# Fabrication of Scaffold in Tissue Engineering using Selective Laser Sintering Process

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## ABSTRACT

Bone defects are serious complications that are caused by extensive trauma or tumor. The traditional therapies fail to repair these defects. Tissue engineering scaffold can be used to regenerate the damaged tissue. This paper describes an experimental investigation of bone scaffold to measure the porosity using gas porosimeter and suggest an alternative to bone scaffold. The prototype was made using additive manufacturing of selective laser sintering technique. The material chosen for the study was synthetic polymer PA12. The behaviour of actual bone and prototype had been observed under compressive load of fixed interval loading condition. Mechanical properties of polymer had been evolved and compared with actual bone.

*Keywords:* Additive Manufacturing (AM), bone scaffold, polymer (PA12), Selective Laser Sintering (SLS), Young's Modulus (E)

## INTRODUCTION

Every day a number of surgical procedures is performed to replace or repair tissue that has been damaged through disease or trauma, combining cells from the body with highly porous scaffold biomaterials. Tissue engineering is a science in which the damaged tissues can be replaced with the porous biomaterials (O'brien, 2011; Atala, 2004; Bonassar & Vacanti, 1998). Literature review shows that researchers are facing problem to replace or regenerate the tissue in the human body.

Surgical treatment which typically focuses on transplanting tissue from one site to another in the same patient is called as autograft and from one individual to another called as a transplant or allograft (O'brien, 2011). Problems faced in these techniques are harvesting autografts. An

autograft which is expensive, painful, constrained by anatomical limitations. Autograft is associated with donor-site morbidity due to infection and hematoma (O'brien, 2011). Similarly, allografts or transplants also have serious constraints due to problems with accessing enough tissue for all of the patients who require them. Also there are risks of rejection by the patient's immune system and the possibility of introducing infection or disease from the donor to the patient.

Alternatively, tissue engineering helps to regenerate damaged tissues, instead of replacing them, by developing biological substitutes that restore, maintain or improve tissue function (O'brien, 2011; Atala, 2004; Bonassar & Vacanti, 1998). The basic requirement of scaffold is that it must be biocompatible and its strength should be in such a way that it should withstand body weight. An attempt has been made to suggest an alternate material which will fulfill basic requirement of scaffold. In this paper an experimental investigation of bone scaffold is carried out to suggest an alternative material (PA12). Mechanical properties of PA12 are compared with actual bone scaffold. The material chosen for the study is PA12, which is a synthetic polymer (Lu et al., 2000; Oh et al., 2003; Rowlands et al., 2007).

As discussed in previous section bone scaffold must be porous. Porosity is the ratio of void volume to the total volume. Porosity is essential in any bone scaffold because it gives strength to the replaced part when the cell grows. Also it goes into the porous structure and provides strength to the scaffold. As porosity increases strength of the bone decreases. Literature shows various techniques to measure porosity of bone (Amziane & Collet, 2017). These techniques can be employed based on the size of the pores. Experimental techniques to measure porosity are as follows.

## Mercury Intrusion Porosimetry (MIP)

In MIP, the volume of liquid that penetrates a solid is measured as a function of applied pressure. The analysis is based on the capillary law governing liquid penetration into small pores. Since mercury is a non-wetting liquid for most materials, an externally imposed pressure is required to force it into the pores of a porous solid. The smaller the pore size, the greater the pressure required to force the mercury, into the pore. This technique is effective when pore size is larger than about 3.5 nm (Lawrence et al., 2007).

## Gas Expansion Method

This is one of the techniques used to measure porosity of bone sample as well as polymer. A sample is placed into an airtight holder and pressure is applied to a reservoir of known volume. After the pressure has stabilized, valve is opened, which permits the gas within the reservoir to expand into the holder. After equilibrium is reached, the new pressure of the system is measured and recorded. It works on the principle of Boyle's law.

