STRUCTURAL, MORPHOLOGICAL AND MECHANICAL PROPERTIES OF LASER IRRADIATED $CaHPO_4$BIOMATERIAL

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Calcium pyrophosphate biomaterials were synthesized using solid state sintering method. Samples were sintered at 450 ^{O}C and 750 ^{O}C to investigate the change in mechanical properties with the increase in sintering temperature. During the heat treatment, the calcium pyrophosphate biomaterials undergo different phases such as alpha and beta pyrophosphates. Structural, morphological and mechanical properties of prepared biomaterials were studied before and after the irradiation of Nd: YAG laser. Samples were characterized using XRD, FTIR and SEM techniques. The increase in intensity of the peaks in XRD spectra of calcium pyrophosphate biomaterials was observed due to the change in crystalinity after laser irradiation. In case of FTIR spectra of the biomaterials the intensity of the peaks after laser irradiation decreased. SEM investigation shows that the morphology of the biomaterials changes due to the Nd: YAG laser and resultantly the rod like structures were obtained. Improvements in the mechanical properties of the biomaterials were observed after the heat treatment but when samples were irradiated by Nd: YAG laser, they lose their mechanical strength. Compressive strength of the biomaterials improved from 0.03-0.24MPa after heat treatment but due to laser effects, compressive strength decreases from 0.19-0.059Mpa.

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1. Introduction

Any material that is man-mad or natural, which makes complete or a part of biomedical device or living structure and also replaces a natural function is known as a biomaterial[1]. In the field of biomaterials, calcium phosphate has a great importance which is the key component of human body bones. This material can be used as the bone repair material[2]. To test the effect on curing of non-unions[3], these materials are injected in animals. First time the hydraulic calcium phosphate cement was devised by Brown and Chow in 1980's[4]. These materials are used in medicines and their compounds, like β-tricalcium phosphate and hydroxyapatite are also under greatattention in medical fields[5]. Hydroxyapatites are mostly used in dental surgery, to fill the bones interstices and as a material for the coating of surface. The mineral phase of human bone consists of "HA" as basic material. Approximately 60 to 70 percent of these materials are present in mineral phase of human bone[6].Such biomaterials which consist of calcium hydroxyapatite biomaterials and bioceramics can be utilized for many applications in human body skeleton treatment and also cover all fields related to human body systems. Their applications include percutaneous devices, periodontal treatment, dental implants, bone defects healing, augmentation of bone, fracture healing, total joint replacement, orthopedics, spinal surgery, cranio-maxillofacial rebuilding and otolaryngology. What type of calcium orthophosphate have to be used depends

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upon desired material either it is bioactive or bioresorbable. In the past, many of the researchers have worked on these materials, however due to less awareness of the toxic effects produced by the use of concerning reagents, they remain unsuccessful. In this regard, their use in teeth and mineral phase of bone is reasonable. However, the first use of CaP as an artificial material was done to repair the surgical defects in rabbits in 1920's[7].In present times due to the properties such asinject ability and in situ setting; calcium phosphate cement is regarded as an efficient biomaterial as compared to various obtainable materials. Calcium phosphate cements having different compositions of powder and liquid are available commercially and some are under experiments. A CPC consists of two components one is solid and other is liquid. When both phases are mixed to each other, a solid paste like material is obtained which gradually sets and hardens. Solid phase contains one or more than one calcium phosphate compounds and the liquid phase may be a saline solution having various pH. Pure water also can be used as liquid phase. The setting time of cement depends upon three factors (1) liquid to powder ratio (2) solid and liquid concentrations and (3) granulometry of powder. Mechanical properties of cement are influenced by setting conditions of cement[8].

