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Preparation, Characterization, and Potential Biomedical Applications of Nanostructured Zirconia Coatings and Films
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7.1
Introduction

Zirconia (ZrO₂) has excellent mechanical strength, thermal stability, chemical inertness, lack of toxicity, and an affinity for groups containing oxygen. These and other favorable attributes make ZrO₂ ceramics, coatings and films potentially useful as biocoatings, femoral heads, orthopedic implants, and biosensors. Following the first report of the biomedical application of zirconia in 1969, its use in the ball heads of total hip replacements (THRs) was introduced in 1988 [1]. In fact, the THR femoral head remains one of zirconia's main uses, with more than 600,000 units having been implanted worldwide, mainly in the US and Europe, up until 2005 [2].

Zirconia coatings and films may also be deposited onto other materials in order to improve their surface properties, such as wear resistance, corrosion resistance, thermal barrier capability, and biocompatibility. Several techniques, including sol–gel processing, plasma spraying, anodic oxidation, magnetron sputtering, electrochemical deposition, and plasma deposition, have been used to prepare ZrO₂ coatings and films. In this chapter, we review the surface morphology, microstructure, crystallite size, phase composition and biomedical characterization of nanostructured ZrO₂ coatings and films prepared using different techniques.

7.2
Preparation and Characterization of Nano-ZrO₂ Films

7.2.1
Cathodic Arc Plasma Deposition

Among others, research groups at the City University of Hong Kong and Shanghai Institute of Ceramics, Chinese Academy of Sciences (SICCAS) have fabricated ZrO₂ thin films with nanosized surfaces on Si (100) wafers using a
Figure 7.1 Schematic diagram of the synthesis of ZrO\(_2\) films using a filtered cathodic arc system [4].

The experimental apparatus includes a magnetic duct and cathodic arc plasma source, with the zirconium discharge being controlled by the main arc current between the cathode and anode. Oxygen gas is bled into the arc region, and the mixed zirconium and oxygen plasma is guided into the vacuum chamber by an electromagnetic field applied to the curved duct. The duct is biased to \(-20\) V in order to exert a lateral electric field, while the external solenoid coils wrapped around the duct produce the axial magnetic field with a magnitude of 100 G. Before deposition, the samples that are typically positioned about 15 cm away from the exit of the plasma stream are sputter-cleaned with an argon plasma for 2 min, using a sample bias of \(-500\) V. The base pressure in the vacuum chamber is approximately \(1 \times 10^{-7}\) Torr, and a radiofrequency (RF) power of 100 W is applied for a deposition time of 120 min. The as-deposited ZrO\(_2\) thin films are subsequently heat-treated at either 800°C or 1000°C for 2 h.

The X-ray diffraction (XRD) results in Figure 7.2 show that the resultant thin film is crystallized, as indicated by the diffraction peaks at 29.83° and 34.85° which can be attributed respectively to the (101) and (002) planes of the tetragonal ZrO\(_2\) phase. The small peak marked by the arrow in Figure 7.2 may stem from the (111) plane of the monoclinic phase. The surface views of the as-deposited and thermally treated ZrO\(_2\) thin films observed using scanning electron microscopy (SEM) and atomic force microscopy (AFM) are shown in Figures 7.3 and 7.4. The surface of the as-deposited ZrO\(_2\) thin film was very smooth, such that the surface features could not easily be distinguished using SEM (Figure 7.3a). However, some very small particles were observed on the surface of the ZrO\(_2\) thin film in the AFM image (Figure 7.3b). Following heat treatment at either 800°C or 1000°C for 2 h, nanosized particles could be observed on the surface of the ZrO\(_2\) thin film (Figure 7.4). The sizes of the particles on the ZrO\(_2\) thin
Figure 7.2: Thin film X-ray diffraction pattern acquired from the ZrO$_2$ thin film deposited on silicon wafer [5].

Figure 7.3: (a) Scanning electron microscopy and (b) atomic force microscopy images of the as-deposited ZrO$_2$ thin film [4].

Figure 7.4: Surface views of the ZrO$_2$ thin films heat-treated at 800°C and 1000°C for 2h [4].
film heat-treated at 800°C or 1000°C were approximately 20 nm and 40 nm, respectively, which indicated that the particle size could be regulated by optimizing the post-treatment processes.

7.2.2 Plasma Spraying

Plasma spraying has been used extensively to prepare ceramic coatings since its development by Union Carbide during the mid-1950s. In plasma spraying, an electrical arc is used to melt and spray the materials onto the surface of a substrate (see Figure 7.5), with the parameters of the density, temperature and velocity of the plasma beam each being important when forming the coatings. Although the temperature in the core region of the plasma beam is relatively constant at approximately 12000 K, it increases dramatically towards the nozzle, to a point where almost any material can be melted in the plasma jet.

