4.1 Introduction

This chapter deliberates the complete description of various raw materials used and experimental methodologies for the synthesis of porous metallic and ceramic based scaffold. The current chapter also discusses about the various modern characterization techniques such as Fourier Transform Infrared (FTIR) Spectroscopy, X-Ray Diffraction (XRD), Transmission Electron Microscopy (TEM), Scanning Electron Microscopy (SEM), Energy-Dispersive X-Ray Spectroscopy (EDAX), and Selected Area Electron Diffraction Pattern (SAED). Fig. 4.1 shows the flow chart of different raw material used and order of fabricating green and sintered scaffold.

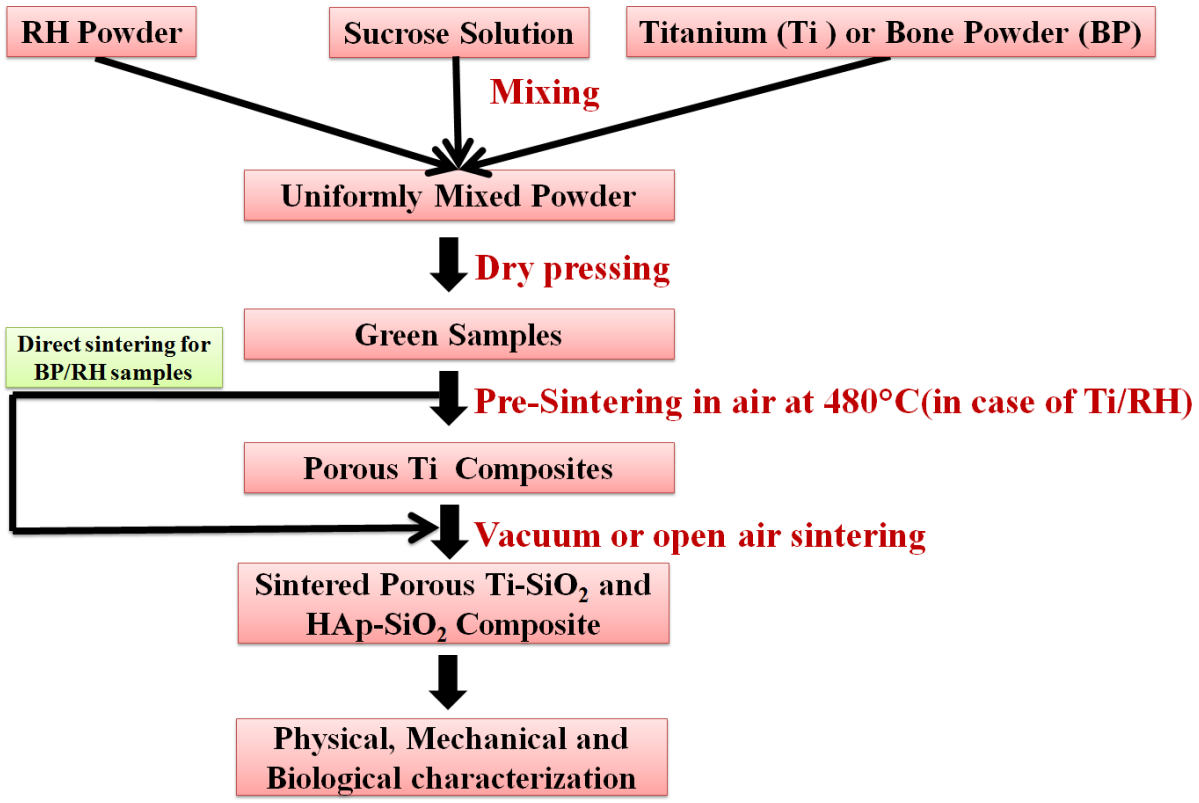

Fig. 4.1 Flow chart describing sequence of raw material used and their order of processing

4.2 Raw Materials Used

Titanium (Ti) powder, Rice Husk (RH) powder, Bone powder (BP) and Sucrose Solution (SS) are the main components used for the fabrication of porous composites. All the materials used are of analytical grade, Ti pwder did'nt required any further purification, but RH abn BP powder was purified manually. The steps involved in the processing of waste RH into RH powder of different mesh size is shown in Fig. 4.2. The bone powder used in the present study was prepared from waste bone left out from restaurants and butcher shops. Waste bone was washed thoroughly so that unwanted materials like bone marrow, spices, etc can be washed away using deionized water. Washed bone was boiled along with common salt in order to degrease it for 2 hours followed by drying and crushing in the oven and grinder respectively. The optical images of Ti powder, RH, RH powder and BP powder is shown in Fig 4.3, Fig 4.4 and Fig 4.5 respectively.

