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"Innovative Implants: Advancements In ZnO Nanoparticle Coated Stainless Steel 316L For Orthopaedic Applications"

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Abstract:
In the field of orthopaedic care, bioimplants play a crucial role in advancing human health. The interaction between bone and implant significantly impacts the healing process, with surface characteristics influencing the behavior of mesenchymal stem cells and their integration with surrounding tissue. Recent advancements have explored the use of nanoparticle surface modifications to enhance the biocompatibility and effectiveness of orthopaedic implants. This study proposes investigating the effectiveness of coating Stainless Steel 316L orthopaedic implants with zinc oxide (ZnO) nanoparticles to improve their biocompatibility, osseointegration, and durability. The research aims to evaluate the physicochemical properties, cytocompatibility, wear rate, and in vitro performance of ZnO nanoparticle-coated Stainless Steel 316L substrates, with the intention of improving orthopaedic operation patient outcomes and implant durability. The hydrothermal method was utilized to synthesize zinc oxide nanoparticles, which were subsequently characterized for composition, crystallinity, and morphology using various analytical techniques such as X-Ray Diffraction, Fourier Transform Infrared Spectroscopy, and Scanning Electron Microscopy with Energy Dispersive X-Ray Analysis, supported by elemental mapping. Analysis revealed that the synthesized nanoparticle exhibited a spherical shape with an average diameter of approximately 18 nm. The coating of ZnO nanoparticles onto Stainless Steel 316L substrates was achieved through the utilization of the spin coating technique, as confirmed by SEM images. Cell viability assessments employing the MTT assay were carried out to assess the cytocompatibility of the synthesized ZnO nanoparticles, both when coated and uncoated onto Stainless Steel 316L substrates, against the NIH-3T3 mouse embryonic fibroblast cell line. Results indicated a significant increase of 24.48% in cell viability for ZnO nanoparticles-coated Stainless Steel 316L substrates compared to uncoated ones. Furthermore, wear and friction resistance were assessed employing ball-on-disc tribometer under various conditions, revealing a substantial improvement in the performance of ZnO nanoparticles-coated Stainless Steel 316L substrates for orthopaedic applications.

Keywords: Orthopaedic implants, nanoparticles, zinc oxide, stainless steel, cytotoxicity, biocompatibility, coated, and durability.

Introduction

Fractures and injuries to bone tissue are significant global public health concerns, leading to reduced quality of life, diminished productivity, and substantial financial strains on individuals. Medical and technological advancements have led to increased life expectancy and higher incidence of age-related conditions. Bio-implants, such as prostheses, are crucial in enhancing the quality of life by advancing therapies for tissue/organ function repair, modification, or preservation (Luo et al., 2023). Orthopedic implants are utilized in addressing various orthopedic issues, including fracture mending, bone substitution, and stabilization of deformities. Biomaterials are commonly utilized for implant manufacturing due to their unique ability to interact with bodily fluids and tissues. However, concerns persist regarding implant rejection triggered by inflammation and infection (Hamelmann and Paulusse, 2023). Orthopedic implants have been extensively researched and developed using various metallic materials, including cobalt-chromium alloys, titanium and its alloys, and stainless steel (Dousari et al., 2023). Biocompatibility, mechanical properties, surface characteristics, and chemical properties are key considerations in implant design. Stainless steel 316L is frequently employed in orthopedic applications due to its cost-effectiveness, excellent mechanical strength, relative flexibility, and ease of fabrication (Dousari et al., 2023). Nonetheless, stainless steel implants are prone to failure due to their low fatigue strength, high elastic modulus, poor wear resistance, and inability to establish direct chemical bonds with native bone tissue. Prolonged use of stainless steel implants can lead to chemical and biological reactions that result in the release of metal ions, corrosion, and inflammation around the implant site. Employing bioactive nanoparticles to modify the surface of stainless steel bio-implants presents a promising solution to these issues (Tamayo et al., 2021). Functionalized nano coatings on implant surfaces can enhance osseointegration, durability, wear resistance, biocompatibility, and cytotoxicity. Nanostructured materials exhibit superior osteoblast adhesion, proliferation, and biocompatibility compared to bulk materials, making them appealing for enhancing implant surface performance in clinical orthopaedic applications (Li et al., 2021). Among various nanomaterials, zinc oxide nanoparticles possess advantageous qualities for coating implants, including a high strength-to-weight ratio, potent antibacterial activity, enhanced biocompatibility, and promotion of osteoblast cell adhesion and proliferation, facilitating implant integration (Zare et al., 2020). Our study introduces a novel approach to coating Stainless Steel 316 substrates with zinc oxide nanoparticles, addressing a gap in existing literature on this subject. In our investigation, we utilized the spin coating method to apply a layer of zinc oxide nanoparticles onto Stainless Steel 316L substrates. The characteristics of the coating, including its mechanical strength, biocompatibility, and antibacterial properties, were thoroughly examined and evaluated. To ensure the safety of the nanocomposite-coated Stainless Steel 316L substrate for orthopedic applications, its cytotoxicity was assessed using the MTT assay. Wear resistance was tested at varying speeds using a ball-on-disc tribometer, while corrosion resistance was analyzed using the linear polarization resistance technique to determine durability.