Nanostructured zirconia coatings have been prepared by the research group at SICCAS since 2002, using plasma spraying [7-11]. The nanostructured zirconia coating fabricated by Zeng et al. possesses two types of structure: (i) a poorly consolidated structure composed of nanosized particles; and (ii) an overlapping structure consisting of micrometer-sized particles [7]. The former structure constitutes the main component of the coating. Whilst the zirconia coating has the same phase composition as the starting powder, its thickness appears to be inconsistent, as shown in the cross-sectional view in Figure 7.6. Subsequently, others have investigated further the preparation and properties of plasma-sprayed nanostructured zirconia coatings, with the result that zirconia coatings with nanostructured surfaces and uniform thicknesses have been successfully fabricated by optimizing the spraying parameters (Figure 7.7).

![Figure 7.5 Schematic diagram of plasma spray torch [6].](image-url)
7.2 Preparation and Characterization of Nano-ZrO$_2$ Films

Figure 7.6 Cross-sectional views of the nanostructured zirconia coating fabricated by Zeng et al. [7].

Figure 7.7 (a) Cross-sectional and (b) Surface views of the nanostructural zirconia coating fabricated by Wang et al. [11]

7.2.3 Sol–Gel Methods

The sol–gel process has been used extensively to deposit thin films (<10 μm). Compared to other, conventional, thin-film processes the sol–gel system allows for a better control of the chemical composition and microstructure of the film, for the preparation of homogeneous films, for a reduction of the densification temperature, and finally the use of simpler equipment and a lower cost. The particular advantages of the sol–gel approach include an easy purification of the precursors (by distillation or crystallization), and the ability to introduce traces of other elements into the film. In addition, the precursors can be mixed at the molecular level in the solution, such that a high degree of homogeneity can be
attained in the films. As a consequence, the sol–gel process permits a lower temperature to be used during the sintering stage. The resultant microstructure depends not only on the treatment of the precursors, but also on the relative rates of condensation and evaporation during film deposition.

In the sol–gel process, the thin films are normally produced using spin- or dip-coating techniques. The spin-coating process consists of four stages: deposition; spin-up; spin-off; and evaporation. However, for complex-shaped substrates, the most frequently used sol–gel technique is dip-coating, where the sample is dipped into a solution containing the precursors and then withdrawn at a constant speed, usually with the aid of a motor. The deposition of a solid film results from gravitational draining and solvent evaporation, accompanied by further condensation reactions.

Chang et al. [12] have proposed the use of a spin-coating sol–gel method to fabricate highly porous or smooth ultra-thin ZrO$_2$ films. The morphology and thickness of these thin films can easily be controlled by adjusting the concentrations of ZrCl$_4$ in the sol solutions. A porous film with a large specific surface area ($180 \text{m}^2 \text{g}^{-1}$) can be obtained using 150 g ZrCl$_4 \cdot l^{-1}$ without the addition of templates. The ZrO$_2$ film prepared under these conditions is composed of nanofibers with a typical width of approximately 100 nm. The entangled and branched nanofibers that constitute the thin film appear porous (Figure 7.8). A type II isotherm is clearly found in the N$_2$ adsorption curve, indicating the macroporous characteristics of the thin film. When the concentration of ZrCl$_4$ is reduced to 17 g l$^{-1}$, the porous surface turns into a smooth thin film. A linear relationship between film thickness and the concentration of ZrCl$_4$, ranging from 17 to 3 g l$^{-1}$, has been observed; for example, an ultrathin film with a thickness of 1.8 nm can be fabricated when a ZrCl$_4$ concentration of 3 g l$^{-1}$ is used.

The texture of sol–gel-derived thin films is mainly controlled by the rates of hydrolysis, condensation, and phase separation. In theory, a smooth morphology
ZrOCl₂·8H₂O + Y(NO₃)₃·6H₂O
or Ce(NO₃)₃·6H₂O or MgCl₂·6H₂O
Anhydrous ethanol
Stirred at 50-70 °C for 1 h
Aged at 30 °C for 72 h, coating sol
Multiple coating
Dip-coating
Calcined at 400 °C for 5 min
Annealed at 600 °C, doped ZrO₂ films

Figure 7.9 Flow diagram demonstrating the preparation of doped zirconia films [14].

should be formed when hydrolysis and condensation are conducted separately at a slow reaction rate. With decreasing ZrCl₂ concentrations, the size of the ZrO₂ fibers increases, however, and the thin films have a smoother morphology due to the slower hydrolytic rate.

Lucca et al. [13] prepared multilayered ZrO₂ thin films by dip-coating and subsequent heat treatment at temperatures ranging from 400 to 700°C, and then investigated the near-surface mechanical responses of the films to nanoindentation. The elastic modulus and hardness are measured at depths which correspond to 7-10% of the film thickness. The temperature of the heat treatment is found to have a significant effect on the resultant near-surface hardness and elastic modulus. Liu et al. [14] have prepared highly oriented CeO₂, Y₂O₃, and MgO-doped ZrO₂ thin films using a sol-gel process by dip-coating in an ethanol solution consisting of zirconium oxychloride octahydrate and inorganic dopants, as shown in the flow diagram in Figure 7.9. The doped ZrO₂ films contained only the zirconia tetragonal phase and exhibited nanoscale morphology (Figure 7.10).