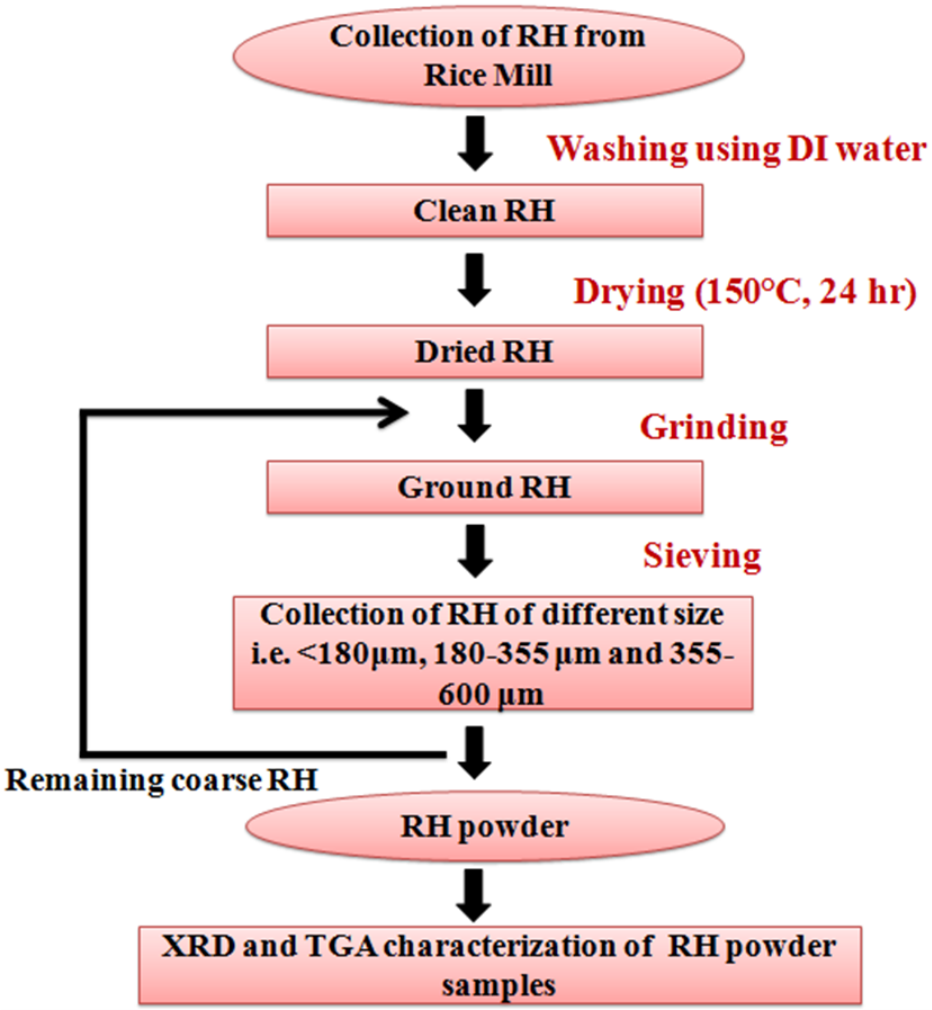

Fig. 4.2 Process flow chart describing the processing of RH-to-RH powder

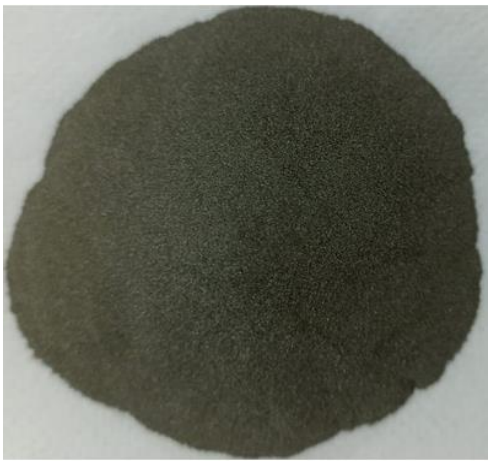

Fig. 4.3 Titanium (Ti) metal powder of 100 mesh particle size

Fig. 4.4 (a) Throughly washed RH and (b) processed RH powder

Fig. 4.5 (a) unsintered bone powder and (b) sintered bone powder

4.3 Synthesis of porous Ti-SiO_2 composite and Silica dopped Tricalcium Phosphate scaffold

The various raw material used are discussed above and complete procedure of fabricating Ti-SiO2 based composite scaffold and Silica dopped Tri-calcium Phosphate scaffold are explained systematically in sub section 5.2 and 6.2 of chapter 5 and 6 respectively. The further physical and mechanical characterizations of synthesized scaffolds are discussed below.