Materials and Methods

Materials

The ZnO powder and KOH solvent were purchased from Sigma Aldrich. These chemicals were used in their original form without further purification.

2.1.2. Synthesis of ZnO Nanoparticles

Dissolve ZnO powder in 100 ml of distilled water to form a zinc precursor solution. Then, dissolve potassium hydroxide (KOH) in another 100 ml of distilled water to prepare a reducing agent solution. Combine the two solutions, maintaining continuous spinning at 400 rpm until complete dissolution

is achieved. To facilitate the generation and substantial growth of ZnO nanoparticles, maintain the reaction mixture in a beaker at 50°C while stirring continuously at 1200 rpm for a duration of 15 hours. White precipitates will form during the reaction process. Allow the beaker to cool naturally to room temperature following the designated reaction period. Subsequently, separate the nanoparticles by filtering and decanting the supernatant solution. Thoroughly cleanse the nanoparticles with a solution comprising ethanol and distilled water, repeating the washing steps to ensure nanoparticle purity. Finally, the obtained nanoparticles were dried at 80°C in oven to obtain a dry powder followed by calcination at 600°C to obtained the desired ZnO nanoparticles (Sumaya et al., 2023).

2.1.3 Preparation of Stainless Steel 316L Substrate

The Stainless Steel 316L substrate was procured Jindal Stainless Hisar Ltd. and served as the base material onto which ZnO nanoparticles were coated. These Stainless Steel 316L substrates were precisely cut into square sheets measuring 20x20x1 mm^3. Surface imperfections were removed by polishing the substrate sheets with aluminum slurry, followed by cleaning with acetone to eliminate any remaining surface contaminants. Subsequently, the samples were washed with deionized water to eliminate any residual before air-drying at 85°C for 10 hours.

2.1.4 Methods for Analyzing Physiological and Morphological Characteristics

The evaluation of synthesized ZnO nanoparticles and their application as coatings on Stainless Steel 316L substrate involves a thorough examination of their physicochemical characteristics. This includes analyzing surface morphology composition using scanning electron microscopy (SEM FESEM: JEOL, JSM 6100, Japan) and chemical Composition and information regarding functional groups were obtained using Fourier transform infrared spectroscopy (FTIR: Perkin Elmer Frontier spectrophotometer, USA). Furthermore, X-ray diffraction (XRD: Rigaku Ultima IV system, USA, with a Cu source at 1.54056 Å and cross-beam optics technology) was employed to determine the crystalline structure of the coated nanoparticles. The wear rate and friction coefficient of the samples were evaluated using a pin-on-disc universal tribometer (DUCOM TR-20 LE).

2.1.5 Synthesis of ZnO Nanoparticles via spin coating

The spin coating method was utilized to achieve a uniform coating of ZnO nanoparticles over the surface of Stainless Steel 316L substrate. Initially, a suspension containing 10 milligrams of nanoparticles dispersed in 100 ml of distilled water was prepared, followed by 24 hours of magnetic stirring to ensure thorough dispersion. Subsequently, the suspension underwent one hour of sonication at room temperature to prevent nanoparticle aggregation. To enhance adhesion and ensure uniform coating application, the Stainless Steel 316L substrate underwent chemical etching before coating, employing a solution composed of 70% nitric acid and 30% hydrogen peroxide. After etching, the substrate was rinsed with distilled water to eliminate any residual etchant and subsequently dried completely. The spin coating process comprised three primary stages: suspension application, spin coating, and drying. The process began by injecting a liquid solution containing nanoparticles into the center of a flat Stainless Steel 316L substrate, which was secured onto a spindle, using a syringe. After that the Stainless Steel 316L substrate was centrifugally spun at 350 rpm to ensure uniform spreading of the liquid across the surface. Any excess material was expelled from the rotating substrate's edge, ensuring an even coating. Finally, the substrate was allowed to air dry at a temperature of 150°C for 2 hours to remove any solvent residue from its surface (Hatamvand et al., 2017).

