaviour. To be noted, wear rate shows a maximal behaviour with respect to the load applied for all the 3 types of samples.

Figure 5 shows the SEM images of wear traces of samples after 2000 cycles at load of 2 N, 5 N, and 7 N values, respectively. Continuous wear tracks with groove structures therein were commonly observed in all case, indicating occurrence of abrasive wear on the samples. At a load of 2 N, tearing of the film and accumulation of wear debris at some location in the trace (marked with a circle), was observed a clear indication of adhesive wear. At a load of 5 N, along with debris, craters, marked in white rectangles in the figure, were observed in the wear track. At the highest applied load of 7 N, extensive wear occurred on the sample surface, and deformations were found on the surfaces, marked with arrows in the figure.

Since the wear resistance of S-200 was superior, EDS analysis of the worn surface was done for this sample, and the results are shown in Fig. 6. The presence of Fe and O in wear trace indicated deposition of debris from SS counterpart on sample after adhesive wear. Furthermore, this result indicated the occurrence of oxidation wear on the sample. The wt% of Fe and O reduced at higher loads while wt% of Ti increased indicating the complete wearing off of the coating at higher load.

### 3.3 Antibacterial properties

The result of the total viable count of *S. aureus* and *K. pneumonia* after exposure to S-P, S-RT and S-200 is shown in Fig. 7a, b, respectively, where higher inhibition of both strains on S-RT and S-200 samples compared to control and S-P was clearly observed. These experiments were repeated 4 times and the statistical parameters were calculated. The S-RT was most resistant to peptidoglycan rich gram positive *S. aureus* while it experiences relatively lesser inhibitory effect on S-200 (Fig. 7a). It is worth noting that the gram negative *K. pneumonia* was equality sensitive to all the treated surfaces with marginally more inhibition on S-200 (Fig. 7b).

To verify our findings at the cellular level, we stained the treated bacteria with LIVE/DEAD BacLight system. Living cells with intact membrane allow only permeable dyes like SYTO9 and exclude membrane impermeable dye like PI, while injured/dead bacteria with compromised membranes allow PI to permeate the cell. Thus living bacteria stain green, while injured/dead cells stain red. It can be clearly observed that, bacterial adhesion, colony and biofilm formation are more on S-P and S-RT samples for both microbial. However, lesser bacteria were adhered on S-200 and the number was even lesser in case of *S. aureus*. We found that, for both *S. aureus* (Fig. 8a) and *K. Pneumonia* (Fig. 8b), the proportion of red
Fig. 5 SEM image of wear track of S-P, S-RT and S-200 samples at (a) 2 N, (b) 5 N and (c) 7 N
stained cells increased after exposure to the coated Ti6Al4V samples, thus indicating that these cells have suffered substantial damage to the cell wall/outer membrane. Thus there was an agreement of our cytological data with the physiological data of the viable count, which confirms the bactericidal properties of the treated surfaces. This indicated ZrO2 coating inhibited the adhesion of bacteria and decimated a larger fraction of it within 4 h.

4 Discussion

Ti6Al4V alloy has been used for the production of orthopaedic and dental implants since decades. However, efforts are being expended to enhance its functionalities and thereby increase the overall lifespan of the implant. We have experimentally shown that PLD of ZrO2 thin film on
Ti6Al4V surface improves its wear resistance and reduces the interfacial surface energy that in turn, results in a superior antibacterial performance. Results are discussed in detail in the following paragraphs.

### 4.1 Tribological behaviour

The value of CoF is an indicator of the tribological behaviour of the material where smaller CoF corresponds to
superior wear resistance [28]. Section 3.1 reported results of CoF and wear rate of S-P, S-RT and S-200 samples characterized using ball-on-disk tribometer under dry sliding condition against SS counterpart ball at different loads.