4.4 Instrumentations

The as-prepared porous composite scaffold was characterized by various advanced characterization techniques which are explained briefly:

4.4.1. X-ray Diffraction (XRD)

X-ray diffraction (XRD) is an advanced material characterization technique for studying crystal structure and interplannar spacing. The basic principle is based on constructive interference of monochromatic X-rays and a crystalline sample. The beam of X-Ray is generated from cathode ray tube, filtered to produce monochromatic radiation, collimated to concentrate, and directed toward the sample. The interaction of the incident rays with the sample produces constructive interference (and a diffracted ray) when conditions satisfy Bragg's Law (equation 4.1).

$$n\lambda=2d \sin \theta \qquad (4.1)$$

Where, n is an integer value, λ represents the wavelength of X-rays, d is interplanar spacing generating the diffraction, and θ is the diffraction angle.

The Bragg’s law relates the wavelength of electromagnetic radiation to the diffraction angle and the lattice spacing in a crystalline sample. These diffracted X-rays are then detected, processed and counted. By scanning the sample through a range of 2θangles, all possible diffraction directions of the lattice should be attained due to the random orientation of the powdered material.

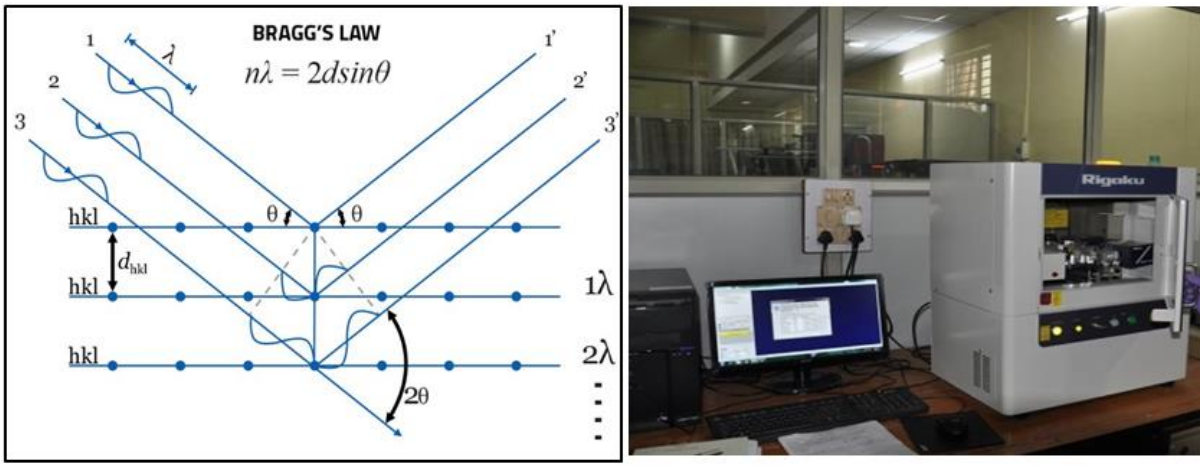

Fig. 4.6 Schematic representation of principle of X-Ray diffraction spectroscopy (XRD) and Photograph of XRD machine

X-ray diffractometers consist of three basic elements: an X-ray tube, a sample holder, and an X-ray detector. X-rays are generated in a cathode ray tube by heating a filament to produce electrons, accelerating the electrons toward a target by applying a voltage, and bombarding the target material with electrons. When electrons have sufficient energy to dislodge inner shell electrons of the target material, characteristic X-ray spectra are produced. These spectra consist of several components, the most common being K_α and K_β. K_α consists, in part, of $K_{\alpha1}$ and $K_{\alpha2}$. $K_{\alpha1}$ has a slightly shorter wavelength and twice the intensity as $K_{\alpha2}$. The specific wavelengths are characteristic of the target material (Cu, Fe, Mo, Cr). Filtering, by foils or crystal monochrometers, is required to produce monochromatic X-rays needed for diffraction. $K_{\alpha1}$and $K_{\alpha2}$ are sufficiently close in wavelength such that a weighted average of the two is used. Copper is the most common target material for single-crystal diffraction, with CuK_α radiation = 1.5418Å. These X-rays are collimated and directed onto the sample. As the sample and detector are rotated, the intensity of the reflected X-rays is recorded. When the geometry of the incident X-rays impinging the sample satisfies the Bragg