2.1.6 Cell Viability Testing

In vitro, an MTT assay employing NIH-3T3 mouse embryonic fibroblast cells was conducted to assess the cytotoxicity of ZnO nanoparticles, as well as coated and untreated Stainless Steel 316L substrates. The cells were cultured in DMEM supplemented with 10% FBS and 100 U/ml of antibiotic solution, and were maintained at 37°C with 5% CO2. Cells were seeded into P24 microtiter plates at a density of 1×10^6 cells per well, and exposed to varying concentrations of ZnO nanoparticles (100-200 µg/ml) for 48 hours. Following incubation, MTT solution (0.5 mg/ml) was added to each well, and the cells were further incubated for 4 hours. Subsequently, the resulting formazan crystals were dissolved in DMSO, and absorbance was measured at 570 nm using a microplate reader. Positive control (untreated cells) and negative control (wells without cells) were included. The same protocol was followed to evaluate cell viability on uncoated and ZnO nanoparticles-coated Stainless Steel 316L substrates (Natarajan et al., 2019). Relative cell viability was determined using the provided formula.

Cell viability (%) = (OD Sample /OD Control) × 100% **(1)**

2.1.7 Wear rate

Using a pin-on-disc tribometer configuration, the wear properties of both uncoated and ZnO nanoparticle-coated Stainless Steel 316L substrates were investigated. The coated and uncoated Stainless Steel 316L substrates were affixed to distinct cylindrical Stainless Steel components using metal-to-metal adhesive. The experiments adhered to ASTM G99 standards, applying a constant normal load of 30 N for three minutes at ambient temperature, while varying the rotational speeds from 200 to 1000 rpm. Prior to each trial, the surfaces of the pin and disc were meticulously cleaned with ethanol to remove any debris or contaminants. Subsequently, the pin was securely inserted into the pin holder, ensuring perpendicular to the rotating disc, and the appropriate load was applied. By setting the load cell and linear variable differential transformer (LVDT) values to zero, the controller was calibrated. Using an LVDT and a load cell sensor, respectively, the wear rate and frictional force on the pin were continually recorded and monitored during the trials (Kalyani et al., 2016).

Result and Discussion

XRD analysis

XRD spectroscopy was employed to analyze the synthesized ZnO nanoparticles. Figure 1 presents the findings from the XRD analysis, indicating the presence of diffraction peaks at specific angles: 32.81°, 35.23°, 41.80°, 51.20°, 58.57°, 63.02°, and 70.24°. These peaks correspond to crystallographic planes 121, 200, 112, 231, 103, 123, and 160, respectively, indicating the presence of a cubic crystalline structure. The consistent with DB Card Number 1011223. Scherrer's formula was used to calculate the nanoparticles' average crystallographic size.

$$D = \beta\cos(\theta)/K\lambda \quad (2)$$

In this equation, D denotes the average crystallite size, K represents the Scherrer constant (typically around 0.9), λ denotes the wavelength of X-ray light (measured in nm), β indicates the full width at half maximum (FWHM) of the diffraction peak (measured in radians), and θ signifies the Bragg angle. The average crystalline size of the synthesized ZnO nanoparticles was determined to be 18 nm (Khiari et al., 2021), (Khiari et al., 2021).

----------**Figure 1**----------

3.1.2. FTIR analysis

The FTIR spectrum of the synthesized ZnO exhibited a transmittance pattern ranging from 4000 cm−1 to 500 cm−1. Through FTIR spectroscopic analysis, the ZnO nanoparticles were examined to

identify potential organic functional groups present in the sample. The depicted peaks (as shown in Figure 2) were identified at distinct frequencies: 3502.05 cm−1, 1632.35 cm−1, 1389.76 cm−1, 1116.05 cm−1, 803.23 cm−1, and 622.09 cm−1, representing OH group, C–H stretching and bending, C=O stretching, C–O vibration, C–N stretching group, respectively. Furthermore, the absorption peak at 599 cm−1 corresponds to the Zn–O stretching modes (Maheswaran et al., 2023).

