Original Article

Surface Characteristics and Bioactivity of Zirconia (Y-TZP) with Different Surface Treatments

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Background: Zirconia being a bio-inert material needs to be surface treated to render it more bioactive and enhance its osseointegration potential. However, bioactivity studies focusing on the ability of sandblasting and ultraviolet photofunctionalization (UVP) surface treatments in inducing apatite precipitation using simulated body fluid (SBF) are lacking. Aim: The aim of the study was to comparatively evaluate the effect of two different surface treatments—sandblasting with 50 µm alumina and UVP with ultraviolet C (UVC) light on the bioactivity of zirconia.

Materials and Methods: A total of 33 discs with dimensions 10 mm × 2 mm were obtained from zirconia blanks (Amann Girrbach, Koblach, Austria) and randomly divided into three groups (n = 11), namely Group I (untreated), Group II (sandblasted), and Group III (UVP). Surface characteristics of representative test samples were analyzed using X-ray diffraction (XRD), atomic force microscopy (AFM), contact angle goniometry, scanning electron microscopy (SEM), and energy dispersive X-ray spectroscopy (EDX), to assess type of crystal phase of zirconia, surface roughness, wettability, surface topography, and elemental composition, respectively. SBF was prepared and calcium content in SBF (Ca-SBF) was determined using inductively coupled plasma mass spectrometry (ICP-MS).

Results: Data were analyzed by one-way analysis of variance (ANOVA), post hoc Tukey honestly significant difference (HSD), and Student’s t test for statistical significance (P < 0.05, significant; P < 0.01, highly significant). Surface characteristics analyses revealed that XRD showed predominant tetragonal (t) zirconia crystal phase for all test groups. Mean surface roughness (Sa) of Group I was 41.83 nm, and it was significantly lesser than that of Group II (115.65 nm) and Group III (102.43 nm). Mean contact angles were 98.26°, 86.77°, and 68.03° for Groups I, II, and III, respectively, and these differences were highly significant. Mean pre-immersion Ca content in SBF was found to be 159 mg/L. Mean post-immersion Ca content was 70.10, 60.80, and 56.20 mg/L for Groups I, II, and III, respectively. Significant differences were found between Group I as compared to both Groups II and III. Bioactivity of Group III was marginally, but insignificantly higher with respect to Group II. Groups II and III were insignificant with respect to each other. Post-immersion XRD revealed predominant “t” phase, and SEM-EDX revealed well-formed, abundant calcium apatite layer on the treated samples as compared to that on untreated samples.

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INTRODUCTION

Developments in clinical prosthodontics are driven by the innovation of new dental materials and processing techniques.\(^1\) The research in implant biomaterials is surging since past few decades due to a continuous increase in the aging population, who demand increasingly functional and aesthetic prosthetic replacements.\(^2\) The criteria for a restorative material to be termed as a “biomaterial” is that it has to be bioinert with excellent aesthetic and mechanical properties.\(^1,3\) Currently, yttria-stabilized zirconia, also known as tetragonal zirconia polycrystal (Y-TZP), is the most used material for fabrication of zirconia-based implants.\(^4\)

Despite addition of stabilizing elements, zirconia is a bio-inert material,\(^5-8\) and this aspect may impact its osseointegration potential.\(^7,9,10\) Hence, studies focusing on surface treatments of zirconia to render the surface more receptive to osseointegration and apatite formation have gained significance.\(^9,11\) However, t-m phase conversions after certain surface treatments that can deleteriously affect the longevity of zirconia as an implant biomaterial have also been reported,\(^1\) and hence ascertaining maintenance of the tetragonal phase following any type of surface treatment of zirconia is crucial in bioactivity studies. Various reports are available stating the importance of surface topography and characteristics, such as surface roughness and wettability on the extent of bioactivity of zirconia.\(^1,2,11\) following different surface treatments. Wettability has been suggested as a key parameter that impacts the chain of processes associated with osseointegration.\(^4,12,13\) The surface topography and elemental composition is also thought to influence the maintenance of the tetragonal phase as well as affects its bioactivity. Thus, bioactivity studies also typically include surface characteristics investigations comparing untreated and treated zirconia surfaces to explain the bioactivity.\(^6,11,14\) Methods such as X-ray diffraction (XRD), atomic force microscopy (AFM), contact angle goniometry, scanning electron microscopy (SEM), and energy dispersive X-ray spectroscopy (EDX) are used by researchers to assess crystal phase, roughness, wettability, topography, and elemental composition, respectively.\(^4,6,9,15,16\)