Equation, constructive interference occurs and a peak in intensity occurs. A detector records and processes this X-ray signal and converts the signal to a count rate which is then output to a device such as a printer or computer monitor. The schematic representation and photograph of XRD are given in Fig 4.6

4.4.2. Scanning Electron Microscopy (SEM)

The scanning electron microscope is used to obtain the surface morphology of a sample at different magnifications, resolutions, and depths of focus. The images are taken at a higher resolution as compared to the optical microscope. A well-focused monoenergetic electron beam is incident on the given solid surface. The interaction between the beam and the surface results in different scattering processes. Secondary electron and backscattered electron are mainly used in the SEM technique to get the surface morphology. This BSE or SE is collected and converted into current signals which are amplified to control the brightness of the cathode ray tube(CRT)/screen of the monitor. SEM works in vacuum conditions. Therefore, special sample preparation is required before putting the sample for imaging. No moisture contents should be there in the sample or the chamber because it would inhibit the vacuum in the chamber. Since the sample should be conducting in nature, therefore metals do not need any preparation before being used. Ceramic samples are coated with hin gold later in order to make their surface conducting. In the present work, SEM analysis of all the sintered samples what carried out to know surface morphology. The SEM was carried out with the help of the instrument model INSPECT 50 FEI. MODEL: EVO-scanning electron microscope MA15/18 of company CARL ZEISS MICROSCOPY LTD. This SEM has equipped with energy dispersive spectroscopy (EDX). EDX and mapping were recorded at specific magnification to get the elemental analysis of prepared samples.

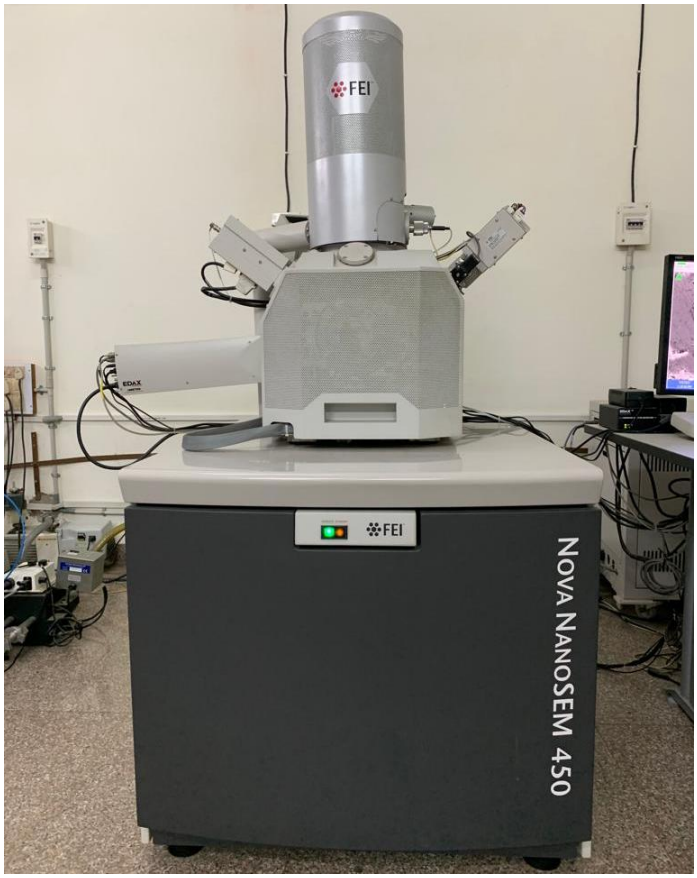

Fig. 4.7 Scanning Electron machine setup

4.4.3. Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectroscopy is a technique which is used to recognize the presence of different functional groups in molecules or compounds either organic or inorganic. It is a powerful tool for identifying types of chemical bonds in a molecule by producing an infrared absorption spectrum that is like a molecular "fingerprint". The term Fourier Transform Infrared Spectroscopy (FTIR) refers to a development in the manner in which the data is collected and converted from an interference pattern to a spectrum. The working principle are based on Michelson interferometer which consist of beam splitter, a fixed mirror and a