Laser irradiation on a solid phase materialresultantly produce plasma phase. Plasma phase that is produced by inducing the laser into the solid phase is transient in nature and its properties depend upon the target material, characteristics or parameters of the irradiated laser, surface morphology and ambient atmosphere. Induced plasma phase can be used for number of applications like laser induced breakdown spectroscopy(LIBS), ion generation,soft and hard X-ray emission,pulsed laser deposition[9].Interaction of laser with matter is not easy to understand it produces many of the complications and it is taken as complex and an interdisciplinary topic. Laser irradiation on a material and interaction of laser with matter covers gap between the research problems and laser applications which provides a comprehensive knowledge to study the structural, optical, mechanical and morphological properties as well as the inherent microstructure of the materials. The effects due to the laser irradiation on a matter specimen is related to the various fields related to electromagnetic, optical, biological and thermodynamic changes in the properties of matter[10]. The changes produced in surface of matter by the irradiation of laser have great importance in laser matter interaction phenomenon and has a number of applicationsuch as thin films deposition, micromachining, photolithography and magnetic materials. Mostly the changes in the surface of material are characterized by spikes, cracks, visual look and ripples etc., observed at low energy, when the material retains its surface but undergoes structural changes. Conversion of laser energy into the formation of ripples or cracks is a special phenomenon that occurs on the surface of target material in ambient conditions[11]. Due to the extraordinary applications of laser its use is increasing day by day for material handling such as automation worthiness, high productivity, non contact handling, reduce handling cost, greater material use and a minimum heat affected area. Every material requires a specific amount of energy for the transformation of its phase. The interaction time and power efficiency of laser are primary parameters for the changes in phase of material for a short time pulse. Because the studiesrelated to structural alteration or changesrequires high energy therefore CO_2 and Nd: YAG lasers are the betteropportunities. Power density and time of irradiation of laser is adjusted in such a way that we could obtain the desired phase transformation and degree of heating[12].Many researchers are working on laser-matter interaction studies of biomaterials. Rodrıguez-Vilchis *et al*.studied the structural and morphological modifications on human dental enamel after Er: YAG laser irradiation[13]. Lin *et al*. discussed the compositional, morphological and phase changes of human dentin after Nd: YAG laser irradiation and he proposed that dentin can be melt and recrystallize by the Nd: YAG laser irradiation[14]. To improve e the performance of materials by laser processing and to study the surface modification applications Brown and his co-workersworked with great devotions[15].

In this present work we take calcium hydrogen phosphate $CaHPO_4$ as a starting material and sintered it at different temperatures such as at 450°Cand 750 °Cfor 6,12,18 and 24 hours respectively to obtain beta and gamma phases of $Ca_2P_2O_7$. Prepared phases are irradiated by Nd: YAG laser with required specifications to study the structural, mechanical and morphological modifications. A comparison of Structural, mechanical and morphological properties have been reported before and after the irradiation of laser shots. Some applications of the biomaterials after

laser exposure and efficiency enhancement in the materials after Nd: YAG laser has been investigated.

2.Experimental work

2.1.Materials and methods

Calcium hydrogen phosphate biomaterials were prepared by solid state sintering method. All the chemicals for this synthesis are used without further purificationand given below.The starting material was reagent grade calcium biphosphate dihydrate ($CaHPO_4.2H_2O$, Merck, 99.9%). CaHPO4 powder was taken and ground using pestle and mortar. Pellets of prepared samples were made using hydraulic press at a pressure of 4 tons to get a disc shaped sample.For drying purpose pellets were placed in oven at 120^oC for4 hours to remove the water molecules. The dried pellets were sintered in a temperature range from 450^oC-750^oC using muffle furnace Vulcan A-550 for various time periods 6,12,18 and 24 hrs to get the gamma and beta phases respectively. Nd: YAG was used as a laser source for laser irradiation on biomaterials. Structural analysis of samples was done before and after laser irradiation using Phillips-X'Pert PRO 3040/60 X-Ray diffractometer which utilize CuKα as radiation source with wavelength 1.542 Å.The surface morphology of biomaterials after and before laser interactionwas investigated by SEM (JEOL 1230 operated at 20 KV). UV-Vis spectra of samples was studied by dual beam Cary 60 (Agilent) spectrophotometer.

