CHAPTER 2

Material and Experimental Details

2.1 INTRODUCTION

This chapter presents the details of Ti-13Nb-13Zr alloy and the procedure of its characterization, before and after heat treatment, and also following ultrasonic shot peening (USSP). The procedures of electrochemical corrosion, static corrosion and low cycle fatigue testing are described. Characterization of the material using optical, scanning electron and transmission electron microscope, electron probe micro-analyzer and X-ray diffractometer, is presented. Procedure of evaluating surface roughness and microhardness is also given. As this alloy is of biomedical importance, the details of bioactivity tests are also given.

2.2 MATERIAL

The alloy Ti-13Nb-13Zr was procured from Baoji Kedipu Sanxxi, China, as rod of 30 mm diameter. Its chemical composition is presented in Table 2.1. Blanks of 110 mm length were prepared from the as-received rod and longitudinally sectioned in two halves along the diameter of the blank. The longitudinally sectioned pieces were machined into cylindrical shape of 12 mm diameter and subjected to two different solution treatment temperatures and quenched at different temperatures.

Table 2.1 Chemical composition of the Ti-13Nb-13Zr alloy (wt%)

Nb	Zr	Fe	O	N	C	Ti
13.5	13.2	0.08	0.11	0.01	0.02	Rest

2.3 ULTRASONIC SHOT PEENING TREATMENT

The equipment used for USSP is shown in Figure 2.1. It consists of acoustic assembly with piezoelectric transducer, booster and sonotrode. It generates mechanical vibrations and transfers them to the hard chrome steel balls of 100C6 grade, inside the vibration chamber. It emits ultrasonic vibrations of 20 kHz frequency and 80 µm amplitude. The steel balls vibrating at high frequency impact the surface of the work material and the surface gets plastically deformed. In the present study, disc-shaped samples of 12 mm diameter and 5 mm thickness, prepared from the optimized heat-treated blanks, were USSP treated for different durations as shown in Table 2.2 using Stress Voyager (SONATS, France) for studying its effect on corrosion and mechanical properties. Part of the USSP treated samples were also stress relieved (SR).

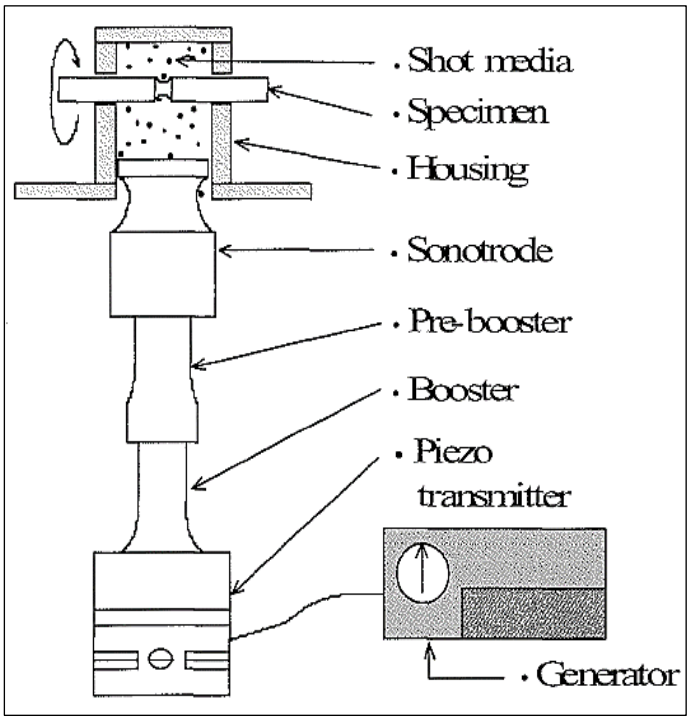

Figure 2.1 Schematic representation of Ultrasonic shot peening set-up [202].

Table 2.2 Processing parameters of USSP.

Sr. No.	Conditions	Ball size	Duration
1	Un-USSP, USSP and USSP-SR	3 mm	15 to 360 s

2.4 MICROSTRUCTURAL CHARACTERIZATION

Characterization of the Ti-13Zr-13Nb alloy was carried out using optical, scanning electron and transmission electron microscope; and X-ray diffractometer (XRD), both in the Un-USSP and USSP treated conditions.