7.2.4
Electrochemical Deposition

Electrochemical deposition is used widely to deposit a thin metallic coating onto the surface of another metal, by the simple electrolysis of an aqueous solution containing the desired metal ion or its complex (Figure 7.11). Electrochemical deposits possess a fine structure and valuable physical properties, such as a high level of hardness and a high reflectivity [15].

In the electrochemical deposition process, reduction takes place when a current is supplied externally, and when the sites for the anodic and cathodic reactions are separate. The reduction mechanism pertaining to the electrochemical deposition
Figure 7.10 Atomic force microscopy images of doped ZrO$_2$ thin films. (a) 13CSZ; (b) 8YSZ; (c) 8MSZ [14].

Figure 7.11 Schematic diagram of the cell used for electrochemical deposition [15].
7.2 Preparation and Characterization of Nano-ZrO₂ Films

Figure 7.12 Reduction mechanism of solvated metal ion in electrochemical deposition [15].

of a simple solvated metal salt (see Figure 7.12) can also be extended to other ligand-coordinated metal systems. The solvated metal ion present in the electrolyte arrives at the cathode under the influence of the imposed electrical field, as well as by diffusion and convection (Figure 7.12a), and enters the diffusion layer. Although the field strength in the diffusion layer is not sufficiently strong to liberate the free metal ion from the solvated state, the solvated water molecules are aligned by the field (Figure 7.12b); the metal ion then passes through the diffused part of the double layer. As the field strength of the double layer is high (on the order of 10⁶ V/cm), the solvated water molecules are removed so as to leave the free metal ion (Figure 7.12c), which is then reduced and deposited at the cathode via an ad-atom mechanism [15].

Electrochemical deposition has been used widely to prepare zirconia and composite films. For example, Stefanov et al. [16] investigated the electrochemical deposition of zirconia film on stainless steel, where the films were obtained in an electrolyte of anhydrous ethyl alcohol. With the rising cathodic voltage, the structure and morphology of the zirconia films exhibited essential changes; those films with the most porous structure were formed at 21 V, and those with the highest density at 25 V. The composition in the bulk of the films was close to the stoichiometric value. Shacham et al. [17] have reported a novel electrochemical method for the deposition of ZrO₂ thin films, where films with thicknesses ranging from 50 to 600 nm were obtained by applying moderate positive or negative potentials (from +2.5 V to -1.5 V versus SHE) on the conducting surfaces, which were immersed in a solution of zirconium tetra-n-propoxide [Zr(OPr)₄] in iso-propanol, in the presence of minute quantities of water (the water/monomer molar ratio ranged from 10⁻³ to 10⁻⁴). The magnitude of the applied potential and its duration were thought to provide a convenient means of controlling the film thickness. Thin films of zirconia and an organoceramic composite which consisted of zirconium hydroxide and poly(diallyldimethylammonium chloride) (PDDA) have been produced using cathodic electrodeposition [18], with films of up to 10 μm thickness obtained on Ni and porous Ni-yttria-stabilized zirconia (YSZ) cermet substrates. The amount of material deposited, and its composition, can be controlled by varying the current density and the concentrations of the PDDA and zirconium salt.
7.2.5
Anodic Oxidation and Micro-Arc Oxidation

Anodic oxidation encompasses electrode reactions in combination with electrical field-driven metal and oxygen ion diffusion, leading to the formation of an oxide film at the anode surface. Anodic oxidation is a well-established method for the production of different types of protective oxide films on metals, and different dilute acids (e.g., H₂SO₄, H₃PO₄, acetic acid) can be used as the electrolyte in the process. The structural and chemical properties of the anodic oxides can be varied over a quite wide range by altering the process parameters, such as the anode potential, electrolyte composition, temperature, and current. The anodizing apparatus is shown schematically in Figure 7.13.

Micro-arc oxidation (MAO), which is also referred to as "anodic spark oxidation" or "plasma electrolytic oxidation," is a novel anodic oxidation technique that is used to deposit ceramic coatings on the surfaces of valve metals such as Al, Ti, Mg, Ta, W, Zn, and Zr, as well as their alloys. In MAO processes, the anode which is composed of the valve metal is immersed in an aqueous solution, and an asymmetric alternating voltage applied between the anode and cathode. In the anodic half-circle, the voltage is usually in the range of 150 to 1000 V, while in the cathodic half-circle it is in the range of 0 to 100 V. MAO processes are typically characterized by the phenomenon of electrical discharge on the anode in aqueous solution, while temperatures of up to 10000 K and local pressures of several hundred bar in the discharge channels have been reported. The quality of the MAO coating is determined by parameters such as the composition and temperature of the electrolyte, the alloy composition, the voltage, current density, and time; high-quality coatings can be created by employing properly selected deposition parameters.