After reviewing extensive literature (Lawrence et al., 2007; OFITE, 2009; Wong & Hernandez, 2012; Slotwinski & Garboczi, 2015; Mueller, 2012; Bidanda & Bártolo, 2007), it is observed that prototype of scaffold can be prepared by AM techniques. Widely used AM techniques are Stereo Lithography (SLA), Fuse Deposition Modelling (FDM), 3D Printing (3DP) and Selective Laser Sintering (SLS) (Salmoria et al., 2008). Using these techniques solid object can be created. Out of these techniques one of the applicable techniques is SLS technique. This technique uses layer by layer through the heating and fusion of powder material using an infrared laser beam. The main advantage of this technique is that porous prototype can be fabricated. This will help get the required strength and surface finish. The other advantage of SLS technique is that no post curing required.

## MATERIALS AND METHODS

### Sample Preparation

Scaffold bone specimen and its prototype (PA12) specimen were chosen for the study. Procedure to prepare samples for the analysis is as follows:

**Procedure for Scaffold Bone.** A fresh, part of femur bone was chosen as a specimen for the study. The size of bone chosen for the study was 2:1 ratio (length is two times its diameter). Figure 1 shows actual bone sample specimen. The size of actual femur goat bone is having outside diameter of 13.47 mm, thickness of 2.04 mm and length of 26.97 mm. Considering limitation of experimental setup and availability of actual bone sample the above size had been chosen for the study.

The specimen dipped into a hydrogen peroxide solution for 12 hours to clear the soft tissue or flesh. Hydrogen peroxide is a natural antiseptic chemical which bleaches the bone without damaging it and also soften the tissue. The mixture of hydrogen peroxide and water was used for cleaning the bone and also less concentric mixture was used, which helped to sterilize the bone and also bone would not dissolve the tissue. Specimen was rinsed well in cold water to remove white powder residue on the bone surface.



*Figure 1*. Actual bone scaffold of goat under investigation

**Procedure for Scaffold Bone Prototype.** The criterion for the selection of material in order to prepare a prototype of bone scaffold is its biocompatibility and strength. A synthetic polymer PA12 material was used to prepare prototype of actual bone. AM technology (SLS) was used with $CO_2$ laser which had a wave length of 10.6 mm and laser beam diameter of 250 mm. The process parameter used to prepare prototype had a laser scan speed of 45 mm/s and chamber temperature 120°C. The property of PA12 material and bio-characteristics are shown in Table 1 and Table 2.

Table 1
*Properties of PA12 material*

| Sr. No | Properties | Values |
|--------|-----------|--------|
| 1 | Tensile modulus | 1500 – 1800 MPa |
| 2 | Ultimate strength | 40 – 45 MPa |
| 3 | Elongation at break | 10 – 20 % |

Table 2
*Bio-characteristic of PA12 material*

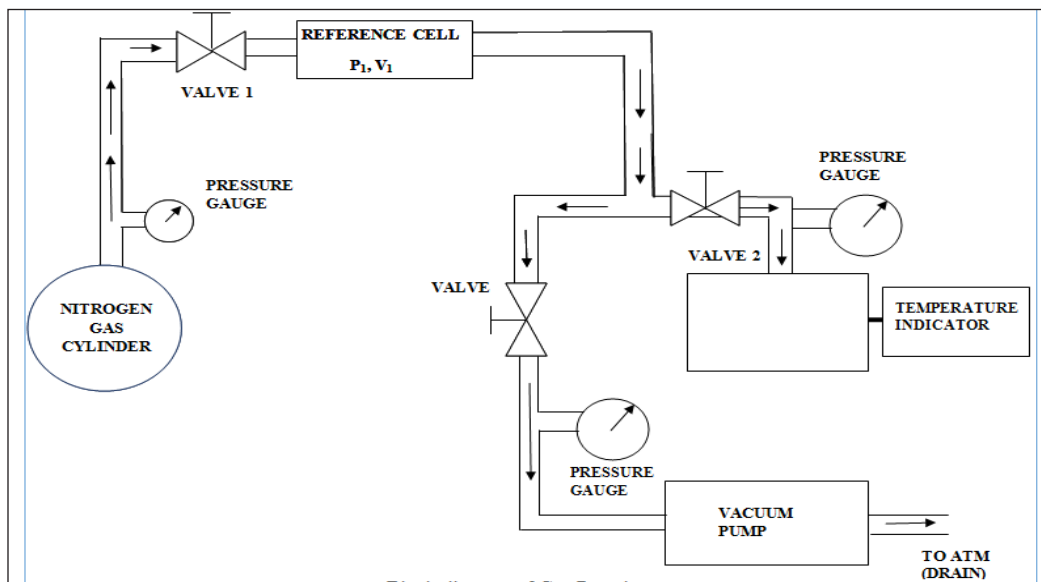| Sr.No. | Bio-characteristic | Human Body Acceptance |
|--------|-------------------|----------------------|
| 1 | Biocompatibility | Yes |
| 2 | Biofunctional | Yes |
| 3 | Bioactive | Yes |
| 4 | Bioinert | Yes |
| 5 | Sterilizable | Yes |