2.2. Synthesis of Calcium pyrophosphate ($Ca_2P_2O_7$) biomaterials

Solid state sintering method was adopted to synthesize calcium pyrophosphate ($Ca_2P_2O_7$)biomaterials. Dicalcium hydrogen phosphate dihydrate ($CaHPO_4.2H_2O$)powder was used as starting reagent for the synthesis of required biomaterials and a disc shaped pellets of taken powder was prepared. To remove water contents from materials pellets were dried at 120^OC for 4 hrs. Samples were annealed at 450^OC and 750 ^OC for 6,12,18 and 24 hrs to get final phases. These synthesized materials was irradiated by Nd-YAG laser source and changes in sample's structural, morphological and mechanical properties after laser irradiation was compared with the before laser properties of samples.

3.Results and discussion

3.1. Colour variations

When calcium biphosphate dihydrate biomaterials were sintered at different temperatures 450^OC and 750 ^OC then two phases (gamma and beta) were obtained respectively. As the temperature was enhanced the hardness of the samples were improved at the same time the physical changes in the biomaterials also were detected such as color variations. Beta phase was observed mechanically soft and brittle as compared to gamma phase which is hard in nature. At temperature of 450^OC color of the synthesized samples becomes grey which represent the gamma phase of material and it change into white color at 750 ^OC that is beta phase of bi phosphate dihydrate biomaterials.

3.2. XRD analysis

XRD analysis of calcium phosphate biomaterials sintered attwo different temperatures 450 ^OC and 750^OC for 6,12,18 and 24 hrs respectively, was performed using X-ray diffractometer(Philips PW 1700). All the peaks were observed at 2θ values 26.71, 29.30, 30.71, 33.29, 40.62, 41.70, 43.47, 50.38, 51.52, 52.96, 61.71and were successfully indexed as002, 112, 120, 102, 122, 221, 130, 321, 023 and 322respectively as shown in Figure.1[16].

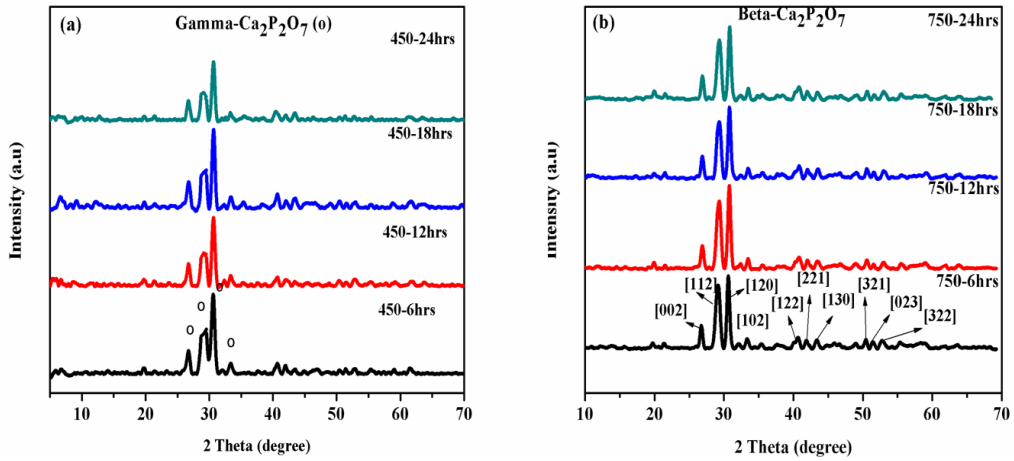

Fig. 1. (a): XRD spectra of Gamma - $Ca_2P_2O_7$ biomaterials before Nd: YAG laser (b): XRD spectra of Beta - $Ca_2P_2O_7$ biomaterials before Nd: YAG laser