For all loads, after initial few cycles, a sharp rise in the CoF for S-P was observed which remained high throughout the test consisting of 2000 cycles. The initial lower value of CoF for S-P is because of thin oxide layer present on its surface that tears of within a few cycles and the counterpart touches Ti6Al4V. The ZrO2 coating seems to have effectively reduced the CoF of Ti6Al4V, more prominently so for S-200. Further, the analysis on S-RT indicated that, the strength and adhesion of ZrO2 coating performed at room temperature could prevent the penetration of the counterpart for some more number of cycles before tearing off. It may be noted that during the initial cycles, the small contact area between the sample and counterpart results in a higher pressure. As soon as counterpart penetrates the surface, the contact area increases and a steep increase in CoF occurs. The non-uniform nature of penetration is reflected in the oscillatory behaviour of CoF the average value of which gradually increases with an increasing number of cycles [29]. The S-200 could withstand with much lower CoF value for more number of cycles at all loads before giving way can be attributed to the superior quality of thin film deposited at higher substrate temperature.

These observations are further corroborated by the results of the scratch test shown in Fig. 2 too where delamination occurs at a much later distance for S-200 as compared to others under identical conditions. ZrO2 (653 HV) is known to possess greater hardness as compared to Ti6Al4V (357 HV) [30]. Our experiments reveal that the coating at an elevated temperature (200 °C in the present case) plays a decisive role in the improving quality of adhesion. It was observed that the number of cycles up to which the coating sustains reduced with increasing load in general. The wear depth measured using a mechanical profilometer (Fig. 4a) revealed a reduction in wear depth in coated samples as the hardness of ZrO2 restricted penetration of counterpart. About 50% reduction in wear rate indicated superior protectiveness of PLD coated ZrO2 film at 200 °C substrate temperature. The SEM images (Fig. 5) showed typical morphologies of the worn surface at two magnifications (300 μm and 50 μm). Continuous sliding marks with grooves and ridges seen in all wear tracks resulted from penetration of counterpart and subsequent scratching on sample surface indicating abrasive wear. During sliding the sample experiences deformations that increases with number of cycles and after a critical accumulation, the nucleated part is removed from the surface. The removal of materials from the surface of the sample in the form of debris and flakes (magnified SEM images in Fig. 5) are an indication of adhesive wear. While with increasing number of cycles the wear track gradually widens in all cases, the wear trace of ZrO2 coated samples was narrower compared to S-P. This is once again attributable to the higher hardness of ZrO2 which restricted penetration of counterpart. The presence of O in the EDS analysis indicated the occurrence of oxidative wear on the sample. The higher oxygen content in S-P sample is indicative of the higher temperature reached by the contact region due to larger friction experienced which promotes the oxidation reaction. Thus the mechanism here can be considered abrasive, adhesive along with oxidation. This is in line with the findings of some other researchers where ZrO2 coating improved the wear properties of the Ti6Al4V [31, 32]. Yuan et al. combined laser induced dimple pattern followed by zirconization of Ti6Al4V with double glow plasma technique to achieve 26.6% reduction in wear rate [31]. Berni et al. could observe 18% reduction in the wear rate in pulsed plasma-deposited zirconia coating on Ti6Al4V [32]. In comparison with these, the wear rate reduced by ~50% for S-200 for certain load conditions in comparison with pristine sample in present case. This clearly reveals that PLD is an excellent and more simple choice for protective coating of biomaterials more so when the coating is done at higher substrate temperature.

4.2 Antibacterial test

Bacterial adhesion during or after implantation can lead to biofilm formation, a complex process influenced mainly by the bacterial properties and material surface characteristics such as chemical composition, surface charge, wettability, and physical configuration [33, 34]. ZrO2 is a biocompatible material with known antibacterial properties. Hence, PLD of ZrO2 on Ti6Al4V is also expected to yield improved antibacterial performance which has been substantiated by our experimental observations.