2.4.1 Optical Microscopy

Specimens for optical microscopy were prepared from the Un-USSP and USSP treated conditions. The USSP treated samples were sectioned; perpendicular to the shot peened surface for characterizing their microstructures along the depth from the USSP treated surfaces. Samples were mechanically polished by emery papers of 200 to 2000 grit. Final polishing was carried out on velvet cloth, mounted on a rotating polishing wheel, using 1 μm alumina suspension in water as abrasive. The mirror-polished samples were etched using Kroll's reagent (5% HNO_3, 10% HF and 85% H_2O) at room temperature and their microstructures were examined using Optical microscope (Leica DM1750M) at different magnifications.

2.4.2 Scanning Electron Microscopy

Surface microstructure and surface morphology of the Un-USSP and USSP treated samples was examined using scanning electron microscope (FEI Quanta 200F) at 30 kV. The surface morphology of corroded samples was also examined. Corrosion products were characterized using SEM energy dispersive spectroscopy (EDS) and X-ray photoelectron spectroscopy (XPS). Fracture behaviour of the samples tested under tensile and cyclic loading was also examined using SEM. Fracture ends of ~4 mm length of the tensile and fatigue tested samples were sectioned transversely and cleaned ultrasonically in acetone.

2.4.3 Transmission Electron Microscopy

In-depth phase characterization of the Un -USSP and USSP treated samples was done using TEM (Technai G^2 20) operating at 200 kV. For TEM foil preparation, thin slice of 500 µm thickness was sectioned from surface region of the samples using a low-speed Buehler saw. The sectioned slices were mechanically polished on emery papers and reduced to 50 µm thickness. Discs of 3 mm diameter were then punched out from the thinned slices. Finally, TEM foils were prepared by electrochemical thinning of the discs, at 22V, using a Struers Tenupol-5 electropolishing unit, in an electrolyte containing 34% butanol and 6% perchloric acid in 60% methanol, cooled to -8°C. The slices from the USSP treated samples were carefully polished, from the untreated side up to 60-65 µm and then the treated side was carefully polished by 2000 grit size emery paper up to 50 µm to have a smooth surface on treated side too. Finally, TEM foils were prepared from the discs punched out from these slices, as mentioned above.

Deformation behaviour of LCF tested samples was also examined using TEM; foils were prepared from the thin slices sectioned transversely from the region close to the fracture end. These slices were mechanically polished and TEM foils were prepared from the discs punched out from these thinned slices, as described above.

2.4.4 X-Ray Diffraction

A Rigaku X-ray diffractometer with Co-Kα radiation and Fe filter was used to characterize phases and; to calculate mean crystallite size and mean microstrain in the Un-USSP and USSP treated samples. It is also used to estimate the volume fractions of the various phases from the respective integrated area of diffraction peaks.

2.5 MECHANICAL PROPERTIES

2.5.1 Evaluation of Elastic Modulus

Elastic modulus of the alloy in all the heat-treated conditions was determined by ultrasonic technique using ultrasonic velocity gauge (Olympus EPOCH 600) Figure 2.2. It contains a longitudinal and a transverse velocity probe of the same frequency for the measurement of longitudinal and transverse velocity. The density measurement was done using the Archimedes principle. Elastic modulus is expressed as,

$$E = \frac{d.V_T\left(3V_L^2 - 4V_T^2\right)}{\left(V_L^2 - V_T^2\right)} \qquad \text{(Equation 2.1)}$$

Where V_L and V_T represent the longitudinal and transverse wave velocities, respectively, and d is the density of the material.