----------**Figure 2**----------

3.1.3. SEM analysis

Surface morphology analysis of the synthesized ZnO nanoparticles was conducted using SEM techniques. SEM micrographs, as illustrated in **Figure 3(a),** indicated the synthesized ZnO nanoparticles exhibited a spherical morphology with an average diameter of 18nm. SEM images of the uncoated Stainless Steel 316L substrate are illustrated in Figure 3(b), while Figure 3(c) displays micrographs depicting the surface of the ZnO nanoparticle coating on the Stainless Steel 316L substrate, demonstrating the uniformity of the coating across the substrate (Khiari et al., 2021).

----------**Figure 3**----------

3.1.4. EDX analysis

The elemental composition of synthesised ZnO nanoparticles was determined using EDX analysis technique. EDX results validate the presence and quantify the amounts of carbon, oxygen, zinc, and gold elements within the ZnO nanoparticles coating on the Stainless Steel 316L substrate. This data is crucial for evaluating the quality and composition of the coated material. The EDX spectrum in **(Figure 4**) confirms the presence of ZnO nanoparticles (a), uncoated Stainless Steel 316L substrate (b), and ZnO nanoparticles coated Stainless Steel 316L substrate. ZnO nanoparticles exhibit the presence of zinc (48.2%), carbon (14.4%), oxygen (31.7%), and gold (5.6%) elements. The uncoated Stainless Steel 316L substrate shows the presence of carbon (5.8%), iron (56.0%), oxygen (1.8%), nickel (7.8%), chromium (13.0%), and gold (15.7%) elements. ZnO nanoparticles coated Stainless Steel 316L substrate exhibits the presence of iron (14.5%), carbon (4.3%), oxygen (4.4%), chromium (2.9%), nickel (2.0%), zinc (66.0%), and gold (5.9%) elements. The spectrum presents precise weight percentage data for all elements, confirming the successful coating of ZnO nanoparticles onto the substrate (Maheswaran et al., 2023).

----------**Figure 4**----------

3.1.5. Cytotoxicity

The MTT assay was employed to evaluate cell viability on coated and uncoated Stainless Steel 316L substrate. Figure (5) illustrates the cell viability result representing control cells, ZnO nanoparticles, uncoated Stainless Steel 316L substrate, and ZnO nanoparticles coated Stainless Steel 316L substrate. Cell viability relative to the control sample was assessed for each substrate (Kalyani et al., 2016). Results revealed that the relative viability of cells on the uncoated Stainless Steel 316L substrate was 54.13%, whereas for substrates coated with ZnO nanoparticles, it was 78.61%, and ZnO nanoparticles 81.42%. Significantly, there was a notable rise (24.48%) in the relative cell viability of the Stainless Steel 316L substrate coated with ZnO nanoparticles in comparison to the uncoated substrate. These findings indicate that the ZnO nanoparticles coating demonstrated superior cell viability compared to the uncoated substrate. The observed enhancement in cell viability on Stainless Steel 316L substrates coated with ZnO nanoparticles suggests the absence of toxic degradation products, highlighting their potential as coating materials for orthopaedic applications (Khiari et al., 2021).

----------Figure 5----------

3.1.6. Wear testing

Wear testing involves assessing the durability and resilience of materials when subjected to mechanical stress and frictional forces. The wear rates of both the ZnO nanoparticle-coated and untreated Stainless Steel 316L surfaces were assessed through a ball-on-disc tribometer examination (Redlich and Tenne, 2019). Figure (6) presents the wear rates of the uncoated Stainless Steel 316L substrate and the ZnO nanoparticles-coated Stainless Steel 316L substrate at speeds of 200 rpm, 400 rpm, 600 rpm, 800 rpm, and 1000 rpm under a 30 N load condition for a duration of 3 minutes. The wear rates of the uncoated Stainless Steel 316L substrate were determined to be 257.36 µm, 293.07 µm, 342.28 µm, 398.21 µm, and 436.74 µm, respectively, while the ZnO nanoparticles-coated SS 316L substrate exhibited wear rates of 109.83 µm, 132.41 µm, 219.16 µm, 268.81 µm, and 317.62 µm (Kalyani et al., 2016). These findings indicate that the wear rate of the coated substrate is lower compared to the uncoated substrate across various speeds under the same load conditions and duration, thus ensuring enhanced overall durability for orthopaedic applications.