Change in phase and appearance of impurity contents in the biomaterials, takes place due to the thermal impact during sintering process.It was notedthat by the increase of sintering temperature for $CaHPO_4.2H_2O$ biomaterials the change in crystalinity of biomaterials is produced at the same time some impurity phases like beta- $Ca_2P_2O_7$ and gamma-$Ca_2P_2O_7$are also observed.The heat treatment of these materials is the reason of cracks formation and hence crystalinity of the biomaterials disturbs[17].It was observed that at 450 ^{O}C gamma phase impurity is produced but as we increase the sintering temperature about 750 ^{O}C a new phase is observed that is beta phase of $Ca_2P_2O_7$. This phase transformation is due to the increase of roughness of biomaterials after heat treatments[18, 19].

$$2CaHPO_4.2H_2O \xrightarrow{120^oC} 2CaHPO_4 \xrightarrow{450^oC} \text{Gamma-}Ca_2P_2O_7 \xrightarrow{750^oC} \text{Beta- } Ca_2P_2O_7$$

XRD spectra of both beta and gamma phases of $Ca_2P_2O_7$ have been given in Fig.1 that shows the confirmation of gamma to beta formation after heat treatment. It also has been observed that as the time of sintering temperature increase a minor change in the intensity of the peaks and d- spacing of planes is produced that confirms the formation of new phase. Furthermore a slight shift in 2θ values of peaks also has been produced after increasing the sintering temperature which shows that the crystalinity of the materials has disturbed.XRD pattern of before and after Nd-YAG laser irradiation was compared. There was no extra peak observed after laser irradiation but the peak intensity of the materials after laser treatment enhances due to formation of cracks and surface roughness. Further, when materials are irradiated by Nd: YAG laser then scattering and diffraction effects takes place within the materials which results the change in intensity[20].XRD pattern of synthesized biomaterials after and before laser treatment has been shown in Figure.1 and Fig.2.

It was observed that peaks become sharper as the laser is irradiated on the biomaterials. The increase in irradiation of energy is directly related to intensity of the peaks[14].No major variations in XRD parameters of the materials were investigated after the laser exposure[12].Initially when no laser was irradiated the homogeneity and crystalinity in the material was noted. After exposing the sample by laser radiation a considerable change in intensity[21].

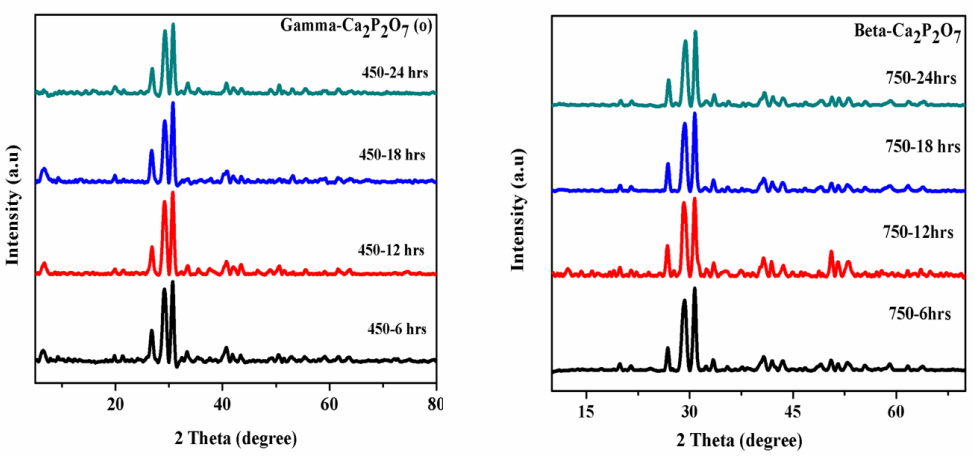

Fig. 2: (a): XRD spectra of Gamma - $Ca_2P_2O_7$ biomaterials before Nd: YAG laser
(b): XRD spectra of Beta - $Ca_2P_2O_7$ biomaterials after Nd: YAG laser