Several reports have summarized different additive and subtractive surface modification methods to improve surface properties of zirconia implant biomaterials. These reports found improved bone bonding, achieved due to surface treatments when compared to untreated surfaces.\(^17,18\)

Airborne particle abrasion known as sandblasting technique has been used to increase surface roughness of zirconia, which has been shown to positively impact osseointegration in cell culture studies.\(^9,11,17,19\) One concern that is often mentioned is that sandblasting could result in damage to the zirconia surface, thereby altering the vital surface characteristics. Airborne particle abrasion with alumina particles lesser than 100 µm in size has been identified as a key factor in achieving an optimum surface roughness to enhance biological response of osteoblasts without causing structural damage to zirconia.\(^9,11,18,20-22\)

Recently, researchers have reported that ultraviolet photofunctionalization (UVP) as a surface modification of zirconia enhances its osseointegration potential.\(^4,12-14\) “Bioactivity” is one of the characteristics of an implant material, which allows it to form a bond with living tissue.\(^6,23\) In vitro testing such as simulated body fluid (SBF) analysis and protein adsorption assays have been used to mimic in vivo conditions, thereby decreasing time, cost, and regulatory issues.\(^24\) and it can be manipulated by researchers in a controlled manner.\(^7,11,23\)

Studies have recommended the use of in vitro bioactivity tests such as immersion of synthetic materials into solutions such as SBF, which replicate the mineral content of human plasma.\(^25,26\) The calcium and phosphorous content in SBF form apatite precipitation on these biomaterials to varying extents, depending on the material, their surface characteristics, duration of immersion environment, and so on.\(^1,26-28\) Thus, immersion in SBF can aid to predict in vivo behavior of a potential implant biomaterial. In vitro testing of
bioactivity in SBF has also minimized the requirement of animal studies.[25,29-31]

Calcium content analysis of the SBF solution by inductively coupled plasma mass spectrometry (ICP-MS) both before and after immersion of samples has been recommended as a reliable method to assess the apatite precipitation, which indicates its bioactivity.[6,26]

Cell culture and protein adsorption studies exploring the bioactive potential of sandblasting and UVP surface treatments on zirconia are available in the literature.[4,14,18,20,32-34]

Thus, studying these characteristics aids in the correlation of bioactivity results and are frequently used as an adjunct in such studies.[11,12,14,28,35,36]

Materials and Methods
Commercially available Y-TZP (Amann Girrbach, Koblach, Austria) was used.

Customizing test samples
Thirty zirconia samples with dimensions of thickness 10 mm × 2 mm diameter with extension tag of 4 × 4 × 2 mm were obtained from copy-milling a customized resin prototype and then sintering it at 1500°C for 8 h.

Grouping of samples and surface treatment
The above processed zirconia were divided and designated as Groups I, II, and III depending on the type of surface treatment (n = 10/group):

Group I (untreated): Samples (n = 10) that were not subjected to any surface treatment
Group II (sandblasted): Samples (n = 10) that were subjected to sandblasting with 50 µm alumina at a distance of 10 mm/at an angle of 90°/and at 2.5 bar pressure for 15 s followed by ultrasonic cleaning for 15 min in de-ionized water to remove alumina particles
Group III (UVP): Samples (n = 10) that were placed in a UV chamber at a distance of 4 cm from the light source of wavelength 254 nm for 15 min

All the samples of the three test groups were subsequently marked, dried, and stored in a desiccator (Borosil, Mumbai, India) until further testing.

Surface characteristic analysis
X-ray diffractometry
X-ray diffractometer (XRD) (Ultima IV, Rigaku, Tokyo, Japan) was used to detect the type of crystal phase present on representative samples (n = 1/group) of all groups.