The strength of bacterial adhesion depends on whether the bacteria donates or accepts electrons from the substrate surface [35]. ZrO2, with a superior electron donor capability, can repel the bacteria leading to lower bacterial adhesion [36–38]. The adhesion, density and overall dose of two chosen bacteria, one gram positive (S. aureus) and one gram negative (K. pneumonia) is summarized in Figs.7 and 8. The total viable count analyzed after 4 h incubation indicated the antimicrobial effect of S-RT and S-200 against both the bacteria to be ~50% more in comparison with S-P. As can be seen from Table 2, the reduced interfacial surface energy of the Ti6Al4V sample following ZrO2 coating results in inferior adherence of bacteria [24, 39, 40]. In live/dead bacteria analysis, there was a qualitative increase in the number of bacteria with compromised membranes on S-RT and S-200 for both S. aureus (Fig. 8a) and K. pneumoniae (Fig. 8b) as revealed by the increased red fluorescence as compared to S-P. The reduced bacterial colony counts on S-200 compared to S-RT can understandably be accounted by its low surface free energy. Thus there is a
definite agreement in the observed cytological data and the corresponding physiological data of the viable count, which confirms the improved bactericidal properties of the ZrO$_2$ coated sample.

5 Conclusion

In this communication, the potential benefits of PLD of ZrO$_2$ thin film on Ti6Al4V sample in terms of superior wear resistance and enhanced bactericidal properties simultaneously in comparison with pristine sample have been addressed. Reduction in surface roughness with better adhesion and lower wettability was observed for the coated samples. The coated sample showed significant improvement in wear resistance for all load conditions of 2 N, 5 N, and 7 N against SS ball as counterpart. The sample showed about 30% reduction in CoF and 50% reduction in wear rate, indicating superior conservational properties of ZrO$_2$ coating by PLD against wear. Among coated samples, the S-200 showed superior adhesion to the substrate with reduced surface wettability in comparison with S-RT. In addition, it also showed a lower CoF value which sustained for larger number of cycles of wear. A clear reduction in wear rate and adhesion of K. Pneumonia bacteria indicated superior quality of S-200 in comparison with S-RT. The improvement in the performance may be attributed to stronger adhesion of deposited film at 200 °C substrate temperature. The wear analysis using SEM and EDS indicated occurrences of abrasive, adhesive and oxidation wear on all the samples at higher load conditions. Reduction in the surface free energy and superior electron donor capability of ZrO$_2$ restricted adhesion and biofilm formation of S. aureus and K. pneumonia bacteria indicated improved antibacterial behaviour of Ti6Al4V post coating. Hence, PLD of ZrO$_2$ coating is an effective method of improving mechanical, tribological and antibacterial properties of widely used Ti6Al4V bio-alloy.

Acknowledgements Authors acknowledge Dr. T. S. R. C. Murthy, MP&CED and Dr. A. K. Sahu, G&AMD, BARC for fruitful discussions.

Declarations

Ethical statement This is to declare that, the submitted work is original and is neither published not submitted for publication elsewhere. The order of authors and the corresponding author are correct in the submission.