Zhao et al. [26] have fabricated zirconia nanotube arrays with a diameter of approximately 130 nm, lengths of up to 190 μm, and aspect ratios of more than 1400, by anodizing the zirconium foil in a mixture of formamide and glycerol (volume ratio = 1:1) containing 1 wt% NH₄F and 3 wt% H₂O (Figure 7.14).

![Figure 7.13 Schematic diagram of the anodizing apparatus](image)
as-prepared nanotube arrays contained amorphous zirconia. Both, monoclinic and tetragonal zirconia coexisted when annealed at 400°C and 600°C, whereas monoclinic zirconia was obtained at 800°C; the ZrO$_2$ nanotubes retained their shape after heating to up to 800°C. The lower dissolution rate of zirconia in organic electrolytes was considered to be the main reason for fabricating zirconia nanotube arrays with high aspect ratios.

Tsuchiya et al. [21] have reported the formation of self-organized porous layer of ZrO$_2$ by the anodization of zirconium in H$_2$SO$_4$ electrolytes containing low concentrations of NH$_4$F. Under optimized electrolyte conditions, and with polarization in the range of several tens of volts, an orderly sponge-like porous ZrO$_2$ was obtained, the layer thickness of which was on the order of several tens of micrometers, while the pore diameter typically varied from 10 to 100 nm. The pore size was almost independent of the applied potential, whereas the amount of well-structured area on the sample surface increased with higher applied potentials.

Yan et al. [22] have reported that zirconia films can also be prepared by the MAO of zirconium in an aqueous solution, using a pulse power supply. The groups
results indicated that the MAO-formed films were nanostructured and porous (Figure 7.15), and were composed of zirconia partially stabilized with CaO, together with a small amount of monoclinic ZrO₂.

7.2.6 Magnetron Sputtering

Magnetron sputtering represents one of the most common methods used for depositing thin films, its popularity stemming from the simplicity of the physical processes involved, combined with versatility and flexibility. Magnetron sputtering sources can be classified as diode devices in which the magnetic fields are used in concert with the cathode surface to form electron traps. The charged particles are confined by a closed magnetic field, such that the high-density plasma is produced in the vicinity of the cathode. An enhanced plasma ionization can then be achieved via either additional gas ionization or plasma confinement. This sputtering system can deliver large ion currents to the substrates, and may be operated over a wide range of pressures. When using this process, the coatings can be produced on substrates that may be very large, and also of complex shape.

Huy et al. [23] have deposited zirconia thin films by using RF magnetron sputtering on zircaloy-4 (Zr-4) substrates directly from the ZrO₂ target. These thin films are deposited at different substrate temperatures (from 40 to 800°C) and
over different times (from 10 to 240 min). By comparing the Raman studies on zirconia thin films and on bulk zirconia, it can be concluded that:

- ZrO₂ is not completely dissociated during the deposition process.
- The zirconia films deposited on different substrates are polycrystalline.
- The structure and phase composition of the films depend on the substrate temperature and on the deposition time, and therefore vary as a function of the distance from the film surface.

7.3 Bioactivity of Nano-ZrO₂ Coatings and Films

Zirconia is generally considered to be a bioinert ceramic because, when implanted, it shows morphological fixation only to the surrounding tissues, without producing any chemical or biological bonding. However, in the past few years many attempts have been made to prepare bioactive ZrO₂ coatings/films, or to improve their bioactivity by means of post-treatment.

The ability to induce apatite formation on a nanocomposite of cerium-stabilized tetragonal zirconia polycrystals (Ce-TZP) and alumina (Al₂O₃) polycrystals via chemical treatment in aqueous solutions of H₃PO₄, H₂SO₄, HCl, or NaOH has been investigated [24]. When this type of nanocomposite is subjected to such treatment at 95°C for 4 h, Zr-OH groups that enhance the formation of apatite in simulated body fluids (SBFs) are first formed on the surface. The apatite-forming ability of zirconium metal pretreated in ≥5 M aqueous NaOH solution has also been demonstrated in SBF immersion tests [25]. Apatite nucleation is believed to be induced by Zr-OH groups in a zirconia hydrogel layer that forms on the metal upon exposure to NaOH.

Both, macro porous and nanocrystalline zirconia films have been prepared by the MAO of zirconium, and the effects of chemical treatments in aqueous H₂SO₄ or NaOH solutions on the microstructure and apatite-forming ability of the films have been investigated [26]. Compared to the MAO film, the chemically treated films did not exhibit any apparent changes in their phase component, morphology, and grain size; however, there were more abundant basic Zr-OH groups. The films treated with H₂SO₄ and NaOH solutions could induce apatite formation on their surfaces in SBFs within 1 day, whereas no apatite was detected on the untreated ZrO₂ surface even after 30 days. Thus, it was considered that the enhanced apatite-forming ability of the chemically treated ZrO₂ films was related to the abundant basic Zr-OH groups on the surface.