----------Figure 6----------

Conclusion

In this research, the potential application of ZnO nanoparticles coating on Stainless Steel 316L substrates to address challenges related to traditional stainless steel orthopedic implant materials was investigated. Various characterization techniques, including X-ray diffraction, Fourier transform infrared spectroscopy, and scanning electron microscopy with energy dispersive X-ray analysis, were utilized to examine the composition, crystallinity, and morphology of the synthesized ZnO nanoparticles and their coating on Stainless Steel 316L substrate. The results revealed that the synthesized ZnO nanoparticles possess a spherical shape with an average size of approximately 18 nm. Through the implementation of the spin coating technique, a uniform distribution of the ZnO nanoparticles coating on the Stainless Steel 316L substrate was achieved, as confirmed by SEM images. Furthermore, cytotoxicity against NIH-3T3 mouse embryonic fibroblast cell line was assessed, and wear and friction resistance of the ZnO nanoparticles coated substrate were evaluated using a ball-on-disc tribometer. The collective findings indicate a significant improvement in the performance of the ZnO nanoparticles coated substrate compared to the uncoated one, particularly in terms of cytotoxicity, wear rate, and friction resistance. Overall, these results suggest that ZnO nanoparticles coating on Stainless Steel 316L substrates has potential for enhancing the suitability of ZnO nanoparticles coating for orthopedic applications, addressing crucial concerns such as biocompatibility, cell viability, tissue integration, and implant durability.

Acknowledgement

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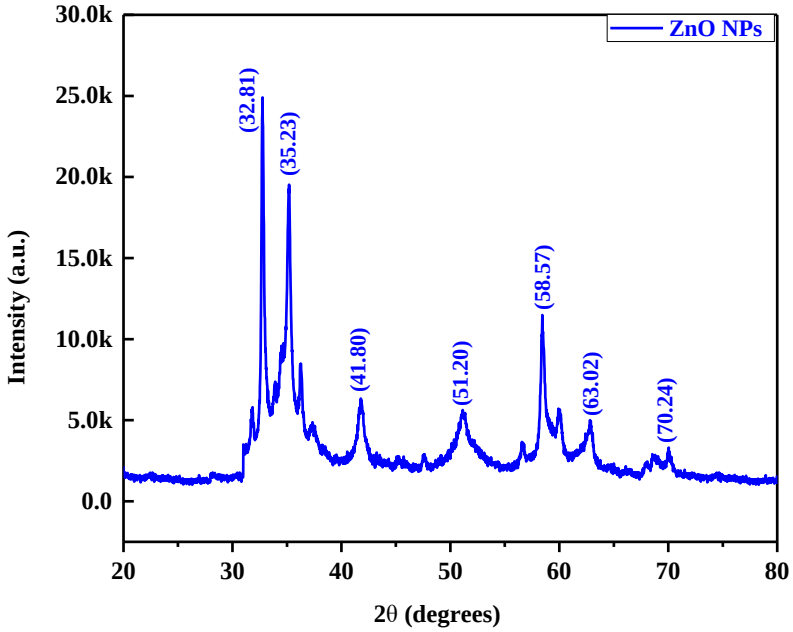

Figure 1: XRD analysis of ZnO NPs

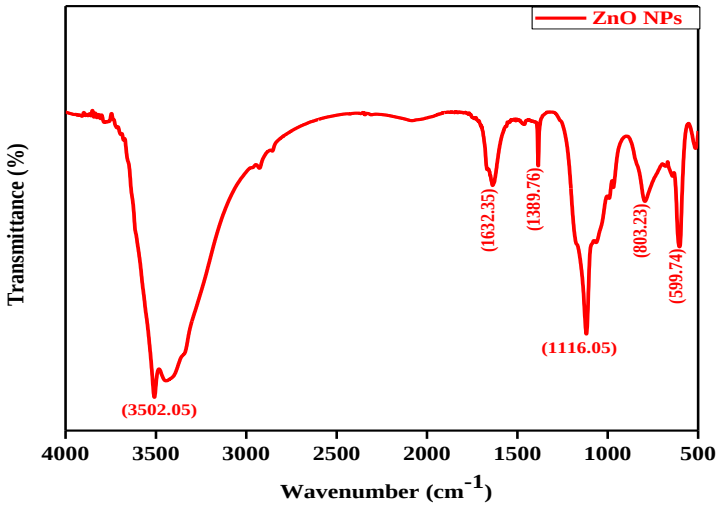

Figure 2: FTIR spectra of ZnO NPs.