Atomic force microscopy
AFM was used to detect surface roughness of representative samples of untreated and treated test groups. The test samples were analyzed in four areas of each sample of each group, by keeping the cantilever tip of the equipment in noncontact mode. Subsequently, 5 µm × 5 µm images with 256 × 256 pixels were taken with a scan rate of 0.5 Hz, to obtain both two- (2D) and three-dimensional (3D) images. The analysis also gave numeric values for surface roughness (Sa) at nanoscale levels for each group.

Contact angle goniometry
The surface wettability (hydrophilicity) of representative samples (n = 5/group) of all three groups was examined by static sessile-drop technique using 1 µL H2O by Universal Goniometer DSA 20E (Kruss, Hamburg, Germany). Five samples per test group were analyzed by measuring five contact angle values per sample, and the overall mean contact angle was recorded.

Scanning electron microscopy coupled with energy dispersive X-ray spectroscopy
The surface topography and surface elemental composition of representative samples (n = 1/group) was analyzed by field emission SEM (Thermo Fisher Scientific, Waltham, Massachusetts) coupled with EDX (Bruker, Billerica, Massachusetts).

The samples were gold-coated using auto-fine coaters and observed with scanning electron microscope (SEM) at ×5000.

Bioactivity test
The samples of all three groups (n = 10/group) were immersed in SBF prepared according to Kokubo and Takadama[25] for 3 weeks (21 days) at 36.5°C.

After ultrasonic washing and rinsing with de-ionized water, zirconia samples were immersed in SBF at 36.5°C without stirring to investigate the bioactivity. The SBF was prepared according to the method proposed by Kokubo and Takadama.[25] The SBF was freshly prepared four times, and the mean pre-immersion calcium content was determined using ICP-MS. After immersion for 21 days, the samples were removed, rinsed with de-ionized water, and dried in a desiccator. The calcium content of SBF solution from each test tube containing samples was assessed by ICP-MS.

Results
Atomic force microscopy
The 3D image of representative sample of Group I (untreated) surface revealed a uniform appearance throughout with lesser number of shallow peaks and valleys, seen at isolated places on the sample surface. The average height of peaks and valleys was found to be 292.13 nm. The 3D image of representative sample of Group II (sandblasted) surface revealed a nonuniform texture, with greater number of very high and
well-defined peaks and valleys distributed throughout the sample surface. The average height of peaks and valleys was 764.06 nm, showing the effect of surface treatment. The 3D image of representative sample of Group III (UVP) surface revealed a nonuniform texture of roughened plains with several deep grooves, along with areas of clustered peaks and valleys, which were well-defined. The average height of peaks and valleys was 541.65 nm. The effect of treatment was reflected in their patterns.

Sandblasting and UV irradiation significantly modified surface roughness \((P < 0.01)\). One-way ANOVA revealed overall significant difference between the mean surface roughness values of the three groups (Groups I, II, and III) [Table 1]. Multiple comparisons using post hoc Tukey HSD analysis showed statistically highly significant increase \((P < 0.01)\) in mean surface roughness for both the surface-treated groups (Group II and Group III) as compared to that of the untreated group (Group I).

Contact angle goniometry

The contact angle measurements and their statistical analyses for different groups (untreated and treated) were analyzed.

Contact angles ranged between 93.60° and 100.66° in Group I; 82.76° and 91.30° in Group II; and 66.12° and 69.48° in Group III. The mean contact angles of Group I, Group II, and Group III test samples were 98.26°, 86.77°, and 68.03°, respectively.

One-way ANOVA revealed overall significant differences between the mean contact angles of the three test groups, indicating highly significant differences between their wettabilities (hydrophilicity). Multiple comparisons using post hoc Tukey HSD analysis showed statistically highly significant decrease in mean contact angles for both the surface-treated groups (Group II and Group III) as compared to that of untreated group (Group I), indicating increased wettability for both the surface-treated groups \((P < 0.01; \text{highly significant})\).

X-ray diffractometry

X-ray diffractograms obtained for all the three test groups (untreated, sandblasted, and UVP samples) before surface treatments, revealed the presence of predominantly tetragonal zirconia peaks, with negligible presence of monoclinic phase, indicating no crystal phase transformation occurring due to any of the surface treatments. The peak of the tetragonal phase was identified at 30° for all three test groups.