Plasma-spray ed nanostructured zirconia coatings stabilized with 3 mol% yttria (3Y-TZP) also exhibited good bioactivity [11]. After immersion in the SBF solution for 28 days, a bone-like apatite was formed on the surface of the plasma-sprayed nanostructured zirconia coatings (Figure 7.16), but not on the surface of the polished coating. The Zr-OH groups formed in the aging process, and the
nanostructured surface of the plasma-sprayed 3Y-TZP coating, were believed to be the keys to the bioactivity of the coating.

The ZrO$_2$ thin films prepared by cathodic arc plasma deposition were immersed in SBF to evaluate their bioactivity; the surface views of the as-deposited and thermally treated ZrO$_2$ thin films, after soaking in SBF for 28 days, are shown in Figure 7.17. After immersion in SBF for 28 days, the surface of the as-deposited ZrO$_2$ thin film was completely covered by the apatite layer, although relatively few apatite structures appeared on the surface of the ZrO$_2$ thin film annealed at 1000°C for 2 h. These results indicated that the bioactivity of the ZrO$_2$ thin film might be degraded after thermal treatment.

A high-resolution transmission electron microscopy (HRTEM) image, which was recorded near the apatite and ZrO$_2$ thin film (Figure 7.18), showed the apatite layer to be in direct contact with the ZrO$_2$ thin film. In this image, the many disordered areas visible around the crystalline apatite indicated that the apatite layer on the ZrO$_2$ thin film had only partially crystallized. The (211) planes of the apatite, with a spacing of approximately 0.28 nm, were well resolved in this region. The HRTEM image of the ZrO$_2$ thin film showed that it consisted of nanosized
crystalline ZrO\textsubscript{2}, with the outermost ZrO\textsubscript{2} crystal smaller than that in the bulk of the ZrO\textsubscript{2} thin film (as denoted by the arrows in Figure 7.18). The several-nanometer size of the particles in the outermost layer was believed to be one of the reasons why the ZrO\textsubscript{2} thin film was bioactive.

The results obtained with the nano-ZrO\textsubscript{2} thin films and coatings may indicate that nanosized surfaces bode well for bioactivity and biocompatibility, when compared to conventional surfaces. The deposition of calcium ions represents the first,
and most crucial, step of carbonate-containing hydroxyapatite nucleation from an ionic solution; indeed, this process is believed to initiate the growth of bone-like apatite on the surface of biocompatible implants [27]. The formation of a negatively charged surface gives rise to apatite precipitation because positive calcium ions are attracted from the solution [28]. The charge densities of the particles are determined by their size; in fact, Vayssieres et al. [29] have suggested that finer nanocrystalline particles might have higher surface charge densities than larger particles. It has also been demonstrated, by using thermodynamic analysis, that the surface or interfacial tension diminishes with decreasing particle size as a result of the increased potential energy of the bulk atoms in the particles [30]. Smaller particles with an increased molar free energy are more likely to adsorb molecules or ions onto the surfaces in order to decrease the total free energy, and thus become more stable. Therefore, the nanosized particles in the outermost layer of the ZrO thin films and coating might represent a key factor for inducing the precipitation of bone-like apatite on surfaces during immersion in SBFs.

Uchida et al. [31] have also reported that pure zirconia gel and zirconia gels containing sodium or calcium induce the formation of apatite in SBFs only when they possess a tetragonal and/or a monoclinic structure. It has been shown previously, that a plasma-sprayed calcium-stabilized zirconia coating is bioactive, with the bioactivity depending on the content of the monoclinic phase in the coating [32].

The bioactivity of zirconia coatings and films is thought to be related to the crystalline structure of zirconia, as well as to the nanostructural surface. When the coatings and films are immersed in a SBF, the water molecules react with zirconia and dissociate to form surface hydroxyl groups. The quantity and nature of the surface hydroxyl groups depend on the crystalline structure of zirconia [33, 34]. Although, the monoclinic phase has the lowest bulk energy among the three zirconia polymorphs [35], its surface energy is higher [36]. However, upon exposure to water molecules there is a greater tendency for this energy to be lowered by the molecular and dissociative adsorption of water, which is exothermic in nature. The adsorption enthalpy of half-monolayer H\textsubscript{2}O coverage on the monoclinic zirconia surface was shown to be $\approx 142$ KJ mol$^{-1}$, whilst that on the tetragonal surface was $\approx 90$ KJ mol$^{-1}$ [37, 38], indicating the higher reactivity of the monoclinic zirconia surface. Accordingly, the surface concentration of the hydroxyl groups on the monoclinic ZrO\textsubscript{2} surface was higher than that on tetragonal ZrO\textsubscript{2} [34]. In fact, the OH concentration of 6.2 molecules per nm$^2$ for monoclinic zirconia with a BET surface area of 19 m$^2$ g$^{-1}$ was much higher than the value of 3.5 molecules per nm$^2$ for tetragonal zirconia with a BET surface area of 20 m$^2$ g$^{-1}$. Moreover, in the former case the dominant species were tribridged hydroxyl groups that were shown to be more acidic, and in the latter case a combination of bibridged and terminal hydroxyl groups. The higher acidity of the (Zr)OH on the monoclinic zirconia surface signified that it would more easily donate protons to form negatively charged (Zr)O$^{-}$. Pettersson et al. [39] have reported that the isoelectric point of the monoclinic zirconia without dopant was 6.4: this indicated that the monoclinic zirconia surface should be negatively charged in an SBF solution with
a pH value of 7.4. The negatively charged surface can attract calcium ions from the SBF solution to its surface.