movable mirror that translate back and forth precisely. The radiation from the source strike to the beam splitter and separate into two beams. One beam is transmitted through the beam splitter and goes to the static mirror whereas second beam is reflected off the beam splitter to the moving mirror. The fixed and moving mirrors reflect the radiation back to the beamsplitter. Again, half of this reflected radiation is transmitted and half is reflected at the beam splitter, resulting in one beam passing through the sample and detected by the detector and spectra are display on computer and the second back to the source.

In our study, the FTIR spectra of the sample were conducted by using the instrument "PerkinElmer Spectrum 100". The radiation sources passed through KBr window and transmitted data were collected by $LiTaO_3$ detector and spectra were displayed in transmitted mode. The pellets of the sample were prepared by mixing the sample with KBr in the ratio of 1:100 and the sample pellets were scanned in the range of 400-4000 cm^{-1} with the spectral resolution of 4.0 cm^{-1} and scan speed 0.2 cm/sec. The working principle and photograph of FTIR spectrophotometer are given in **Figure 2.6**.

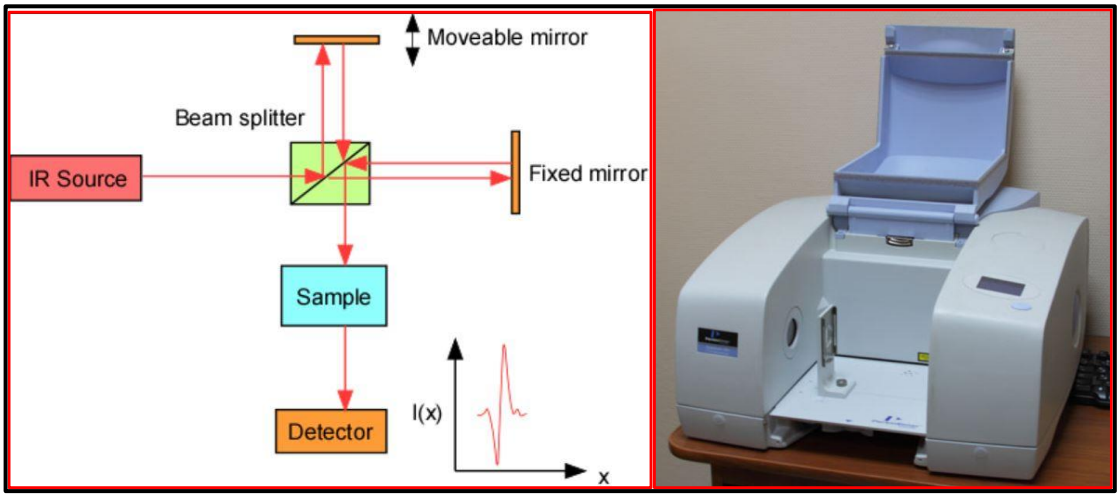

Fig. 4.8 Schematic representation of working of Fourier transforms infrared (FTIR) spectrophotometer and its photograph

4.4.4. Transmission Electron Microscopy (TEM)

TEM is a powerful electron microscope that uses a beam of electrons to focus on a specimen producing a highly magnified and detailed image of the specimen. The magnification power is over 2 million times better than that of the light microscope, producing the image of the specimen which enables easy characterization of the image in its morphological features, compositions and crystallization information is also detailed. In this technique, a heated tungsten filament in the electron gun produces electrons that get focus on the specimen by the condenser lenses (Fig. 4.9). On reaching the specimen, the specimen scatters the electrons focusing them on the magnetic lenses forming a large clear image, and if it passes through a fluorescent screen, it forms a polychromatic image. The denser the specimen, the more the electrons are scattered forming a darker image because fewer electron reaches the screen for visualization while thinner, more transparent specimens appear brighter.