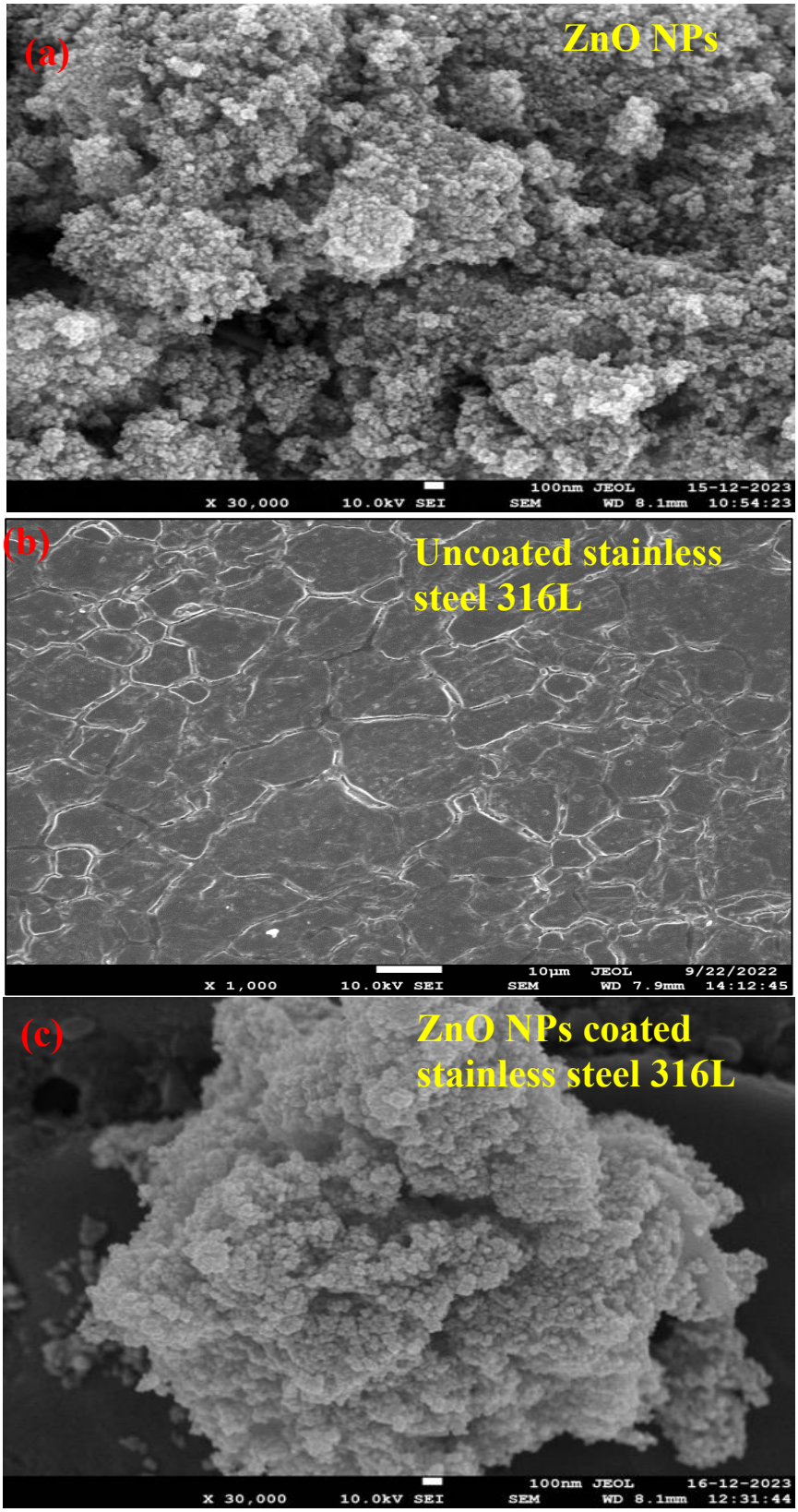

Figure 3: SEM images of ZnO nanoparticles (a), Uncoated stainless steel 316L substrate (b), and ZnO nanoparticles coating on stainless steel 316L substrate (c).

