CHAPTER 2

METHODS AND MATERIALS

This chapter deals with the description of various methods involved and the materials used, in achieving the objectives of the investigation.

2.1 MATERIALS

The surgical grade 316L Stainless Steel was used as the substrate for present investigation. The elemental compositions of the alloy are given in the Table 2.1.

Table 2.1 Chemical composition of 316L stainless steel

<table>
<thead>
<tr>
<th>Alloy</th>
<th>Main alloying elements (wt. %)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cr</td>
</tr>
<tr>
<td>316L SS</td>
<td>17.20</td>
</tr>
</tbody>
</table>

2.2 SPECIMENS PREPARATION

The surgical grade 316L Stainless Steel plates were used as the substrate. The sample sizes of 10 × 25 mm were polished using silicon carbide waterproof papers up to 2500 grit. Final polishing was done using 3 microns diamond paste, in order to obtain a scratch-free and mirror-finished surface. The polished specimens were washed with detergent solution, degreased with acetone and thoroughly washed with distilled water. This was
followed by ultrasonic cleaning in acetone for 10 minutes and finally the sample was rinsed in deionised water and dried.

2.3 SYNTHESIS AND COATING

The sol-gel method was adopted for ceramic oxide synthesis.

2.3.1 Titanium dioxide on 316L SS

Titanium isopropoxide (TIP, Aldrich), triethanolamine (TEA, Alfaacer), ethanol (EtOH, Merck) and polyethylene glycol (PEG, Merck) were used as starting materials. The relative volume ratio of each chemical in the sol was 3:1:20:0.5 (TIP: TEA: EtOH: PEG). Introduction of ethanol prior to triethanolamine induces immediate precipitation due to the highly reactive alkoxide and to avoid this, triethanolamine has been added as a stabilising agent for hydrolysis. The mixture was initially stirred with 10 ml of ethanol followed by the addition of titanium isopropoxide and PEG. After stirring the solution for 30 min, remaining ethanol was added and then the stirring was continued for further 3 hours at room temperature. These substrates were then dip coated with the sol solution with a withdrawal speed of 1 cm/min. Sol–gel titanium dioxide coating thus obtained on surgical grade 316L SS was later dried and sintered at 450°C for one hour.

2.3.2 Zirconium dioxide on 316L SS

Zirconium propoxide (ZrP, Aldrich), triethanolamine (TEA, Alfaacer), ethanol (EtOH, Merck) and polyethylene glycol (PEG, Merck) were used as the starting materials. The relative volume ratio of each chemical in the sol was 3:1:20:0.5 (ZrP: TEA: EtOH: PEG) for the synthesis of porous zirconium dioxide thin films. The earlier synthesis procedure and coating method was followed to prepare the samples.
2.3.3 Mixed titanium – zirconium dioxide on 316L SS

2.3.3.1 Preparation of TiO\textsubscript{2} 80\% - ZrO\textsubscript{2} 20\% (MOA)

The relative volume ratio of each chemical in the sol was 2.4: 0.6: 1: 20: 0.5 (TIP: ZrP: TEA: EtOH: PEG) for the synthesis of MOA thin films coated on 316L stainless steel. The earlier synthesis procedure and coating method is followed to prepare the MOA ZrO\textsubscript{2} mixed oxide.

2.3.3.2 Preparation of TiO\textsubscript{2} 60\% - ZrO\textsubscript{2} 40\% (MOB)

The relative volume ratio of each chemical in the sol was 1.8: 1.2: 1: 20: 0.5 (TIP: ZrP: TEA: EtOH: PEG) for the synthesis of MOB thin films coated on 316L stainless steel. The earlier synthesis procedure and coating method was followed to prepare the MOB mixed oxide.

2.3.3.3 Preparation of TiO\textsubscript{2} 40\% - ZrO\textsubscript{2} 60\% (MOC)

The relative volume ratio of each chemical in the sol was 1.2: 1.8: 1: 20: 0.5 (TIP: ZrP: TEA: EtOH: PEG) for the synthesis of MOC thin films coated on 316L stainless steel. The earlier synthesis procedure and coating method was followed to prepare the MOC ZrO\textsubscript{2} mixed oxide.