*Figure 2*. Schematic of Gas Porosimeter for measurement of grain volume of bone scaffold

The procedure to measure the porosity is as follows:

Boyle's law used to find the grain volume ($V_g$) of the scaffold (Figure 2). Bulk volume ($V_b$) can be obtained by using hand held scanner and difference of bulk volume and grain volume to the bulk volume gives the porosity.

Porosity is calculated by using following formula

% Porosity = $[ (V_b - V_g) \div V_b ] \times 100$

## Experimental Setup

An experimental set up shown in Figure 3 had been developed to measure the porosity of bone scaffold and its prototype. The test was carried out at the constant temperature. Nitrogen gas was used to carry out test on goat sample with a pressure of up to 1.37895 $N/mm^2$ (200 PSI). A reference cell was having a known volume, $V_1$ and pressure, $P_1$. The sample cell was for testing core samples of approximately 4 cm in diameter by 5 cm long with volume $V_2$ and pressure $P_2$. A digital pressure gauge with 0.01 $N/mm^2$ accuracy was attached to find the pressure difference between sample cell and reference cell. The vacuum pump was used for evacuation of nitrogen gas.



*Figure 3*. Gas porosimeter setup to measure the grain volume  of bone scaffold

Two chambers with known volumes were connected by a non return valve. The tested sample was placed in the sample cell whose volume was $V_2$. The pressure in this chamber was $P_2$. The second reference cell (volume $V_1$), initially at pressure $P_1$, was connected to the first by opening the valve between them, thus permitting the gas to expand isothermally. $P_3$ was the equilibrium Pressure in the system with Sample. Volume $V_3$ was obtained by Boyle's law ($P_1V_1 = P_3V_3$) Grain volume ($V_g$) was obtained by difference of volume of the system ($V_2$) without sample and the volume of system was with sample ($V_3$). By scanning

the bulk volume ($V_b$) was obtained as 2.13 cm$^3$. The procedure to obtain bulk volume ($V_b$) is shown in sec 2.1.2. This bulk volume ($V_b$) was required to compute percentage porosity of bone scaffold. For the prototype of bone scaffold the percentage of porosity was obtained as 16.94%.Whereas the percentage of scaffold bone was 14.04 %. To evolve mechanical properties the percentage porosity plays a key role which is discussed in next section.

## RESULTS

### Structural Analysis of Bone Scaffold and Polymer

Table 3 shows test result for bone sample and PA12 polymer prototype. Test was conducted on universal testing machine (UTM) under uniaxial compressive load. Compressive load was applied in the interval of 100 N. Behaviour of polymer specimen had been compared with bone scaffold. Evolution of material properties like young's modulus (E), stiffness are discussed in the following paragraph.

Figure 4 shows the load versus deflection for the actual bone and PA12 polymer. The behaviour of actual bone and PA12 material was observed at an interval of 100 N. It was observed that initially up to 600 N deflections were directly proportional to load. Only 7.34 % variation was observed in actual bone and polymer. After 600 N, 16.31 % variation in deflection was observed. Maximum load i.e. at (2280N) the deflection of 4.112 mm was for actual bone sample and for polymer prototype 4.511 mm was observed.