References

1. T. Kim, C.W. Seea, X. Li, D. Zhu, Orthopedic implants and devices for bone fractures and defects: Past, present and perspectives. Eng. Regen. 1, 6–18 (2020)
2. K. Colic, A. Sedmak, The current approach to research and design of the artificial hip prosthesis: A review. Rheumatol. Orthop. Med. 1, 1–7 (2016)
3. M. Saini, Y. Singh, P. Arora, V. Arora, K. Jain, Implant biomaterials: A comprehensive review. World J. Clin. Cases. 3, 52–57 (2015)
4. G. Galagali, E.S. Reddy, P. Nidawani, S.S.P. Behera, P. Preetham, M. Sarangpala, Implant failures: A comprehensive review. Int. J. Prevent. Clin. Dental Res. 1, 11–17 (2014)
5. M. Kaur, K. Singh, Review on titanium and titanium based alloys as biomaterials for orthopaedic applications. Mater. Sci. Eng. C. 102, 844–862 (2019)
6. V. Sansone, D. Pagani, M. Melato, The effect on bone cells of metal ions released from orthopaedic implants. A review. Clin. Cases Miner. Bone Metab. 10, 34–40 (2013)
7. Z. Khatoon, C.D. Mctiernan, E.J. Suuronen, T.F. Mah, E.I. Alarcon, Bacterial biofilm formation on implantable devices and approaches to its treatment and prevention. Heliyon 4, e01067 (2018)
8. M. Katsikogianni, Y.F. Missirlis, Concise review of mechanisms of bacterial adhesion to biomaterials and of techniques used in estimating bacteria-material interactions. Eur. Cell Mater. 8, 37–57 (2004)
9. M. Kaur, K. Singh, Review on titanium and titanium based alloys as biomaterials for orthopaedic applications. Mater. Sci. Eng. C 102, 844–862 (2019)
10. G. Shen, F. Fang, C. Kang, Tribological performance of bioimplants: A comprehensive review. Nanotechnol. Precis. Eng. 1, 107–122 (2018)
11. H. Dong, W. Shi, T. Bell, Potential of improving tribological performance of UHMWPE by engineering the Ti6Al4V counterparts. Wear 225, 146–153 (1999)
12. S. Shaikh, S. Kedia, D. Singh, M. Subramanian, S. Sinha, Surface texturing of Ti6Al4V using femtosecond laser for superior antibacterial performance. J. Laser Appl. 31, 22011 (2019)
13. S. Shaikh, S. Kedia, A.G. Majumdar, M. Subramanian, S. Sinha, 45S5 bioactive glass coating on Ti6Al4V alloy using pulsed laser deposition technique. Mater. Res. Express. 6, 125428 (2019)
14. D. Ege, I. Duru, A.R. Kamali, A.R. Boccaccini, Nitride, zirconia, alumina, and carbide coating on Ti6Al4V femoral heads: Effect of deposition techniques on Mechanical and tribological properties. Adv. Eng. Mater. 17, 1701177 (2017)
15. A.C. Hee, Y. Zhao, S. Jamali, A. Bendavid, P.J. Martin, H. Guo, Characterization of tantalum and tantalum nitride films on Ti6Al4V substrate prepared by filtered cathodic vacuum arc deposition for biomedical applications. Surf. Coat. Technol. 365, 24–32 (2018)
16. A. Hatema, J. Lin, R. Wei, R.D. Torresa, C. Laurindoa, P. Soaresa, Tribocorrosion behavior of DLC-coated Ti-6Al-4V alloy deposited by PIID and PEMS + PIID techniques for biomedical applications. Surf. Coat. Technol. 332, 223–232 (2017)
17. W. Zhang, C.T. Wang, W. Liu, Characterization and tribological investigation of sol-gel ceramic films on Ti-6Al-4V. Wear 260, 379–386 (2006)
18. G. Pradhaban, G.S. Kaliaraj, V. Vishwakarma, Antibacterial effects of silver-zirconia composite coating using pulsed laser deposition onto 316L SS for bio implants. Prog. Biomater. 3, 123–130 (2014)
19. G. Heinrich, T. Grogler, S.M. Rosiwal, R.F. Singer, CVD diamond coated titanium alloys for biomedical and aerospace applications. Surf. Coat. Technol. 94, 514–520 (1997)
20. K. Chauhan, D. Subhedar, R. Prajapati, D. Dave, Experimental investigation of wettability properties for zirconia based coatings by RF magnetron sputtering. Mater. Today Proc. 26, 2447–2451 (2020)
21. E. Nikoomanzari, A.F. Alhosseini, M.R.P. Alamoti, M.K. Keshavarz, Effect of ZrO2 nanoparticles addition to PEO coatings on Ti–6Al–4V substrate: Microstructural analysis, corrosion behavior and antibacterial effect of coatings in Hank’s physiological solution. Ceram. Int. 46, 13114–13124 (2020)

22. J. Chevalier, What future for zirconia as a biomaterial? Biomater. 27, 535–543 (2006)

23. S. Sultana, A. Rafiuddin, M.Z. Khan, M. Shahadat, Development of ZnO and ZrO2 nanoparticles: Their photocatalytic and bactericidal activity. J. Environ. Chem. Eng. 3, 886–891 (2015)

24. Y. Liu, Q. Zhao, Influence of surface energy of modified surfaces on bacterial adhesion. Biophys. Chem. 117, 39–45 (2005)

25. W. Chen, Y. Liu, H.S. Courtney, M. Bettenga, C.M. Agrawal, J.D. Bumgardner, J.L. Ong. In vitro anti-bacterial and biological properties of magnetron co-sputtered silver-containing hydroxyapatite coating. Biomaterials 27, 5512–5517 (2006)