4.Scanning electron microscopy

4.1. Analysis of the morphology features at 450°C and 750°C (Before Nd: YAG laser)

Laser effects, phase changes, thermal diffusion, adhesion, nucleation process, Surface morphology, structural analysis and the grain size of the materials can be investigated using scanning electron microscopy technique[12].Before laser treatment the surface morphology of the fabricated calcium phosphate material was studied with the help of scanning electron microscopy(XL30; Philips, Eindhoven, Netherlands)[22]and the scale of magnification was adopted 10um. Two different series of samples were prepared for this purpose sintered at 450 ^{O}C and 750 ^{O}C respectively.SEM images of both series are shown in Figure. 3.It was observed that before heating the surface of the samples is almost smooth, grains were compacted and size of grains was very large. As the temperature of sintering increased the physical appearance of the samples changed and micro crackswere produced due to thermal shocks.At 450 ^{O}C there are some micro cracks but at 750 ^{O}Ccompactness of material and crystalline growth disturbed hardly.Literature study reveals that density of the materials depends upon crystalline growth In other words we can say that the temperature changes has a great impact on densification of the materials[23].

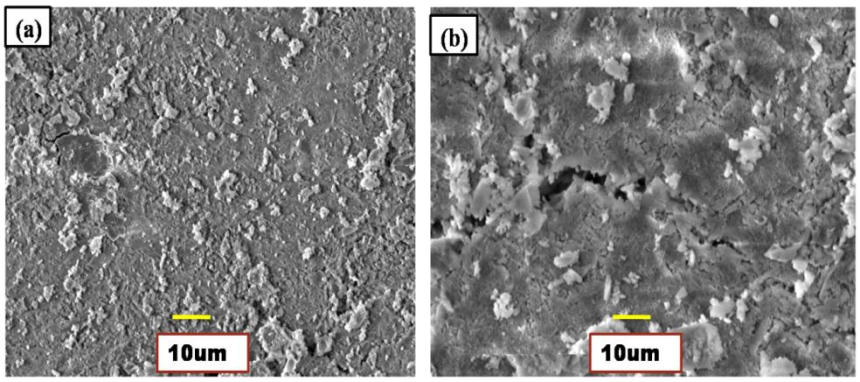

Fig. 3 (a): SEM image of $Ca_2P_2O_7$ biomaterials sintered at 450 °C before Nd: YAG laser
(b): SEM image of $Ca_2P_2O_7$ biomaterials sintered at 750 °C before Nd: YAG laser

4.2. Analysis of the morphology features at 450°C and 750°C (With Nd: YAG laser)

After the irradiation of Nd-YAG laserthe morphological and structural changes in $Ca_2P_2O_7$ biomaterials sintered at 450 ^oC and 750 ^oC was analyzed. Laser having characteristics pulse energy of 34.6mJ at wavelength of 532nm was irradiated and frequency was adjusted at 10 Hz with pulse width 4msec.The materials are recrystallize and the reduction of organic and moisture contents take place after irradiation of Nd-YAG laser with mentioned characteristics. Due to the gradient of temperature after Nd: YAG irradiation the biomaterials melt and rod like nanocrystalline structure is obtained as shown in Figure. 4. It also was observed that some part of the surface has relatively larger sized crystals surrounded by small sized grains, and cracks at the place of laser connection were present.When the laser was removed the upper surface of biomaterials becomes in make contact with air and nucleation sites are appeared at interface between air and $Ca_2P_2O_7$.Hence, this effect results to formation of columnar structures shown in figures. Nd: YAG laser did not affect on porosityof the materials due to the minor change in surface volume after irradiation.Absence of pores is beneficial because porescause reduction in mechanical strength of biomaterial and structural fractures may be produce[24].In this case the changes due to Nd: YAG laser on $Ca_2P_2O_7$ biomaterial overcomes on the changes produced due to sintering temperature.