The behaviour of both samples under specific interval loading condition was observed by plotting stress versus strain graph. Figure 5 shows the stress versus strain plot for the bone and PA12 polymer. Initially stress is directly proportional to strain for both the samples, this shows elasticity of material. From the above Figure 4, it is observed that
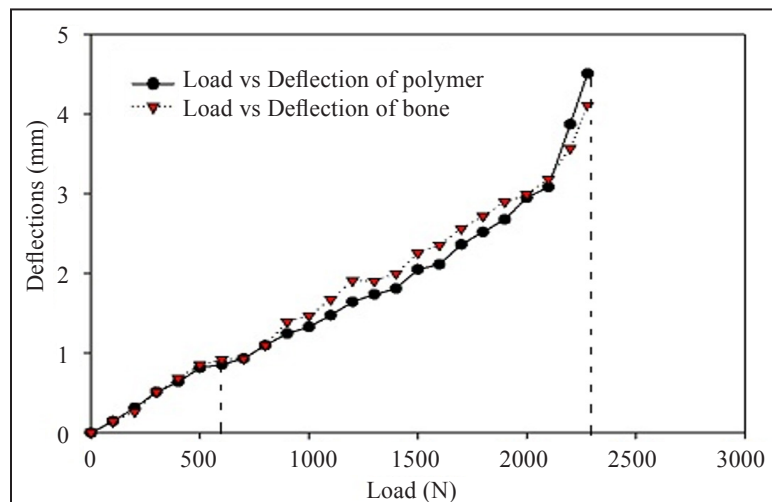


*Figure 4*. Load Vs deflection curves for the polymer prototype and bone sample with fixed interval of load

Table 3
*Test results for PA12 specimen and bone scaffold*

| Sr. No | Load (N) | PA12 sample size, diameter of 13.47 mm, thickness of 2.04 mm and length 26.97 mm | | | | | Bone sample size, diameter of bone 13.47 mm, thickness of 2.04 mm and length 26.97 mm | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Deflection (mm) | Strain | Stress (MPa) | Youngs Modulus MPa | Stiffness (N/mm) | Deflection (mm) | Strain | Stress (MPa) | Youngs Modulus (MPa) | Stiffness (N/mm) |
| 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.000 |
| 2 | 100 | 0.145 | 0.005 | 1.366 | 273.2 | 689.655 | 0.142 | 0.005 | 1.366 | 273.2 | 704.225 |
| 3 | 200 | 0.313 | 0.012 | 2.732 | 235.427 | 638.978 | 0.263 | 0.013 | 2.732 | 210.172 | 760.456 |
| 4 | 300 | 0.512 | 0.019 | 4.098 | 215.884 | 585.938 | 0.512 | 0.020 | 4.098 | 204.918 | 585.938 |
| 5 | 400 | 0.638 | 0.024 | 5.464 | 230.999 | 626.959 | 0.683 | 0.023 | 5.464 | 237.586 | 585.652 |
| 6 | 500 | 0.816 | 0.030 | 6.831 | 225.761 | 612.745 | 0.851 | 0.032 | 6.831 | 213.456 | 587.544 |
| 7 | 600 | 0.858 | 0.032 | 8.197 | 257.652 | 699.301 | 0.921 | 0.033 | 8.197 | 248.385 | 651.466 |
| 8 | 700 | 0.931 | 0.035 | 9.563 | 277.025 | 751.880 | 0.931 | 0.036 | 9.563 | 265.634 | 751.880 |
| 9 | 800 | 1.098 | 0.041 | 10.929 | 268.446 | 728.597 | 1.098 | 0.039 | 10.929 | 280.230 | 728.597 |
| 10 | 900 | 1.245 | 0.046 | 12.295 | 266.344 | 722.892 | 1.392 | 0.048 | 12.295 | 256.148 | 646.552 |
| 11 | 1000 | 1.329 | 0.049 | 13.661 | 277.233 | 752.445 | 1.469 | 0.051 | 13.661 | 267.867 | 680.735 |
| 12 | 1100 | 1.475 | 0.055 | 15.027 | 274.771 | 745.763 | 1.675 | 0.056 | 15.027 | 268.345 | 656.716 |
| 13 | 1200 | 1.643 | 0.061 | 16.393 | 269.100 | 730.371 | 1.911 | 0.062 | 16.393 | 264.410 | 627.943 |
| 14 | 1300 | 1.737 | 0.064 | 17.760 | 275.749 | 748.417 | 1.905 | 0.066 | 17.760 | 269.084 | 682.415 |
| 15 | 1400 | 1.81 | 0.067 | 19.126 | 284.983 | 773.481 | 1.999 | 0.068 | 19.126 | 281.260 | 700.350 |
| 16 | 1500 | 2.051 | 0.076 | 20.492 | 269.461 | 731.351 | 2.259 | 0.077 | 20.492 | 266.127 | 664.011 |
| 17 | 1600 | 2.113 | 0.078 | 21.858 | 278.991 | 757.217 | 2.359 | 0.080 | 21.858 | 273.224 | 678.253 |
| 18 | 1700 | 2.365 | 0.088 | 23.224 | 264.842 | 718.816 | 2.565 | 0.089 | 23.224 | 260.944 | 662.768 |
| 19 | 1800 | 2.522 | 0.094 | 24.590 | 262.965 | 713.719 | 2.722 | 0.095 | 24.590 | 258.844 | 661.278 |
| 20 | 1900 | 2.679 | 0.099 | 25.956 | 261.307 | 709.220 | 2.899 | 0.101 | 25.956 | 256.993 | 655.398 |
| 21 | 2000 | 2.951 | 0.109 | 27.322 | 249.707 | 677.736 | 2.997 | 0.111 | 27.322 | 246.148 | 667.334 |
| 22 | 2100 | 3.087 | 0.114 | 28.689 | 250.641 | 680.272 | 3.187 | 0.116 | 28.689 | 247.315 | 658.927 |
| 23 | 2200 | 3.874 | 0.144 | 30.055 | 209.234 | 567.888 | 3.574 | 0.127 | 30.055 | 236.651 | 615.557 |
| 24 | 2280 | 4.511 | 0.167 | 31.148 | 186.222 | 505.431 | 4.112 | 0.149 | 31.148 | 209.044 | 554.475 |

