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Design and 3D printing of non-stochastic polylactic acid structures for biomedical applications

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Abstract

The mechanical characteristics of Polylactic acid (PLA) created using the fused deposition modeling (FDM) technique for biomedical applications are investigated in this paper. PLA is a low-cost, biocompatible polymer that may be employed in a variety of biomedical applications. Bone scaffolds must have a porosity range that is optimal for tissue development, injection of rheumatic agents, and tailoring of the mechanical characteristics of the wounded area. The most serious issue with polymeric implants is a mismatch in mechanical characteristics between bone and the implant, which leads to degeneration of the surrounding bone structure, implant disassociation, and implant deformation. The present work covers three-dimensional (3D) printing and mechanical characterization of PLA manufactured by using the FDM process. It also comprises the design and modelling of PLA structures with porosities ranging from 10% to 60% and research into their impact on mechanical characteristics. Mechanical parameters were measured, such as compressive strength and elastic modulus. The elastic modulus of the planned PLA structure was determined to be 6.5 GPa at 0% porosity, while 40% porosity was found to be the optimal amount. The mechanical characteristics of the real cancellous screw are used to create and customize Cancellous Screws with varying porosity ranges.

Keywords: Polylactic acid, Fused deposition modeling, Elastic modulus, Porosity, Additive manufacturing

1. Introduction

Additive manufacturing (AM) is a method of producing items by layering materials together, usually layer by layer, using three-dimensional (3D) computer-aided design (CAD) data. AM technology enables sophisticated designs and has an influence on production processes in a variety of industries, including automotive, aerospace, and biomedical engineering. AM technology is being used to construct orthopaedic implant structures and scaffolds due to advancements in additive manufacturing technology and the availability of numerous materials. In general, the implants are used as structural reinforcements inside the human body for load-bearing applications. The temporary implants include screws and plates, whereas the permanent implants include hip and knee joints and require more toughness, sufficient strength, and resistance to wear between the joints. Selective laser sintering, selective laser melting, direct metal laser sintering, fused deposition modeling (FDM), and electron beam melting are among the AM technologies that may produce functioning components for orthopaedic applications in a single step. Furthermore, the dense implant has a lower rate of bone production and in-growth [1-2]. These problems can be minimized by using porous implant structures. The screws that are implanted need to be temporary and degradable to remove the need for a secondary operation. Dense screws cannot be degraded like plates that can be manufactured with voids, and temporary implants, such as porous screws, cannot sustain the strength requirement capabilities. To match the required properties, nonstochastic structures have been developed and the porosity provided is developed by the means of these structures. One such example of 3D printed PLA screws is shown in Figure 1. Various authors have been studying the development of these implants and scaffolds.

Figure 1 3D printed PLA screws.