2.3.3.4 Preparation of TiO\textsubscript{2} 20\% - ZrO\textsubscript{2} 80\% (MOD)

The relative volume ratio of each chemical in the sol was 0.6: 2.4: 1: 20: 0.5 (TIP: ZrP: TEA: EtOH: PEG) for the synthesis of MOD thin films coated on 316L stainless steel. The earlier synthesis procedure and coating method was followed to prepare the MOD mixed oxide.
2.4 PREPARATION OF OPTIMISED MIXED TITANIUM – ZIRCONIUM DIOXIDE COATING ON 316L SS

The relative volume ratio of each chemical in the sol was 1.5: 1.5: 1: 20: 0.5 (TIP: ZrP: TEA: EtOH: PEG) for the synthesis of titanium dioxide 50% - zirconium dioxide 50% thin films coated on 316L stainless steel. The earlier synthesis procedure and coating method was followed to prepare optimised samples.

2.5 PREPARATION OF SIMULATED BODY FLUID (SBF)

SBF being a meta stable solution containing calcium and phosphate ions already saturated with respect to the apatite, any irregularity in the preparation lead to precipitation of Ca and PO₄ ions. Hence, it is required to take utmost care in the preparation of SBF. In the preparation of SBF the following procedure was adapted. All the apparatus used in preparing the SBF was cleaned with dilute hydrochloric acid solution and rinsed with ultra-pure water and again immersed in dilute hydrochloric acid over night. The next day the apparatus was washed several time with ion-exchanged water and the mouths of the apparatus were covered with wrapping film and were placed in a drier below 50°C. Further, in a 1000 ml beaker, 750 ml of water was taken and was maintained at 36.5°C with continuous stirring over a clean bench. Additions of reagents in the Table 2.1 were done in the same order as in the table. All the chemicals were weighed in weighing bottle. No chemical was added before the complete dissolution of the previous chemical.

Calibration of the pH meter was done before each fresh solution preparation. After the addition of (CH₂OH)₃CNH₂, the temperature of the solution was accurately maintained at 36.5°C and the pH was adjusted to 7.4 using the 1 kmol/dm³ HCl solution. After the adjustment of the pH, the solution was transferred to a volumetric flask of 1000 mL. The beaker was
rinsed several times with ultra-pure water and the solution was added in the flask. The volume of the solution was made up only after the temperature of the solution was dropped to 20°C. The flask was shook well after the make-up. The prepared solution was stored in polyethylene bottle in a refrigerator maintained at 3-5°C.

Table 2.2 Various reagents used in the preparation of SBF solution

<table>
<thead>
<tr>
<th>Order</th>
<th>Reagents</th>
<th>Grams in 1000 mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>NaCl</td>
<td>8.035</td>
</tr>
<tr>
<td>2</td>
<td>NaHCO₃</td>
<td>0.355</td>
</tr>
<tr>
<td>3</td>
<td>KCl</td>
<td>0.225</td>
</tr>
<tr>
<td>4</td>
<td>K₂HPO₄·3H₂O</td>
<td>0.231</td>
</tr>
<tr>
<td>5</td>
<td>MgCl₂·6H₂O</td>
<td>0.311</td>
</tr>
<tr>
<td>6</td>
<td>1 Kmol/cm³ HCl</td>
<td>40 cm³</td>
</tr>
<tr>
<td>7</td>
<td>CaCl₂</td>
<td>0.292</td>
</tr>
<tr>
<td>8</td>
<td>Na₂SO₄</td>
<td>0.072</td>
</tr>
<tr>
<td>9</td>
<td>((HOCH₂)₃CNH₂)</td>
<td>6.118</td>
</tr>
<tr>
<td>10</td>
<td>1 Kmol/cm³ HCl</td>
<td>Appropriate amount for adjusting the pH</td>
</tr>
</tbody>
</table>

Stability of the solution was examined before using. This was done by keeping 50 mL of the solution in an incubator at a temperature of 36.5°C for 2-3 days. Lack of any precipitation was an identity for stable solution. The solution was discarded and fresh solutions were prepared in case of any suspensions.
2.6 IMMERSION TEST IN SIMULATED BODY FLUID

The coated specimens subjected were soaked in 30 ml of SBF. After being soaked for certain given periods, the specimens were removed from SBF, washed with distilled water and dried in a clean bench at room temperature.