26. J. Robertson, C. McGoverin, J.R. White, F. Vanholsbeeck, S. Swift, Rapid detection of Escherichia coli antibiotic susceptibility using live/dead spectrometry for lytic agents. Microorganisms. 26, 924 (2021)

27. S. Kota, V.K. Charaka, S. Ringgaard, M.K. Waldor, H.S. Misra, A contributes to Deinococcus radiodurans resistance to nalidixic acid, genome maintenance after DNA damage and interacts with deinococcal topoisomerases. PLoS ONE 9(1), e85288 (2014). https://doi.org/10.1371/journal.pone.0085288

28. J. Zhou, Y. Sun, S. Huang, J. Sheng, J. Li, E.A. Boateng, Effect of laser peening on friction and wear behavior of medical Ti6Al4V alloy. Opt. Laser Technol. 109, 263–269 (2019)

29. E. Avcu, The Influences of ECAP on dry sliding wear behaviour of AA7075 aluminium alloy. Tribol. Int. 110, 173–184 (2017)

30. P. Popoola, F. Ochonogor, M. Abdulwahab, S. Pityana, C. Macco, Microhardness and wear behaviour of surface modified Ti6Al4V/Zr-TiC metal matrix composite for advanced material. J. Optoelectron. Adv. Mater. 14, 991–997 (2012)

31. S. Yuan, N. Lin, J. Zou, Z. Liu, Z. Wang, L. Tian, L. Qin, H. Zhang, Z. Wang, B. Tang, Y. Wu. Effect of laser surface texturing (LST) on tribological behaviour of double glow plasma surface zirconizing coating on Ti6Al4V alloy. Surf. Coat. Technol. 368, 97–109 (2019)

32. M. Berni, N. Lopomo, G. Marchiori, A. Gambardella, M. Boi, M. Bianchi, A. Visani, P. Pavan, A. Russo, M. Marcacci, Tribological characterization of zirconia coating deposited on Ti6Al4V components for orthopaedic applications. Mater. Sci. Eng., C 62, 643–655 (2016)

33. M. Ribeiro, F.J. Monteiro, M.P. Ferraz, Infection of orthopedic implants with emphasis on bacterial adhesion process and techniques used in studying bacterial-material interactions. Biomatt. 2, 176–194 (2012)

34. S. BinAhmed, A. Hasane, Z. Wang, A. Mansurov, S.R.V. Castillón, Bacterial adhesion to ultrafiltration membranes: Role of hydrophilicity, natural organic matter, and cell-surface macromolecules. Environ. Sci. Technol. 52, 162–172 (2018)

35. A.T. Poortinga, R. Bos, H.J. Busscher, Charge transfer during staphylococcal adhesion to TiNOX coatings with different specific resistivity. Biophys. Chem. 91, 273–279 (2001)

36. M.L.G. Martin, L.L. Broncano, B. Janczuk, Wettability and surface free energy of zirconia ceramics and their constituents. J. Mater. Sci. 34, 5923–5926 (1999)

37. R.P. Tanoira, D. Horwat, T.J. Kinnari, C.P. Jorge, E.G. Barrena, S. Migot, J. Esteban, Bacterial adhesion on biomedical surfaces covered by yttria stabilized zirconia. J. Mater. Sci. Mater. Med 27, 1–9 (2016)

38. G.S. Kaliaraj, V. Vishwakarma, K. Alagarsamy, A.M.K. Kirubaran, Biological and corrosion behaviour of m-ZrO2 and t-ZrO2 coated 316L SS for potential biomedical applications. Ceram. Int. 44, 14940–14946 (2018)

39. S.C. Dexter, Influence of substratum critical surface tension on bacteria adhesion-in situ studies. J. Colloid Interface Sci. 70, 346–354 (1979)

40. A. Milne, M.E. Callow, Non-biocidal antifouling process, in Polymer in Marine Environment. ed. by R. Smith (The institute of Marine Engineers, London, 1985), pp. 229–233

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.