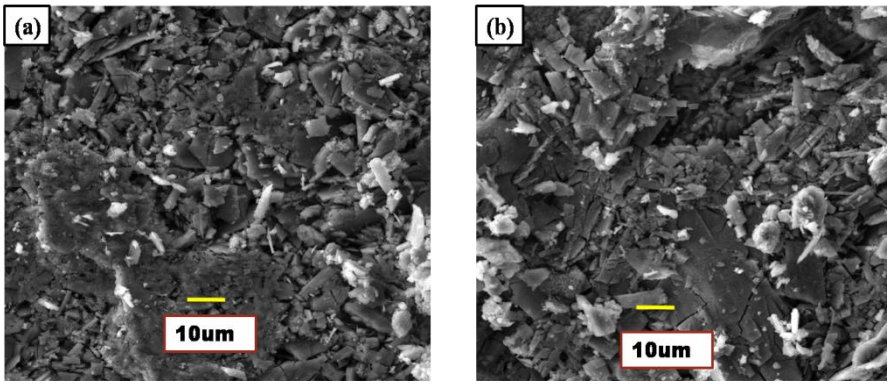

Fig. 4 (a): SEM image of $Ca_2P_2O_7$ biomaterials sintered at 450 ^oC after Nd: YAG laser
(b): SEM image of $Ca_2P_2O_7$ biomaterials sintered at 750 ^oC after Nd: YAG laser

5.Fourier Transform Infrared Spectroscopy

Fourier transform infrared spectroscopic analysis of prepared calcium pyrophosphate samples at different sintering temperature 450^{o}C and 750^{o}C was done in the range of 500-4000cm^{-1}. Samples were analyzed before and after Nd: YAG laser irradiation and their functional groups studies were done.Different pyrophosphates absorbs in the range of 720cm^{-1}-1211 cm^{-1}[25-28].It was observed that before laser irradiation at 450 ^{o}C and 750 ^{o}C,both series shows absorbance bands at 700 cm^{-1} and 697 cm^{-1} due to the presence of P-O-P linkage stretching mode in $Ca_2P_2O_7$. At 450 ^{o}C the unique absorbance of gamma pyrophosphate occur at 1180cm^{-1} and as the sintering temperature increased up to 750^{o}C the two bands of absorption occur at 1213 cm^{-1} and 1138 cm^{-1} that are characteristic bands for beta pyrophosphates[29].Presence of major peak in the range of 946 cm^{-1} -955 cm^{-1} was observed in both series of samples which is the characteristic value of absorbance for the existence of phosphate groups[30] as shown in Figure.5.