In conclusion, nanosized ZrO$_2$ coatings and films composed of tetragonal or monoclinic phases can be prepared using a variety of technologies. Moreover, such coatings and films are generally bioactive and can induce the precipitation of apatite onto the surface when they are soaked in SBFs for a certain period of time.

7.4
Cell Behavior on Nano-ZrO$_2$ Coatings and Films

It has been observed that nano-ZrO$_2$ had no cytotoxic properties when cells were cocultured on its surface. Likewise, it has been shown that nano-ZrO$_2$ is unable to generate mutations of the cellular genome, and no adverse responses have been reported following the insertion of nano-ZrO$_2$ into bone or muscle in "in vivo" models. This lack of cytotoxicity is illustrated in Figure 7.19, which shows bone marrow mesenchymal stem cells (BMMSCs) seeded onto ZrO$_2$ films after different culture times. At one day, the BMMSCs were seen to grow and proliferate very well on the film surface (Figure 7.19a), which became partially covered by the cells

Figure 7.19 Bone marrow mesenchymal stem cells seeded onto the ZrO$_2$ films after different times. (a) 1 day; (b) 4 days; (c) Higher magnification of 4-day sample [5].
and the extracellular matrix. After a longer seeding time of four days the cells had fused to form a complete layer on the thin film surface (Figure 7.19b). An examination at higher magnification (Figure 7.19c) showed the cells to possess a good morphology, with abundant dorsal ruffles and filopodia.

The morphology of MG63 cells seeded onto the surface of a plasma-sprayed nanostructured zirconia coating after one, three, and seven days, is shown in Figure 7.20. The cells exhibited good adhesion and spreading after one day, with dorsal surface ruffles and filopodia seen clearly after three days of culture. After seven days, the coating surface was completely covered by MG63 cells, which indicated that this surface favored their adhesion and growth. Cell attachment, adhesion and spreading represent the first phase of cell–implant interaction, and the quality of this phase is known to influence the capacity of cells subsequently to proliferate and to differentiate. When the proliferation and vitality of MG63 cells cultured on the 3Y-TZP coating and the polystyrene (PS) control were monitored using the alamarBlue™ assay, the results (Figure 7.21) confirmed cell proliferation on both surfaces (albeit with a longer culture time for PS). The fact that no significant differences were identified between the 3Y-TZP coating and PS control indicated that the cytocompatibility of both materials was similar.
Vincenzo et al. [40] attempted to address the genetic effects of zirconia coatings on osteoblast-like cells by using a DNA microarray technique. The study results showed clearly that ZrO₂ could upregulate/downregulate certain functional activities of the osteoblast-like cells, such as cell cycle regulation, signal transduction, immunity, and the cytoskeleton components. The surface morphology of the sample was shown to have a major influence on the gene expression of proteins for cellular binding, with cell adhesion/anchoring being improved by the presence of surface canyons (~1 μm deep) placed between the large crystals that were distributed homogeneously over the surface.

7.5 Applications of Nano-ZrO₂ Films to Biosensors

Nanostructural materials have been used extensively for both the efficient transport of electrons and optical excitation, based on their high surface-to-volume ratio and tunable electron-transport properties—two factors which are crucial to the function and integration of nanoscale devices. Since the sizes of biological macromolecules such as proteins, enzymes, and nucleic acids are comparable to those of nanoscale building blocks, any interaction between these biomolecules should induce significant changes in the electrical properties of the nanostructured bio-interfaces. A range of nanostructured materials, such as nanoparticles, nanotubes, and nanowires—all of which have been produced from metals, semiconductors, carbon, or polymeric species—have been investigated intensely for their ability to
fabricate the functional biointerfaces and to enhance the response of biosensors. Nanoscale biointerfaces on electrode surfaces can be obtained in a variety of ways, including modification of the electrode surfaces using biological receptor molecules such as enzymes, antibodies, or oligonucleotides or modification with nanostructured materials. As a result, nanostructured biointerfaces have been recognized as having impressive functional properties that point naturally towards bioelectrochemical applications.