After immersion in SBF for 21 days, X-ray diffractograms obtained for all the three test groups (untreated, sandblasted, and UVP samples) revealed the presence of predominantly tetragonal zirconia peaks, with negligible presence of monoclinic phase, indicating no crystal phase transformation occurring due to any of the surface treatments. The peak of the tetragonal phase was identified at 30° for all three test groups.

Energy dispersive X-ray spectroscopy

Pre-immersion EDX spectrums of untreated and respective surface-treated samples revealed the presence of the elements, zirconium (Zr) (32.66%–40.26%), oxygen (O2) (53.59%–61.31%), yttrium (Y) (5.28%–5.71%), aluminum (Al) (0.13%–0.38%), and hafnium (Hf) (0.16%–0.19%) in all test groups.

Post-immersion EDX results revealed a higher Ca/P ratio for both the surface-treated groups as compared to the untreated group [Table 2]. Group III (UVP) showed the highest Ca/P ratio of 2.08, followed by Group II (sandblasted) and Group I (untreated) with ratios of 1.79 and 1.15, respectively.

Bioactivity

The results of bioactivity tests are represented in Table 3.

Ca-SBF analysis to detect calcium content in SBF before the immersion of test samples revealed calcium content to be in the range of 153–163 mg/L with a mean of 159 mg/L. This mean value was considered as the pre-immersion reference value and used for comparing with the post-immersion calcium content in SBF obtained for the three test groups.

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**Table 1: Comparative evaluation of mean surface roughness (Sa in nm) between Groups I, II, and III for overall significance by one-way analysis of variance**

| Test groups | No. of samples | Mean (nm) | \(P\) value |
|-------------|----------------|-----------|-------------|
| Group I     | 1              | 41.83     | 0.001**     |
| Group II    | 1              | 115.65    |             |
| Group III   | 1              | 102.43    |             |

**\(P\) value < 0.01; highly significant**

Inference: One-way ANOVA revealed overall significant difference between the mean surface roughness values of the three test groups.
Student’s paired *t* test revealed significant reduction (*P* < 0.05) in the mean post-immersion calcium content of Groups I, II, and III as compared to the pre-immersion calcium content (reference value), indicating significant bioactivity for untreated as well as both the surface-treated groups [Table 3]. One-way ANOVA revealed overall significant difference between the mean post-immersion calcium content values of three test groups (*P* < 0.01). Multiple comparisons using post hoc Tukey HSD analysis showed statistically significant and greater decrease (*P* < 0.05) in the mean post-immersion calcium contents in SBF for both the surface-treated groups (Groups II and III) as compared to that of the untreated group (Group I).

**DISCUSSION**

Zirconia ceramics became a prevalent biomaterial in restorative dentistry, and subsequently, research for using it as a nonmetallic implant material is on the rise.[1,37-39] Sandblasting (airborne particle abrasion) is a subtractive method and is used to improve the surface area available for bonding. The major advantage of sandblasting is that it not only cleans organic contaminants from zirconia surface but also modifies its energy, wettability, and microroughness, thus increasing bonding area and promoting osseointegration.[40,41] However, studies exploring sandblasting as a surface treatment method have yielded mixed results. The main shortcoming with sandblasting is the appearance of flaws, pits, and microcracks on the surface that can induce t–m phase changes.[17,42,43] These shortcomings can be overcome by using low blasting pressure, low particle size, and short blasting distances.[9,17] Controlled sandblasting technique results in microroughened surface that has been suggested to improve the osseointegration potential. Most studies have tested bioactivity of zirconia by coupling sandblasting with acid etching.[20,44,45]

Studies focusing on the effect of sandblasting alone on improving the bioactivity of zirconia are sparse,[11,18,20,46] and hence merit further investigation. Sandblasting was done using 50-µm-sized alumina, as particle sizes >100 µm are reported to cause reduction in flexural strength, induce t–m phase transformation, and material loss.[9] The blasting procedure was carried out as per recommendations in the literature.[17,18,20,47-49]