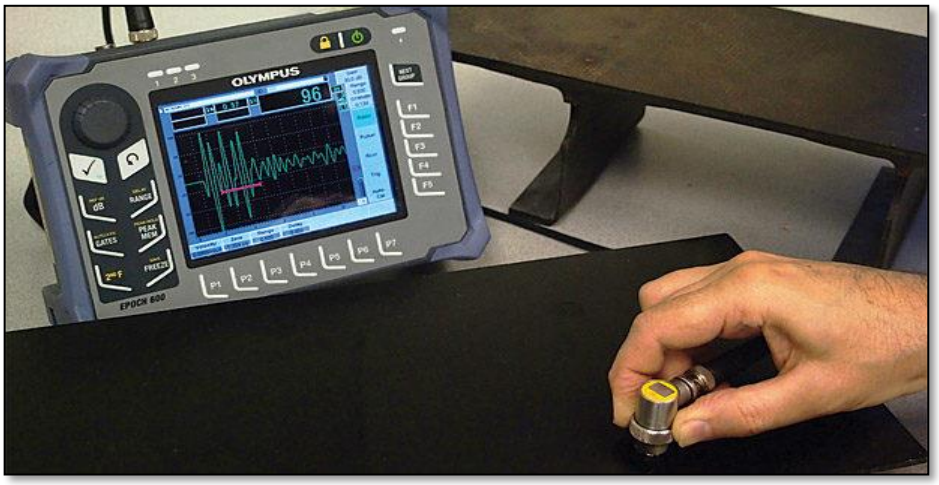

Figure 2.2 Ultrasonic velocity gauge for elastic modulus measurement [203]

2.5.2 Roughness Measurement

Surface roughness of the different Un-USSP and USSP treated samples was measured by Mitutoyo surface profilometer (model. SJ-410). Surface roughness was measured in different regions and the average value was taken.

2.5.3 Microhardness

Microhardness of the specimens was measured using a microhardness tester (Shimadzu) at an applied load of 100 g for 10 s. Microhardness measurement was conducted on top surface of the Un-USSP sample and on the sectioned transverse surface of the USSP treated samples. The microhardness was measured at three different locations, and their average was taken.

2.5.4 Tensile tests

Tensile tests were performed by mounting axial extensometer (Model 2630-102) and using 100 kN Instron^{TM} (Model 5982) universal testing machine. The tensile samples had gauge diameter of 6.25 mm and gauge length of 25 mm according to ASTM A 370 (shown in Figure 2.3). The tensile tests were conducted at a nominal strain rate of 10^{-3}/s at room temperature.

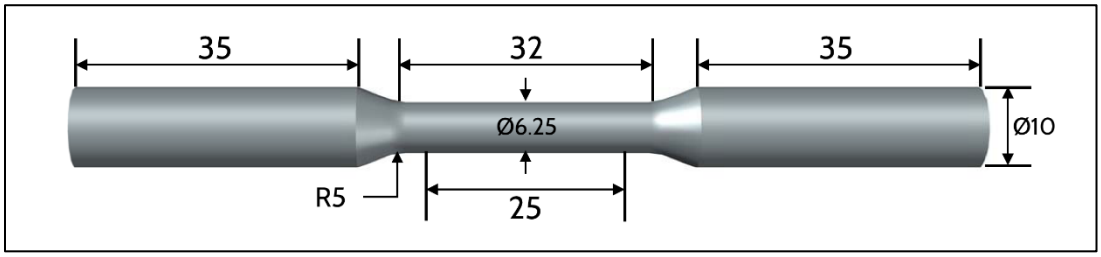

Figure 2.3 Geometry of tensile test sample (all dimensions in mm).

2.5.5 Low Cycle Fatigue tests

The as heat-treated blanks were machined into fatigue samples of gauge length of 15 mm and gauge diameter of 5.5 mm as shown in Figure 2.4. The gauge section of samples was mechanically polished with emery papers up to 2500 grit size to remove machining marks, if any. Final polishing was done using 0.5 µm alumina powder suspension in water as abrasive. The polished gauge surface of fatigue samples was subjected to USSP treatment with hard steel balls of 3 mm diameter at a frequency of 20

kHz and amplitude of 80 µm using Stress Voyager (SONATS) uniformly, for different durations rotating the sample to ensure uniform peening along circumference of the cylindrical gauge section. A MTS^{TM} servo-hydraulic machine (Model 810) of 50 kN capacity, equipped with fully automatic Flex Text 40 controller was used to conduct total strain controlled low cycle fatigue (LCF) tests with fully reversible (R = –1) axial loading with triangular waveform. All the tests were repeated to ensure reproducibility of results.