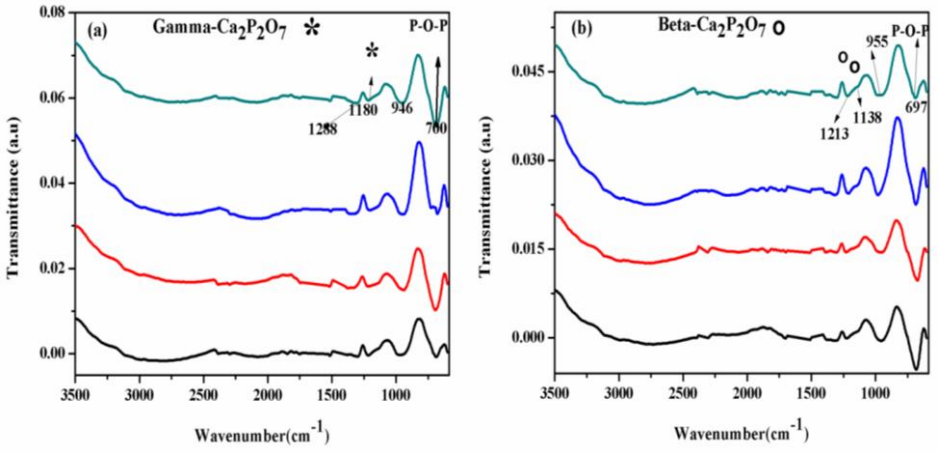

Fig. 5(a): FTIR spectra of $Ca_2P_2O_7$ biomaterials sintered at 450 ^oC before Nd: YAG laser
(b): FTIR spectra of $Ca_2P_2O_7$ biomaterials sintered at 750 ^oC before Nd: YAG laser

After the Nd: YAG laser irradiation there was no major change in the FTIR spectra of calcium pyrophosphate biomaterials were observed. The upper surface of the materials after the laser irradiation becomes rough and melting bubbles are appeared on the surface due to this, Some of the peaks drop their intensity as compared to the without laser irradiated samples[31]. The FTIR spectra of the samples with Nd: YAG laser has been shown below in Fig.6.

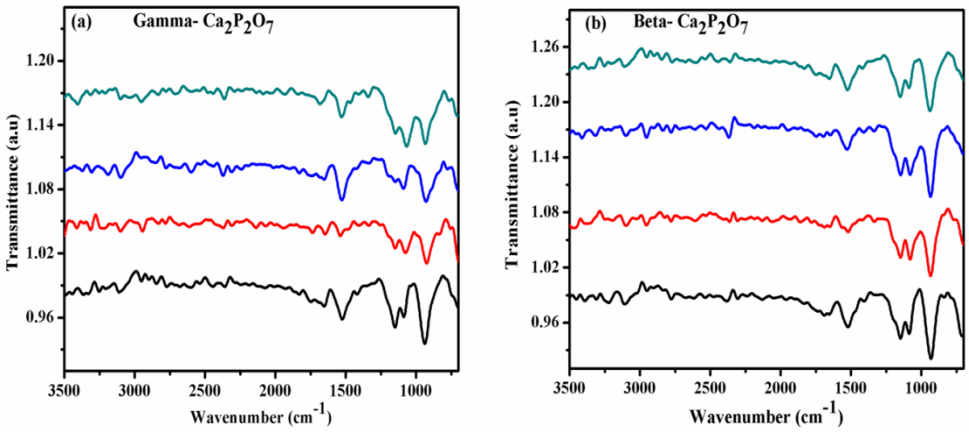

Fig. 6(a): FTIR spectra of $Ca_2P_2O_7$ biomaterials sintered at 450 ^oC after Nd: YAG laser
(b): FTIR spectra of $Ca_2P_2O_7$ biomaterials sintered at 750 ^oC after Nd: YAG laser

6. Mechanical behavior of Calcium Pyrophosphate biomaterials

Mechanical testing of the synthesized calcium pyrophosphate biomaterials was made using Autograph-AGS-J Shimadzu 5KN model.For the mechanical testing of the materials pallets of the samples sintered at 450 ^oC and 750 $^oC$was prepared using hydraulic press system. To test the Compressive strength diameter of the pallet was calculated through vernier caliper and then pallet was placed parallel to the piston of testing machine.It was proposed that as we increase the sintering temperature the material hardness and the compressive strength enhance. Plots between applied force and stroke for both series of the biomaterials at 450 ^oC and 750 ^oC are shown in figure. A point at which breakdown of material takes place is shown. Maximum stress that a material can bear before fracture is related to its compressive strength. It is clear from Table 1 and Table 2 that at 450 ^oC the maximum bearable force value for biomaterial is less as compared to

biomaterial sintered at 750 ^{o}C, which shows that the hardness of material depends upon sintering temperature[20].