polymer specimen shows 9 % deviation with the bone sample. This difference may be because of porosity difference in the samples. Maximum ultimate stress for bone sample and prototype is 31.148 MPa. As discussed in above section, approximately 20% porosity difference is observed in prototype, which affects strength of the polymer. Hence this study can be further extended to improve strength of the polymer material by using composite material, which should be biocompatible and should give adequate strength, to withstand load.
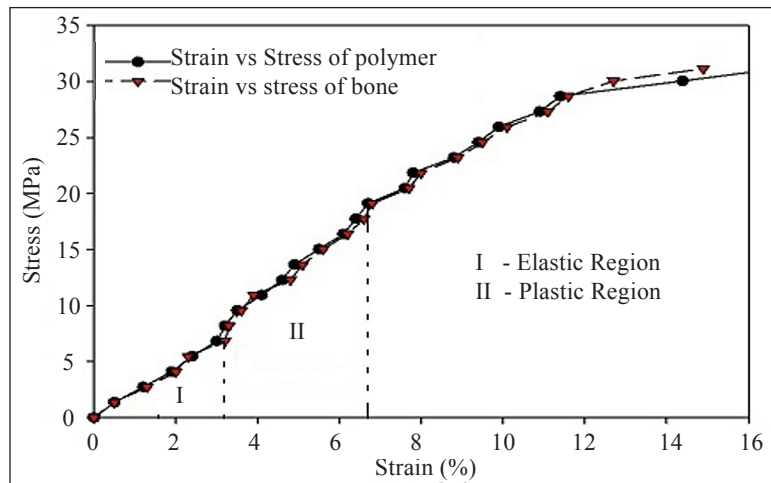


*Figure 5*. Stress-strain curve for polymer prototype and bone sample

Figure 6 shows the deflection versus Young's modulus for the polymer (PA12) and bone. At each point young's modulus of PA12 is observed. It is also seen that PA12 material does not have adequate strength as compared to bone material. It is observed that at maximum deflection of bone is at 4.511 mm, the young' modulus is a 186.22 MPa and the deflection for PA12 is 4.112 mm and the Young's modulus is 209.04 MPa. The difference between PA12 is 10.44 % difference with the bone sample. Further study had been extended to observe the characteristic property of material i.e. stiffness. The effect of various stiffness ratios on change in deflection had been studied.