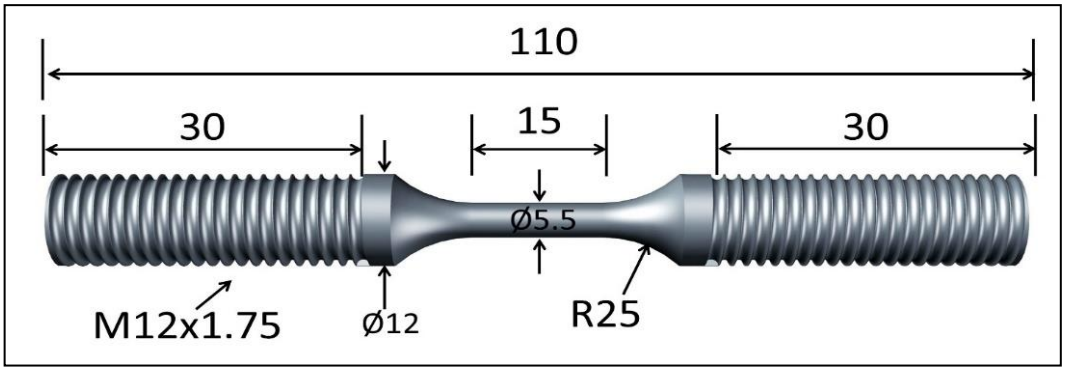

Figure 2.4 Geometry of LCF test sample (all dimensions in mm).

For optimizing the USSP condition, fatigue tests were first conducted on the samples USSP treated for 2, 4 and 6 minutes, at the fixed total strain amplitude of ±0.70%. Fatigue life was found highest of the sample USSP treated for 4 minutes. Samples were stress relieved after USSP treatment of 4 minutes to examine the effect of stress relieving treatment (USSP240-SR) on LCF behaviour of the alloy. Later, LCF tests were conducted at total strain amplitudes of ±0.55%, ±0.60%, ±0.70%, ±0.80%, and ±0.90%, at a fixed strain rate of 0.005 s^{-1}. The test matrix of low cycle fatigue tests is shown in Table 2.3. Cyclic strain was controlled mounting an extensometer of 10 mm gauge length (Model: MTS 632.13C-20) on gauge section of the fatigue samples. The elastic, plastic and total strain components along with stress amplitudes, both in tensile and compressive part of each fatigue cycle, were displayed and stored during test by the

controller software attached to the machine. The microstructure and fracture morphology of the fatigue-tested specimens were analyzed using scanning electron microscope.

Table 2.3 Test matrix for LCF tests.

SR. No.	Sample Condition	Total strain amplitudes (%)	Strain rate (s^{-1})
1	Un-USSP	±0.55, ±0.60, ±0.70, ±0.80 and ±0.90	$5x10^{-3}$
2	USSP240		
3	USSP240-SR		

*SR=Stress relieving

2.6 ELECTROCHEMICAL CORROSION MEASUREMENTS

Electrochemical tests were performed on flat surface of the Un-USSP (as heat treated) and the USSP treated disc-shaped samples of 3 mm thickness and 12 mm diameter in a potentiostat (Biologic Science-SP 200) using a three-electrode cell. The working electrode was the test material with an immersed area of 1.0 cm^2. Platinum wire mesh and saturated calomel electrodes (SCE) were used as the counter and reference electrodes, respectively. Electrochemical measurements were performed at room temperature in a naturally aerated Ringer's solution at a fixed and neutral pH. The composition of the Ringer's solution is 6.8 g/L of NaCl, 0.38 g/L of KCl, 0.3 g/L of $CaCl_2$, 0.14 g/L of $NaH_2PO_4.H_2O$, and 1 g/L of glucose. In order to obtain steady open circuit potential (OCP), all the samples were immersed in Ringer's solution for 2000 seconds.

2.6.1 Potentiodynamic Polarization tests

Potentiodynamic polarization scans were recorded at a 1 mV/sec scan rate, ranging from 200 mV below OCP to +2400 mV above OCP. To ensure reproducibility, experiments were done at least thrice in similar conditions, using fresh electrolyte for each experiment, as per the method described in ASTM G5-94.

2.6.2 Electrochemical Impedance tests

Electrochemical impedance spectroscopy (EIS) measurements were carried out for the Un-USSP and USSP treated samples after stabilizing the OCP in a three-electrode cell arrangement. The frequency ranged from 10 mHz to 100 kHz, and the amplitude of the sinusoidal potential signal was 10 mV with respect to the OCP. The cathodic slopes in all the cases were in the range of 714-259 mV/decade. The impedance spectra were collected after 60 minutes of immersion inside the corrosion media, and the experimental results were fitted using the EC-Lab (V11.27) data analysis software.

2.6.3 Static Immersion tests

For static immersion tests, small disc-shaped samples of 12 mm diameter and 3 mm thickness were machined from the initial solution treated and water quenched sample (Un-USSP). Samples in the Un-USSP condition were mechanically polished up to 1500 grit size of emery paper and subsequently, USSP treated on both the sides.