2.7 SURFACE CHARACTERISATION

2.7.1 X-ray diffraction analysis

The ceramic oxide coating on 316L SS confirmed by X-ray diffraction patterns were recorded with a Pan Analytical X-pert pro diffractometer using Cu K\(_\alpha\) radiation, with 40 KV and 30 mA, at a scan rate of (2\(\theta\)) 0.02°/min\(^{-1}\).

2.7.2 Fourier Transform Infra Red Spectroscopic studies

The ceramic oxide surface and the ceramic oxide immersed in SBF solution were characterized using Thermo Electron Corporation, USA. The specimens were recorded in the range of 400 – 4000 cm\(^{-1}\) wave number with a resolution of 4 cm\(^{-1}\).

2.7.3 Atomic Force Microscopy analysis

The surface morphology and roughness of the coated ceramic oxide were recorded by using Atomic Force Microscopy (AFM) technique. The nanosurf easy scan 2 (Nanosurf AG, Grammetstrasse 14, CH-4410 Liestal, Switzerland) instrument was used to record AFM. The images were acquired by contact mode using silicon nitride cantilevers with a spring constant of 0.15 N/m at a resonance frequency of 13 kHz. All the images were recorded under air atmosphere at room temperature.
2.7.4 Scanning electron microscopy / Energy Dispersive X-ray analysis

The ceramic oxide coated specimens before and after immersion in SBF solution were analysed using scanning electron microscope (SEM). Then the instrument used was an (Hitachi Model-S 3400) in combination with an energy dispersive X-ray analysis (EDAX).

2.8 ELECTROCHEMICAL STUDIES

2.8.1 Cell assembly

Figure 2.1 shows the electrochemical set up conventionally used for polarisation and electrochemical impedance studies. The cell set up was made of a 500 ml capacity flask equipped with a platinum (mesh) electrode as the counter electrode, saturated calomel electrode as the reference electrode and ceramic oxide coated 316L SS as a working electrode with the size of 1 cm² were exposed in electrolyte solution.
2.8.2 Open circuit potential – time measurement

The change in potential with respect to time was monitored for a period of time. The potential of the working electrode with respect to reference electrode after stabilization period was termed as open circuit potential (OCP) or corrosion potential ($E_{corr}$).

2.8.3 Potentiodynamic anodic cyclic polarisation studies

Potentiodynamic anodic cyclic polarisation studies were carried out for uncoated, ceramic oxide coated 316L SS immersed in SBF solution. All the potential measurements were carried out at a scan rate of 0.167 mV s$^{-1}$ in an aerated medium. The sweep direction was reversed at a current density of 3 mA cm$^{-2}$ and scanning was continued till potential at which the current density in the forward scan was equal to that in the reverse scan. Potentiostat (model PGSTAT 12 with FRA, Autolab, The Netherlands B.V.) controlled by a personnel computer with dedicated software viz., General Purpose Electrochemical System (GPES version 6.0) was used for conducting the polarisation experiments. In order to obtain reliable results, polarization experiments were triplicated in SBF solution.

2.8.4 Electrochemical impedance spectroscopic studies

EIS studies were carried out at open circuit potential using an electrochemical system Frequency Response Analyser (FRA), which included a potentiostat model PGSTAT 12. The ceramic oxide coated specimens with an exposed surface area of 1 cm$^2$ were used as the working electrode. The impedance spectra were acquired in the frequency range of $10^4$ Hz to $10^{-2}$ Hz with a 10 mV amplitude sine wave generated by a frequency response analyzer.
Impedance spectra were represented in both complex impedance diagram (Nyquist plot) and bode amplitude and phase angle plots. In Nyquist graph, the imaginary component (-Z’’ ohm, out-of-phase) of impedance was plotted as a function of the real component (Z’ ohm, in-phase), whereas the Bode representation shows the phase angle (θ) vs logarithm of the frequency f. The impedance spectra were modeled by fitting these data with an equivalent circuit. The software {EQUIVCRT} model was used in this study for EIS data modeling (Boukamp 1986). The impedance spectra were fitted using a non-linear least square (NLLS) fitting procedure for ceramic oxide coated 316L SS in SBF solution.