Methods for preparing nanostructured biointerfaces on electrodes include physical or chemical adsorption, self-assembly, sol-gel processes, electrochemical deposition, and electrochemical polymerization. Nanoscale inorganic matrices such as nano Au, Ag, ZrO₂, SiO₂, TiO₂, ZnO, MnO₂, aluminum silicate, magnesium silicate, phyllosilicate sol-gel matrices, and magnetic iron oxide nanoparticles have good mechanical properties and thermal stability, and are also resistant to microbial attack and organic solvents. Therefore, most of these are used as carriers and supports (especially the nanostructured biocomposites), and provide a well-defined recognition interface. For these reasons they have become the ideal materials for the immobilization of biomolecules.

Nanostructural zirconia particles and films have recently been considered as a potential solid support for the immobilization of bioactive molecules in biosensor applications, due to their large specific surface area, high thermal/mechanical/chemical resistance, excellent biocompatibility, and their affinity towards groups containing oxygen. During the past few years, both functionalized and unfunctionalized zirconia nanoparticles have been developed as solid supports for enzyme immobilization and biocatalysis. For example, nanosized zirconia gel has been used to form a reproducible and reversible adsorption-desorption interface for DNA, to immobilize hemoglobin for a novel hydrogen peroxide biosensor, to immobilize DNA for investigating the effects of lanthanide on its electron transfer behavior, for the detection of DNA hybridization, and to be grafted with phosphoric and benzenephosphonic acids for the immobilization of myoglobin. Zirconia films are also able to bond to nonbiomolecules such as PDDA by coelectrodeposition.

Tong et al. have reported a novel and simple immobilization method for the fabrication of hydrogen peroxide \(\{\text{H}_2\text{O}_2\}\) biosensors using horseradish peroxidase (HRP)-ZrO₂ composite films [41]. Such composite films are synthesized on a gold electrode surface based on the electrodeposition of zirconia doped with HRP by cyclic voltammetric scanning in a KCl solution containing ZrO₂ and HRP. Within the HRP-ZrO₂ film, the HRP retain its bioactivity and exhibits an excellent electrocatalytic response to the reduction of \(\text{H}_2\text{O}_2\). The results have indicated that ZrO₂ is a good biomaterial capable of retaining the bioactivity of biomolecules, while the HRP doped in biological HRP-ZrO₂ films exhibit a good electrocatalytic response to the reduction of \(\text{H}_2\text{O}_2\).

A highly reproducible and reversible adsorption-desorption interface of DNA based on the nanosized zirconia film in solutions of different pH values has been successfully fabricated by Liu et al. [42]. Their results showed that DNA could adsorb onto the nanosized zirconia film from its solution, yet desorb from the
nanoparticles in 0.1 M KOH solution. The ZrO$_2$ film serves as a bridge for the immobilization of DNA on the surface of the glassy carbon (GC) electrode. This reversible adsorption–desorption performance of the nanosized zirconia film gives rise to potential applications in the preparation of removable and reproducible biochips and information storage devices.

The direct electrochemistry and thermal stability of hemoglobin (Hb), when immobilized on a nanometer-sized, zirconium dioxide (ZrO$_2$)/dimethyl sulfoxide (DMSO)-modified pyrolytic graphite (PG) electrode, have been studied by Liu et al. [43]. Here, Hb showed a high affinity to H$_2$O$_2$, and both nanosized ZrO$_2$ and DMSO could accelerate electron transfer between Hb and the electrode. The ZrO$_2$ nanoparticles were more important in facilitating the electron exchange than was DMSO, as they provided a three-dimensional stage, while some of the restricted orientations also favored a direct electron transfer between the protein molecules and the conductor surface. The particles were able to stabilize the oxidized form of Hb, to decrease the polarization impedance, and to enhance the thermal stability of the biosensor. These findings may lead to a new approach for the construction of mediator-free sensors by immobilizing proteins or enzymes on the ZrO$_2$ nanoparticles, enabling the determination of different substrates such as glucose, by using glucose oxidase.

The detection of DNA hybridization is of paramount importance for the diagnosis and treatment of genetic diseases, the detection of infectious agents, and for reliable forensic analyses. A novel electrochemical DNA biosensor based on methylene blue (MB) and zirconia (ZrO$_2$) film-modified gold electrode for DNA hybridization detection was described by Zhu et al. [44], in which the ZrO$_2$ film was electrodeposited onto a bare Au electrode surface. This was considered to be a simple, yet practical, means of creating inorganic material microstructures, notably because it overcomes the inherent drawback of sol–gel materials, namely brittleness. The DNA probe, with a phosphate group at its 5' end, can be attached onto the ZrO$_2$ film surface, which has a high affinity towards phosphate groups. As the electroactive MB is able to bind specifically to the guanine bases on the DNA molecules, it can be used as an indicator in the electrochemical DNA hybridization assay. The steps involved in the fabrication of the probe DNA-modified electrode, and the detection of a target sequence, are illustrated schematically in Figure 7.22.