TEM operates on the same basic principles as the light microscope but uses electrons instead of light. Since the electrons have both wave and particle nature and the de Broglie wavelength of electrons are significantly smaller than that of light and so they have higher resolution capability. This enables the instrument's user to examine fine detail-even as small as a single column of atoms, which is tens of thousands of times smaller than the smallest resolvable object in a light microscope.TEM forms a major analysis method in a range of scientific fields, in physical, chemical and biological sciences. At smaller magnifications TEM image is contrast due to absorption of electrons in the material, as well as thickness and composition of the material. At higher magnifications complex wave interactions modulate the intensity of the image, requiring expert analysis of observed images. Alternate modes of use allow for the TEM to observe modulations in chemical identity, crystal orientation,

electronic structure and sample induced electron phase shiftas well as the regular absorption-based imaging.

In the present study, the TEM analysis of the sample was performed on TECNAI 20 G2-Electron Microscope operated at accelerating voltage 200 kV (Fig. 4.9) The samples were prepared by simply mounted the dilute solution of the sample on carbon coated TEM grid and dried under table lamp for 5 h after that vacuum dried overnight.

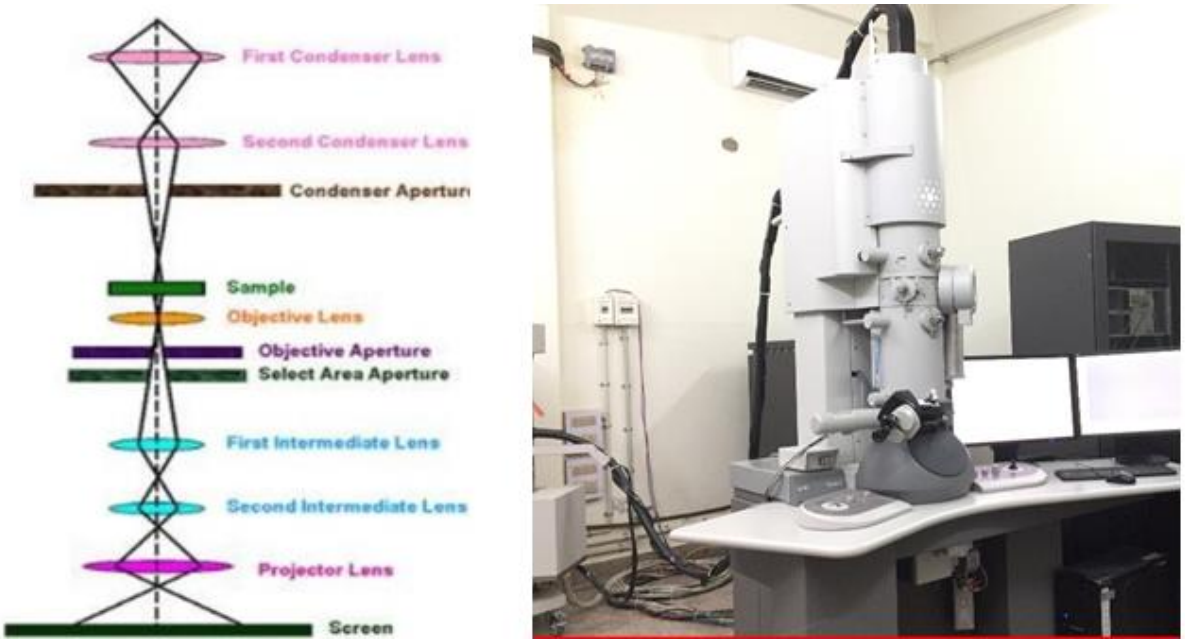

Fig. 4.9 Schematic representation of principle of Transmission electron microscopy (TEM) and Photograph of TEM

4.4.5. Physical and Mechanical Characterization

The density of the develop composite scaffold was calculated to understand sintering behaviour. The actual density of samples was measured by the Archimedes method in which the weight of the samples is measured in air and the liquid used here was water. The weight of samples you are measured by OHAUS AX324 precision balance. To estimate the

mechanical properties like compressive strength and elastic modulus some mechanical test were performed. The ability of the material to sustain mechanical load under compression is termed as compressive strength of that material. Compressive strength of the developed composite scaffold was measured by using cylindrical samples. The compression test of the cylindrical samples was done on Universal testing machine (H10KL, Tinus Olsen, USA). The cylindrical shape samples were tested by applying a load and the the values were obtained from the UTM. The value was obtained for three samples at each temperature and their values were noted. The Elastic modulus of Titanium-based composite was obtained directly from the elastic part of stress strain curve.

Fig. 4.10 UTM machine for measurement of compressive strength