Recently, researchers have turned their focus on the development of UVP for surface modification of zirconia as it is a simple and inexpensive surface treatment modality to enhance the osseointegration potential of zirconia without compromising its structural changes.[4,12,13,15,50] It not only imparts Table 2: Microchemical surface elemental composition of zirconia disc samples in Group I (untreated), Group II (sandblasted), and Group III (UV irradiated) before and after immersion in simulated body fluid

| El  | AN  | Series | Pre-immersion C atom (at %) | Post-immersion C atom (at %) | Ca/P ratio |
|-----|-----|--------|-------------------------------|-------------------------------|------------|
|     |     |        | Group I | Group II | Group III | Group I | Group II | Group III |          |
| Zr  | 40  | L      | 40.26   | 39.20   | 32.66     | 11.17    | 14.38    | 10.56     | 1.15     |
| Ca  | 20  | K      | -       | -       | -         | 21.00    | 13.00    | 16.39     | 1.79     |
| O   | 8   | K      | 53.59   | 55.16   | 61.31     | 47.85    | 64.17    | 64.19     | 2.08     |
| P   | 15  | K      | -       | -       | -         | 18.18    | 7.23     | 7.86      |          |
| Y   | 39  | L      | 5.61    | 5.28    | 5.71      | 1.69     | 1.10     | 0.95      |          |
| Hf  | 72  | L      | 0.17    | 0.16    | 0.19      | 0.11     | 0.12     | 0.05      |          |
| Al  | 13  | K      | 0.38    | 0.20    | 0.13      | -        | -        | -         |          |
|     |     |        | 100     | 100     | 100       | 100      | 100      | 100       |          |

El = element, AN = atomic number, series = characteristic X-ray lines, unn. C [wt %]= the unnormalised concentration in weight percent of the element norm, C [wt %]= the normalised concentration in weight percent of the element, C atom [at %]= the atomic weight percent, C error (1 Sigma) [wt %]= the error in the weight percent concentration at the 1 sigma level

Table 3: Comparative evaluation of the difference between the pre-immersion calcium content (reference value) and the mean post-immersion calcium content obtained for Groups I, II, and III, using Student’s paired *t* test

| Pre-immersion calcium content in mg/L (reference value) | Test groups | Mean post-immersion calcium content in mg/L | Mean difference of calcium content in mg/L | *P* value |
|--------------------------------------------------------|-------------|--------------------------------------------|-------------------------------------------|----------|
| 159 mg/L                                               | Group I     | 70.10                                      | 88.90                                     | 0.039*   |
|                                                        | Group II    | 60.80                                      | 98.20                                     |          |
|                                                        | Group III   | 56.20                                      | 102.80                                    |          |

*P* value < 0.05; significant

Inference: Student’s paired *t* test revealed significant reduction (*P* < 0.05) in the mean post-immersion calcium content of Groups I, II, and III as compared to the pre-immersion calcium content (reference value), indicating significant bioactivity for untreated as well as both the surface treated groups.

**Table 2:** Microchemical surface elemental composition of zirconia disc samples in Group I (untreated), Group II (sandblasted), and Group III (UV irradiated) before and after immersion in simulated body fluid

**Table 3:** Comparative evaluation of the difference between the pre-immersion calcium content (reference value) and the mean post-immersion calcium content obtained for Groups I, II, and III, using Student’s paired *t* test
changes in the surface roughness and topography, but also makes the zirconia surface “superhydrophilic” by reducing the hydrocarbon contamination of surfaces to very low levels, which are prime factors for bioactivity and enhanced osseointegration.\cite{4,12,13,15,50} UVP can be accomplished by ultraviolet A (UVA) and ultraviolet C (UVC) types of irradiation. In this study, UVC irradiation was selected as it has been reported to enhance the bioactivity by altering the biological properties without compromising the physical characteristics and mechanical properties. UVC irradiation works through photocatalytic degradation mechanism/concept unlike the photocatalytic degradation in UVA treatment. The UVP protocols in this study were as per those stated in literature.\cite{12,15}