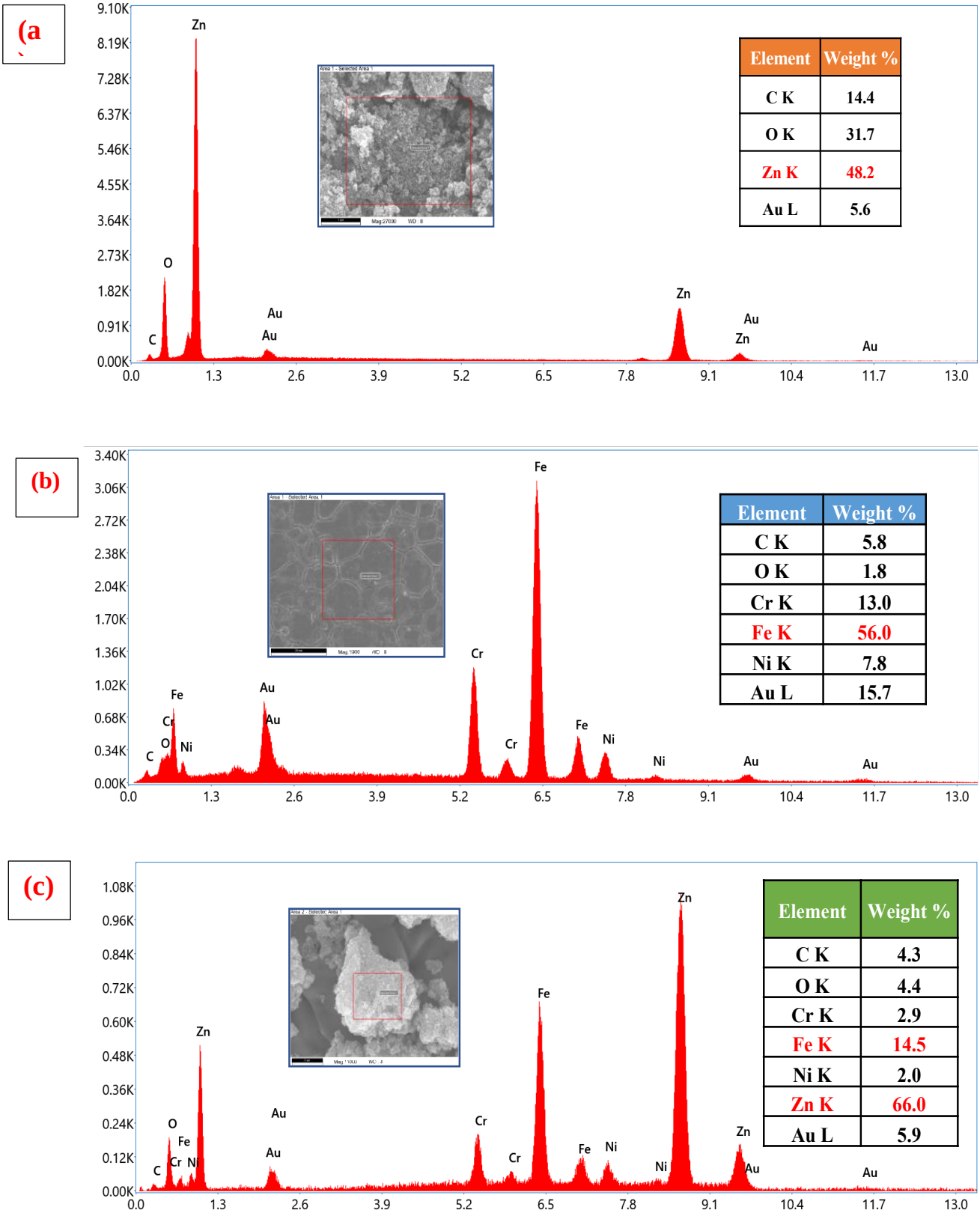

Figure 4: EDX spectra of ZnO nanoparticles (a), uncoated stainless steel 316L substrate (b), and ZnO nanoparticles coating on stainless steel 316L substrate (c).