Table1: Mechanical parameters of Calcium Pyrophosphate biomaterials before laser irradiation

Sample ID	Area $(mm)^2$	Young's Modulus (a.u)	UTS MPa	Fracture Point MPa	Compressive Strength MPa
450 ^{o}C/6 hours	165.95	0.99	1.06	1.06	0.030
450 ^{o}C/18 hours	174.34	0.89	0.69	0.69	0.086
450 ^{o}C/24 hours	174.27	0.64	0.98	0.98	0.11
750 ^{o}C/6 hours	165.95	1.31	3.05	3.05	0.09
750 ^{o}C/18 hours	167.7	0.25	0.76	0.76	0.124
750 ^{o}C/24 hours	167.33	0.47	0.26	0.26	0.245

Table 2: Mechanical parameters of Calcium pyrophosphate biomaterials with Nd: YAG laser irradiation

Sample ID	Area $(mm)^2$	Young's Modulus (a.u)	UTS MPa	Fracture Point MPa	Compressive Strength MPa
450 ^{o}C/6 hours	165.95	1.83	0.33	0.13	0.193
450 ^{o}C/18 hours	167.33	1.06	1.99	0.01	0.191
450 ^{o}C/24 hours	167.7	0.31	0.144	0.10	0.143
750 ^{o}C/6 hours	165.95	0.84	1.006	0.7	0.134
750 ^{o}C/18 hours	174.34	0.057	0.71	0.4	0.085
750 ^{o}C/24 hours	174.27	0.059	0.05	0.02	0.059

Different mechanical parameters of synthesized biomaterials were calculated using following mathematical formulas.

$$\text{Young's Modulus (Y)} = \frac{\text{Stress}}{\text{Strain}} \qquad \text{Equation (1)}$$

$$\text{Fractural strength } (f) = \frac{\text{Fractural force}}{\text{Area of pellet}} \qquad \text{Equation (2)}$$

$$\text{Ultimate Tensile Strength (UTS)} = \frac{\text{Ultimate force}}{\text{Area of pellet}} \qquad \text{Equation (3)}$$

$$\text{Compressive Strength (CS)} = \frac{\text{Compressional force}}{\text{Area of pellet}} \qquad \text{Equation (4)}$$

Table.1 shows the variations in the mechanical properties of calcium pyrophosphate biomaterials as the temperature and the time of applied sintering temperature on the samples increases. Furthermore it has been reported by Laasri and co workers that the mechanical characteristics of the biomaterials are related to the density of the materials and the densification is directly related to the sintering temperature and grain size of the materials. In other words the sintering temperature affects the mechanical properties of the materials. Loss in mechanical properties was observed when the sample of the calcium pyrophosphate was sintered in the dry form but in case of humid samples, the increase in sintering temperature causes to increase in the strength or the mechanical properties of biomaterials[32]. It is revealed from the literature study that the mechanical properties of biomaterials also affected by the laser irradiation. It was investigated that after the laser irradiation the mechanical parameters of the synthesized biomaterials have been decreased due to the structural defects and high intensity laser irradiation. It has been reported by Dergal *et al*. that if we use high intensity laser beam then the mechanical properties of the biomaterials decreased due to the disturbance of atomic packing inside the materials[33]. Table.2 shows the trend of mechanical parameters of the calcium pyrophosphate biomaterials after the irradiation of Nd: YAG laser.

5. Conclusions

The Calcium phosphate biomaterials were synthesized by solid state sintering technique. Compressive strength of the biomaterials improved from 0.03-0.24MPa as the sintering temperature increased but after the laser exposure the material becomes mechanically weak and its compressive strength decreases from 0.19-0.059Mpa.

XRD results confirm the formation of gamma- $Ca_2P_2O_7$ and beta- $Ca_2P_2O_7$ phases at 450 ^oC and 750 ^oC respectively. SEM investigations showed the appearance of microcracks and rod like structures of the bulk materials. There was no removal of functional groups after the laser irradiation but decrease in intensity of peaks in the FTIR spectra of biomaterials was pointed out.

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