Kokubo and Takadama\cite{25} had reported that in vivo apatite formation could be replicated appreciably under in vitro conditions by immersing samples in SBF at physiologic temperature (37°C). In such studies, the calcium content of freshly prepared SBF before immersion of test samples and the post-immersion calcium content in SBF are assessed. The extent of calcium depletion in SBF is indicative of the test sample's bioactive potential. This method is also referred to as the “biomimetic” method.\cite{6} The bioactivity findings are also corroborated by surface characteristics analysis of test samples to assess apatite formation, topography, composition, and so on, for correlation of test results.\cite{6,26}

Thus, the assessment of crystal phase after surface treatments assumes importance and significance because any phase change implies that the material is more prone to surface degradation. XRD analysis was used to assess the types of crystalline phase on the zirconia surfaces as recommended in the literature.\cite{7,12,13}

Surface texture or roughness is an important parameter affecting osseointegration. In vitro studies have shown increased osteoblast proliferation\cite{11} and apatite formation\cite{18} on roughened surfaces. Surface roughness at a micrometer resolution has been studied in previous studies for various implant biomaterials.\cite{6,50} However, the implant surface 3D topography at a nanolevel has been suggested to be important in determining the extent of bioactivity, and to eliminate implant rejection.\cite{6,13,15,34,37} The effect of surface treatments of zirconia on its bioactivity was evaluated in this study, by using the SBF method, due to previously mentioned advantages of testing bioactivity in SBF, by following the recommended protocols.\cite{7,10,27,30,35} Considering the impact of the composition and preparation of SBF on test outcomes, the guidelines for the same as recommended in the literature were strictly adhered to.\cite{25} In the preparation of SBF, it has been reported that apatite nucleation can be induced at the surface of a glass container or the edge of scratches in such containers, which could mask the actual test results.\cite{25} Hence, new plastic containers and polypropylene test tubes with smooth surfaces were used for preparation and immersion of the test samples in SBF. All test samples were individually immersed in test tubes containing equal volumes of SBF, and incubated at 37°C for 3 weeks to ensure standardized study conditions.\cite{6}

In this study, calcium content in SBF was assessed by ICP-MS,\cite{36} as this equipment has an accuracy to detect and automatically compute the percentage of any given ion concentration in a known quantity of a solution, from a 1-mL sample dose. Multiple measurements of the calcium ion concentration in SBF were randomly done in this study to ascertain the standardization of SBF preparation procedure, and the mean pre-immersion Ca content was obtained and kept as the reference value for comparison with the post-immersion calcium content.

Different studies have tested bioactivity of zirconia in SBF by using various periods of immersion ranging from 2 days to several weeks.\cite{6,7,23,26,35} In a related pilot study, a 2-week immersion protocol was initially tested. However, no appreciable calcium depletion in SBF or formation of apatite on the test samples of all test groups was observed at the end of this period. This could perhaps be caused due to inherent bio-inert nature of zirconia. Hence, in this study, the immersion of test samples was done for a period of 3 weeks, which has also been considered as the minimum immersion time in previous studies.\cite{6,7,27,36} The respective mean post-immersion calcium content in SBF was derived and compared with the pre-immersion calcium value to arrive at each group’s bioactivity potential. The respective mean post-immersion calcium contents in SBF of the test groups were compared to determine if there were any significant differences in their bioactivity with respect to each other. In addition, the surface characteristics of post-immersion test samples of each group were assessed to see the impact of immersion aging and apatite formation on the zirconia surfaces.

Pre-immersion XRD revealed strong tetragonal (t) peaks of zirconia with negligible “m” phase in representative diffractograms of all test groups. The highest peak of ZrO₂ appeared at 30° (2θ value), indicating no phase transformation due to either sandblasting or UVP. These results are suggestive of maintenance of the mechanically superior “t” zirconia crystal phase following either surface treatments and
are in agreement with that observed in previous reports. The crystal phase was rechecked using XRD to determine whether immersion aging had resulted in any t–m transformation.