Figure 7 shows effect of various stiffness ratios ($K_{poly}/K_{bone}$) on percentage change in deflection. From the Figure 7, it is observed that polymer material shows 30.23 % difference with the bone sample. From the graph it is observed that with increase in stiffness ratio percentage, difference of polymer with the bone material is less than 9 %. As brought out in above section. This study could be useful to get exact value of stiffness of the PA12 material. It is seen that the % deflection in case of bone is 30% more compared to that of bone scaffold.
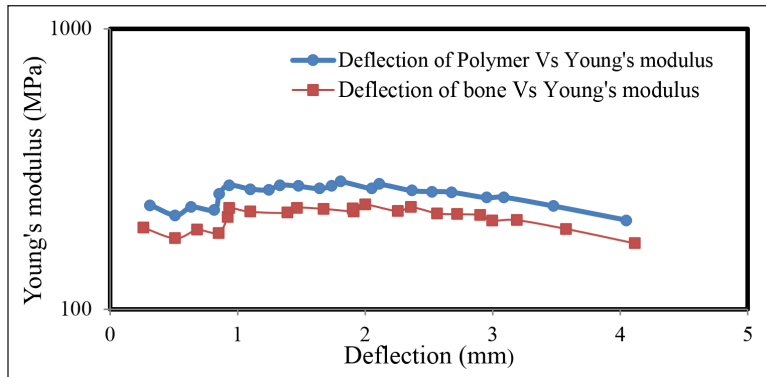
*Figure 6.* Deflection Vs Young's modulus of polymer prototype and bone sample
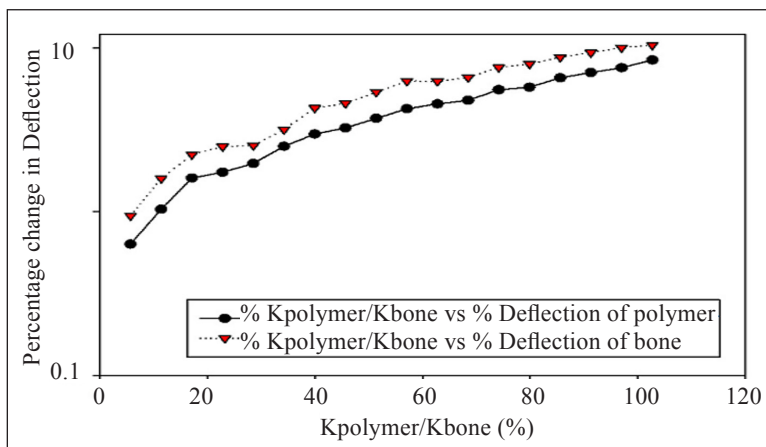


*Figure 7*. Effect of various stiffness ratio on deflection

## DISCUSSIONS

Recently biomechanical science is an emerging area to regenerate damaged tissue. Hence study has been performed to suggest an alternate for human scaffold using PA12 biocompatible material. An important property of PA12 polymer is porosity, which gives strength to the replaced part. An experimental investigation has been performed to measure porosity of bone scaffold and its prototype. Behaviour of bone scaffold and its prototype has been analyzed. It is observed that strength of the polymer can be enhanced by using composite biomaterials.

## CONCLUSION

Biocompatible polymers are synthetic or natural polymers used to replace part of a living system or to function in intimate contact with living tissue. It intended to interface with biological system to evaluate, treat or replace any tissue, organ or function of the body. A

polymer will be considered biocompatible, if it allows the body to function without any complications such as allergic reactions or other side effects. Considering this issue polymer PA12 had been selected which was compatible with human body.

The study reveals that to replace actual bone, synthetic polymer can be used. The material should be biocompatible and should have sufficient mechanical strength to withstand load. The material chosen for the study is PA12 synthetic polymer material. The prototype of scaffold bone has been manufactured using selective laser sintering, which is an additive manufacturing technique. An experimental set-up has been developed to measure porosity of actual bone and polymer scaffold.

The structural analysis of bone and polymer has been performed. Material property of polymer like young's modulus (E) shows 1.03% difference with the bone sample. The prototype shows more deformation before ultimate load. It is seen that polymer material does not have adequate strength as compare to bone material. The study can be further extended to get required strength in the polymer. Therefore there is need to have composite biomaterials for the human scaffold like chitin, chitosan, nanocrystalline cellulose. This can enhance the mechanical properties of bone.

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