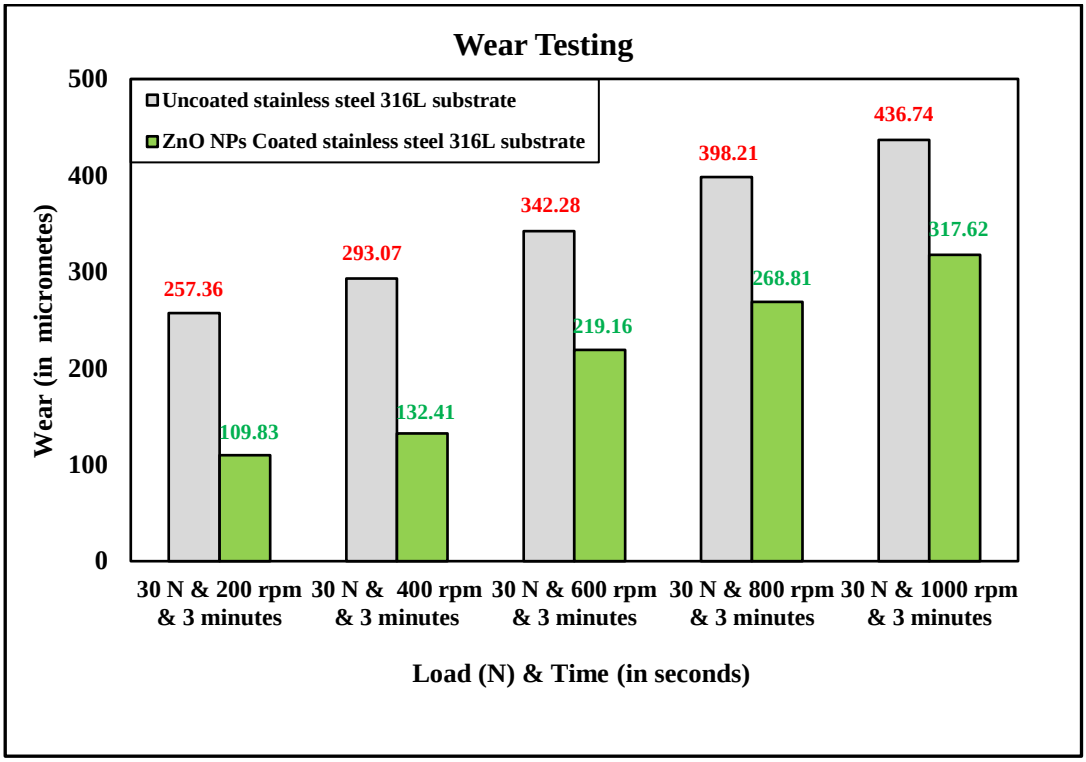

Figure 5: Wear testing for uncoated stainless steel 316L samples, and ZnO nanoparticles coated stainless steel 316L substrate different speed 200, 400, 600, 800 rpm, and 1000 rpm, 30 N and 3 minutes.

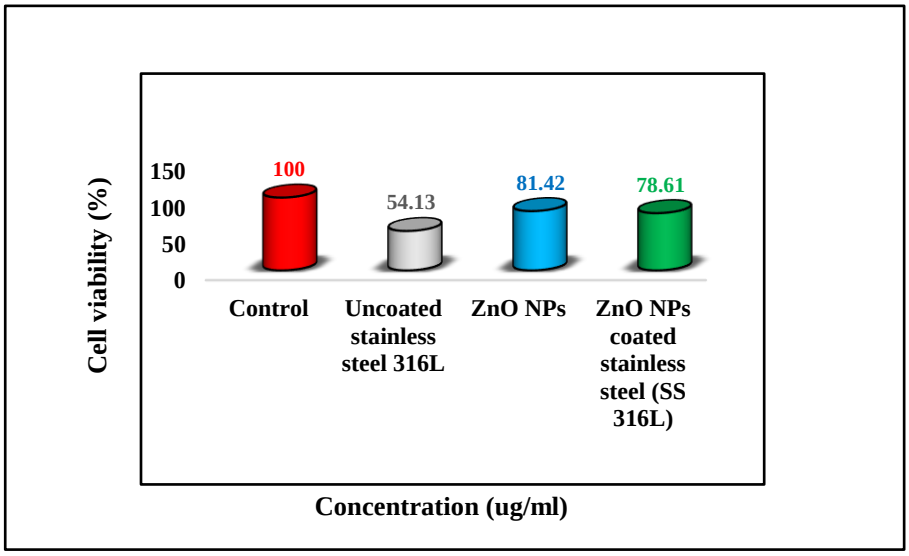

Figure 6: Cell viability for uncoated stainless steel 316L samples, ZnO nanoparticles, ZnO nanoparticles coated stainless steel 316L substrate.