Surface roughness evaluation on a nanoscale by AFM of representative samples of all test groups revealed average surface roughness of 41.83 nm for Group I (untreated), 115.65 nm for Group II (sandblasted), and 102.43 nm for Group III (UVP) [Table 1]. Both types of surface treatments resulted in significantly higher surface roughness as compared to the untreated sample ($P < 0.05$). (Although the surface roughness achieved by sandblasting was slightly higher than that achieved by UVP, this difference was found to be statistically insignificant [$P > 0.05$]. These results were correlated with the respective 2D and 3D images). These findings indicate that both types of surface treatments improve the surface roughness similarly. Previous standalone studies using sandblasting or UVP have reported significant increase in surface roughness as compared to untreated surfaces, and the results obtained in this study are in line with these findings. Comparative studies on surface roughness caused by these surface treatments are lacking, and hence further correlations on this aspect of this study results cannot be drawn.

The mean contact angles of 98.26° for Group I (untreated) samples, 86.77° for Group II (sandblasted) samples, and 68.03° for Groups III (UVP) samples, respectively, were obtained in this study. On comparison, these differences were found to be highly significant ($P$ value $<0.01$) between all the three test groups. These results indicate that both types of surface treatments significantly improve the wettability as compared to that of the untreated surface, and are in accordance with those reported in previous studies. Respective EDX spectrums revealed the presence of the elements, Zr (32.66%–40.26%), O$_2$ (53.59%–61.31%), Y (5.28%–5.71%), Al (0.13%–0.38%), and Hf (0.16%–0.19%) in all test groups [Table 2], indicating that both surface treatments do not alter the elemental composition of zirconia, as compared to the untreated sample. Thus, the stabilizing elements added by the manufacturer have been retained even after the surface treatment procedures. These findings are in line with those reported in previous studies.

The mean pre-immersion Ca content of SBF was found to be 159 mg/L, and this was used as the reference value for calculating bioactivity. Previous bioactivity studies using SBF have reported a mean calcium content of 100–160 mg/L in freshly prepared SBF. The reference value obtained in this study was within the literature reported range. Group I (untreated) showed a mean post-immersion Ca content of 70.10 mg/L, Group II (sandblasted) showed a mean post-immersion Ca content of 60.80 mg/L, and Group III (UVP) showed a mean post-immersion Ca content of 56.20 mg/L at the end of 3 weeks. The difference between the mean pre- and post-immersion Ca contents in SBF, which is observed is due to the precipitation of calcium-rich apatite phase on the zirconia test surfaces. The lower the post-immersion Ca content in SBF, the higher the bioactivity for that particular test group.

On comparison, the respective mean post-immersion Ca content in SBF for all the three test groups showed statistically significant calcium depletion when compared with the pre-immersion Ca content, indicating highly significant bioactivity for untreated as well as
both the surface test groups [Table 3] (*P* value <0.01). Although the results of this study indicate significant bioactive potential for untreated zirconia by virtue of the calcium depletion observed after immersion, this bioactivity was found to be significantly lesser as compared to that of both the surface-treated groups (*P* value <0.01). On comparison between the two types of surface treatments, Group II (sandblasted) had lesser post-immersion calcium depletion in SBF than that of Group III (UVP). However, this difference was found to be statistically insignificant (*P* value >0.01), indicating similar bioactive potential for both the types of surface treatments used in this study.