Liu et al. [45] have also developed a new method of immobilizing DNA, based on the sol–gel technique. In this experiment, a ZrO$_2$ gel-derived, DNA-modified electrode was used as the working electrode, with studies being conducted of the electron transfer of DNA in 1.0 mM potassium ferricyanide system in different concentrations of lanthanum(III), europium(III), and calcium(II). The results showed that lanthanide ions could greatly expedite the electron transfer rate of DNA in the Fe(CN)$_6^{3-}$ solution, the order of effect being Eu$^{3+} >$ La$^{3+} >$ Ca$^{2+}$; this indicated that the lanthanide ions had a stronger interaction with the immobilized DNA than did the calcium ions, with the order being consistent with the charge-ionic radius ratio. The suggested reasons for the effect that lanthanide ions had on current enhancement included an electrostatic effect and a weak
coordination of the La$^{3+}$ ions to DNA. The negative charge on the phosphate backbones of DNA was seen to be neutralized by the metals with positive charges, which in turn decreased the repulsion of the DNA-modified electrode surface to Fe(CN)$_6$$^{3-}$, and led to an increase in the current.

Four different types of layer-by-layer (LBL) film have been assembled on solid surfaces. These have been designated as PDDA/$\{\text{ZrO}_x\}_n$, $\{\text{PDDA/ZrO}_x\}_n$, $\{\text{PDDA/NPZrO}_x\}_n$, and $\{\text{PDDA/PSS}\}_n$, where ZrO$_x$ represents the zirconia sol-gel formed by a vapor-surface, sol-gel deposition [46]. Among the four film types, PDDA/$\{\text{ZrO}_x\}_n$ demonstrated a better porosity and permeability, and was capable of loading greater quantities of myoglobin (Mb) from solution. Because of the better porosity of this film, the small counterions in the buffer solution could enter and exit it more easily, and this resulted in the film having a better cyclic voltammetry response, according to the mechanism of "electron hopping." The fact that the PDDA/$\{\text{ZrO}_x\}_n$-Mb films showed a larger surface concentration of electroactive Mb ($I^r$) than did the $\{\text{PDDA/ZrO}_x\}_n$-Mb films, was probably because the "soft" polyelectrolyte PDDA in the PDDA/$\{\text{ZrO}_x\}_n$ films "blocked" some pores or channels in the ZrO$_x$ layers. Compared to the $\{\text{ZrO}_x/\text{Mb}\}_n$ films, which were directly assembled LBL by Mb and ZrO$_x$ sol-gel, the advantage of the PDDA/$\{\text{ZrO}_x\}_n$-Mb films is that any possible denaturation of Mb could be prevented as its loading into the films was completely spontaneous. Moreover, any possible direct contact of Mb with the organic solvent used in the vapor-surface, sol-gel deposition of ZrO$_x$ could be avoided. In this way, Mb was able to retain its original structure and bioactivity.

Today, the interfaces between protein molecules and inorganic materials are among the "hottest" research topics in biomedicine, biochemistry, biophysics, and even in industrial applications. If there is a need to modify a substrate surface with

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**Figure 7.22** Steps involved in the fabrication of the probe DNA-modified electrode, and the detection of a target sequence.
protein molecules, the first step is to acquire a complete understanding of the mechanism that underlies any specific binding between the target-specific protein and the target in a direct way. Tomohiro Hayashi et al. [47] have analyzed the adhesion force using AFM, and revealed for the first time the mechanism that underlies the specific binding between a titanium surface and ferritin that possesses the sequence of a Ti-binding peptide in its N-terminal domain. Based on such experimental data, it can be concluded that the electrostatic interaction between charged groups, the hydrophobicity or hydrophilicity of the surface, and the surfactant addition, each play important roles in the specific binding between the Ti-binding peptide (minTBP-1) and Ti. Although the adhesion force decreases dramatically in solution with the surfactant, the nonspecific interactions are suppressed and the specificity and selectivity of the minTi-LF are enhanced due to accretion of the surfactant.

In conclusion, nano-ZrO$_2$ films have been utilized as a solid support for the immobilization of bioactive molecules in biosensor applications due to their high thermal/mechanical/chemical resistance, excellent biocompatibility, and their affinity to certain functional groups. It is possible that the phase (whether tetragonal or monoclinic), morphology, surface microstructure, and crystallite size of ZrO$_2$ thin films could vary with under different synthesis processes, and this would result in differences between the electrochemical properties and the biocompatibility of the ZrO$_2$ films. Although nano-ZrO$_2$ exhibits many excellent characteristics, the electrochemical properties and biocompatibility of nano-ZrO$_2$ in biosensors remain inadequate. It is important, therefore, to explore new methods that can be used to improve the electrochemical properties and biocompatibility of the nano-ZrO$_2$ surfaces.

References


References