Figure 2.5 Test samples suspended in Ringer's solution for static immersion test.

After USSP treatment, the samples were ultrasonically cleaned for 5 minutes in deionized water and acetone. Each sample was weighed three times with an accuracy level of 10^{-4} g just before immersion in the Ringer's solution. Three test samples in each condition were suspended vertically in a beaker containing 200 ml of Ringer's solution

at room temperature, as shown in Figure 2.5, for 35 weeks. All the beakers were covered with paraffin film, and holes were punched to maintain the airflow. The level of the solution was checked every third day and maintained by adding deionized water. The final weight loss was measured using the same scale.

2.7 BIOACTIVITY TESTS

2.7.1 Wettability tests

Disc-shaped samples of 3 mm diameter and 2 mm thickness were prepared from as heat-treated alloy with low elastic modulus and optimal mechanical properties. The surface wettability of these discs was measured using contact angle measuring equipment (three samples for each group). Before measuring the water contact angle, the discs were washed in acetone for 5 minutes each, then completely dried. Forceps and gloves were employed to prevent contaminants and oil from being transferred from the skin to the samples. The contact angle measuring device was linked to a computerised digital camera, which captures the image when a single drop of distilled water (approximately 10 µl volume) was dropped on the surface of the examining disc using syringe. The photos were captured 30 seconds after the drop was placed on the sample surface, and the measurements were taken at room temperature.

2.7.2 Cell proliferation/viability tests

MTT (3-[4,5-dimethyl-thiazol-2-yl]-2,5-diphenyltetrazolium bromide, HiMedia) reduction assay using MG63 cell lines was used to study cell proliferation. It is a simple colorimetric technique for checking cell viability by using a standard microplate absorbance reader. The principle involves reduction of yellow coloured MTT to blue coloured formazan crystals. The increase in cellular activity increases the formation of formazan crystal, thereby increasing the absorbance value, which is directly proportional

to the active cells. The MTT assay was performed by psyllium husk-gelatin composite scaffolds placed at the bottom of the 96-well plate following the standardized method. The 3 mm sample discs of Un-USSP, USSP, and USSP-SR conditions were then sterilized by washing them in ethanol and exposing them to ultra-violet radiation for 30 minutes inside a biosafety cabinet. Cells cultured in the wells without any test samples were taken as a positive control, whereas the cell culture medium unaided was taken as the negative control for the experiments. MG63 cells were seeded on each scaffold at a density of 1×10^4 cells/mL and maintained in a CO_2 incubator (Galaxy® 170 S, Eppendorf, Germany) for 48 hours. After incubation, the culture medium was removed from each well, and a total of 100 μL solution of medium and MTT (5 mg/mL in PBS) solution was added to each well. The formazan crystals formed after 4 hours of incubation with MTT inside the wells were solubilized using a 100 μL dimethyl sulfoxide (DMSO, HiMedia) solution for 15 minutes. The optical absorbance was recorded at 570 nm on a multimode plate reader (Synergy H1 hybrid, Biotek, USA). All the experiments were performed in triplicates.

2.7.3 Statistical Analysis

All the experiments were performed in triplicate unless otherwise stated, and the results were expressed as mean with standard deviation. The one-way analysis of variance (ANOVA) was performed for all the samples [204]. To evaluate the difference between groups, one-way ANOVA was followed by Turkey's multiple comparison tests. Two sets of data were considered statistically different when $p < 0.05$.

2.7.4 Cell Adhesion

In vitro biocompatibility or cell adhesion was also carried out on similar disc-shaped samples of 3 mm diameter and 2 mm thickness. Samples in Un-USSP, USSP and USSP-SR conditions were again sterilized by exposing them to ultra-violet radiation for

30 minutes inside a biosafety cabinet. Human bone osteosarcoma cells MG-63 cells were seeded on the surface of sterilized samples and incubated at 37 °C in the incubator. After 2 and 4 days of incubation, cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, followed by washing with phosphate buffer saline (PBS). Cells were permeabilized with 0.5% Triton X-100 in PBS for 10 minutes. The cells were blocked and actin cytoskeleton filaments of cells were stained. After washing in 1µl/mL DAPI (4,6-diamidino-2phenylinodole) solution in 1%, PBS was added and kept in dark for 45 minutes to counterstain the nuclei and then final washing was given to samples before imaging. In the end, the samples were viewed using a fluorescence microscope. These experiments were also performed in triplicates.