Thus, despite being categorized as a bio-inert ceramic, there is a definite apatite-forming tendency on untreated zirconia at the end of a 3-week immersion period.[6,7,26] However, this apatite layer formed on the untreated sample was found to be a poorly defined, discontinuous layer of bone-like apatite in the form of scattered crystals, with the evidence of uncovered zirconia substrate at certain locations, as evidenced in the post-immersion SEM image. The post-immersion SEM images for Groups II and III also corroborate this finding of superior bioactivity, in that their apatite layer was made of denser, larger crystals with a continuous surface topography, and the zirconia substrate was not visible in any of the observed fields. Group II (sandblasted) sample showed dense, large, irregular, crystal-like calcium apatite deposits of irregular density and distribution, and Group III (UVP) showed profuse, rectangular crystal-like calcium apatite deposits with greater uniformity in size, density, and distribution. Moreover, the post-immersion EDX results revealed a higher Ca/P ratio for both the surface-treated groups as compared to the untreated group [Table 2]. Group III (UVP) showed the highest Ca/P ratio of 2.08, followed by Group II (sandblasted) and Group I (untreated) with ratios of 1.79 and 1.15, respectively. It has been reported in the literature that Ca/P ratio of 1.50 indicates apatite formation similar to trabecular bone, whereas values upward of 1.60 indicate cortical bone-like apatite formation.[25] When viewed in this perspective, the low Ca/P ratio for the untreated group is indicative of trabecular bone formation, whereas both surface-treated groups in this study had Ca/P ratios indicative of cortical bone formation, indicating their superiority as surface treatment methods. All these findings suggest that surface treatment of zirconia serves to significantly enhance its bioactive potential and also results in apatite layer of superior quality as compared to an untreated surface. These findings are echoed in the results of previous studies that have evaluated the bioactivity of zirconia following surface treatment by either sandblasting or UVP.[6,7,10,26] These results are also in agreement with literature quoting that untreated zirconia surfaces also attract the calcium present in SBF, but to a diminished extent, as compared to any type of surface treatment.[23] Surface treatments are said to promote bioactivity, as they remove impurities, reduce surface hydrocarbons, increase surface energy, thereby providing improved surface characteristics such as roughness and wettability, which are critical in promoting cell adhesion and calcium apatite formation. Previous studies evaluating the efficacy of sandblasting and UVP surface treatments have reported improved cell adhesion and osseointegration.[13] The results of superior bioactivity in SBF obtained after these two types of surface treatments in this study complement the results obtained from previous cell culture studies.[4,11,12,14,18,20]

Further, post-immersion X-ray diffractograms revealed that there was no detectable crystalline phase change from tetragonal to monoclinic (t–m transformation), following a 3-week immersion. The post-immersion EDX revealed the presence of all the surface elements that were detected in the pre-immersion EDX, and additionally calcium and phosphorus were also detected attributable to apatite formation. This prevention of t–m transformation, even after immersion, can be attributed to the maintained presence of the stabilizing elements such as yttrium and hafnium that were added by the manufacturer. Thus, even after a 3-week immersion or aging, there was no detectable low temperature degradation (LTD), which is usually the area of concern with using zirconia ceramic as an implant biomaterial. The results obtained with this study serve as an encouragement for use of zirconia as an implant biomaterial, with respect to this finding.

In this study, both sandblasting and UVP surface treatments resulted in insignificant differences with respect to their bioactivity in SBF, and hence the null hypothesis of this study is validated. Therefore, it can reasonably be assumed within the limitations of this study that either of these surface treatments can be used to significantly and probably, similarly, improve the bioactivity of zirconia. This is especially so, given that both these types of surface treatments significantly improved surface roughness and wettability, without deleteriously affecting their surface crystalline phase (“t” phase) and their elemental composition. Although in this study, superior surface wettability was observed after UVP as compared to sandblasting, the bioactivity of the UVP samples was only marginally and insignificantly higher in comparison. Wettability has been repeatedly emphasized as one of the key determinants in deciding the bioactive potential of
a material, both with respect to the apatite-forming ability as well as for improving cellular adhesion.\textsuperscript{[4,12,15,50]} Hence, the apparent lack of a significantly superior bioactive potential after UVP surface treatment, over that obtained after sandblasting surface treatment needs to be considered, especially given the fact that UVP-treated samples showed significantly superior wettability as compared to the sandblasted samples. This can be attributed to the lesser number of samples that were investigated in this study and merits further investigation. Moreover, bioactivity studies in SBF comparing these type of surface treatments are lacking in the literature, and hence further correlations with the results obtained in this study cannot be drawn to arrive at better conclusions. Though it is well accepted that chemical and topographical aspects of surface texture are important in playing a vital role in osseointegration,\textsuperscript{[18,27]} the exact effect of this aspect on bioactivity is still the object of investigation by researchers.

**CONCLUSION**

Both surface treatments, sandblasting with 50 µm alumina and UVP irradiation improved the bioactivity as compared to the untreated group, and this is supported by results of surface characteristic analyses. Both surface treatments did not alter the structure and mechanical property of zirconia.

**Clinical Implications**

Enhanced bioactivity due to surface treatments in vitro signifies enhanced osseointegration potential of surface-treated implants in vivo.

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**Conflicts of interest**

There are no conflicts of interest.

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