Agarwal et al. [3] studied biodegradable materials for biomedical use in the fabrication of plates, screws, clips, sutures, hooks, and most recently, tissue engineering scaffolds. Since bone is a living tissue that remodels in response to the strain it bears, it might have an impact over time, which is known as stress shielding. Fixation devices made of biodegradable materials can decay and their mechanical qualities diminish, allowing the broken bone to be reloaded until its full load-bearing capability is restored [3]. A studied biodegradable polymer especially Polylactic Acid (PLA) and Polyglycolic acid (PGA), are two polyesters that are important in the biomedical field. These polymers may be made to have specific mechanical and physical characteristics for specific applications. It was found that degradation characteristics are influenced by several factors, including molecular structure, crystallinity, and copolymer ratio. The use of these materials in the orthopaedic sector and biocompatibility experiments have been conducted in this study. It has been pointed out that PLA and PGA are not the same material and have distinct biotechnological features [4]. studied thermal properties, PLA properties include mechanical properties, swelling behaviour, degradation rates, and cytocompatibility. According to the findings of the assessment, it is conceivable to create a family of degradable elastomers that cover a wide range of qualities. The usual values obtained from the investigation are 2-5 MPa for elastic modulus and 5-30% yield stresses, with degradation rates of 80-100% after 6 months [5]. studied the PLA production for tissue engineering. In this study, he noted that biodegradable polymers are recognized as one of the best possibilities for biomedical devices since they are destroyed by simple hydrolysis into compounds that the human body can metabolize [6]. the fabrication of biodegradable materials that can guide and encourage tissue regeneration has been researched. In this work, the fabricated scaffolds with suitable biodegradability, interconnectivity, and mechanical properties have been studied. The compressive strength of the scaffold was determined by the geometry of the scaffold as well as the compatibility of technology and materials for improved scaffold development, according to the findings [7]. investigated the fabrication of scaffolds using a variety of input parameters supplied to the printing machine. In this study, changes in various parameters including print speed, pressure, and hatch spacing were investigated. The mechanical compressive strength of uniform and gradient scaffolds was investigated in this work [8]. investigated fully dense PLA bricks produced by 3D printing. The blocks are made using a single fused deposition modelling process in a single direction. The specimens are tested in tension, compression, and fracture after the blocks have been manufactured. The elastoplastic material reaction was greater in the extrusion direction than in the transverse direction, according to the findings [9]. investigated the porous scaffolds, which can be utilized to treat trabecular bone deficiencies. The examples are printed in three dimensions and evaluated for creep and impact resistance. The test findings demonstrated that the maximum force of destruction at an impact of 119 MPa may function under a load of up to 10MPa without changing form or losing mechanical qualities. The scaffolds that are created can be utilized to repair minor bone deficiencies [10]. investigated the construction of a 3D printed PLA scaffold structure's suitability for bone tissue engineering. Different geometrical constructions have been built and described utilizing 3D printing. The results revealed that fabricating a scaffold with 90% porosity is not advised since it may cause the scaffold's mechanical qualities to deteriorate [11]. investigated the low cycle characteristics of 60% porosity 3D printed scaffolds. Two distinct geometrical pores have been investigated in this study. It has been stated that the circular pore scaffold has a steady resistance to fatigue damage, which is advantageous for bone healing. The fatigue behaviour of circular pores is consistent throughout the structure, and the stress concentration is homogenous [12]. have studied the influence of pore shape and size on specimen mechanical strength Low mechanical strength is a significant obstacle for porous scaffolds in bone tissue creation, according to the findings. When compared to other constructions, the honeycomb structure has superior mechanical qualities. The influence of structural geometry has been shown to play a crucial part in the specimen's structural integrity in this investigation. In comparison to other structures created by other methods, the structure created by 3D printing has great strength. Smaller pore sizes are recommended for bone restoration since they retain high strength while also allowing for tissue growth [13]. studied the physicochemical and biological characteristics of 3D printed specimens with different pore sizes. Structural, chemical, and biological investigations were conducted to evaluate these qualities. The 3D printing procedure reduced the molecular weight of PLA and the temperature at which it degraded in this study but had no effect on the polymer's semi-crystalline structure. FDM technology is a quick and repeatable method for creating tridimensional custom-made scaffolds for tissue engineering [14]. have adjusted the Pore size values to adjust porosity, and porous scaffolds were 3D printed using printer shown in Figure 2.

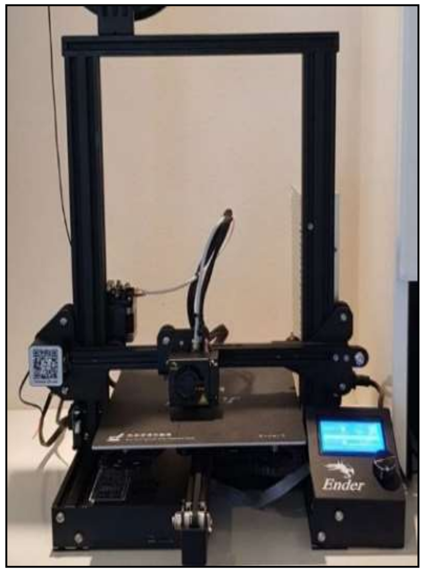

Figure 2 3D printing FDM machine.

The porosity range was set between 0.5 and 0.75, and the mean pore size was set between 0.1 and 0.3 mm to achieve the best results. The ideal option was found to be a strut with a height between layers of 0.72m and a radius of 0.15 mm [15]. utilized Fused deposition modeling to create gyroid-based scaffolds with a spring back effect. Porosity is assessed using micro- computerized tomography (CT), and mechanical characteristics are established using compression tests that take into account geometry, printing conditions, and PLA crystallization. Gyroid structures exhibited isotropic behaviour when compared to strut-based scaffolds. The gyroid structure was chosen as one of the finest architectures for tissue engineering based on the findings [16]. have investigated the fabrication of a high-strength biodegradable thermoset polymer for internal fixing. In his work, it has been stated that screws made of poly glycerol sebacate (PGS) had excellent osteointegration. A lathe machine has been used to effectively make bone screws made of PGS polymer. The results demonstrate that PGS has a bending strength of 122.01, 8.82 MPa, indicating that it has a lot of promise for fixation devices. Changing the reaction time and monomer ratio can alter the polymer's degrading characteristics [17]. studied the influence of form on 3D printed lattice structures. Different non-stochastic lattice structures are built and characterized, each lattice construction is put through its paces in terms of compression, shear, and bending. Because of their high-shear and out-of-plane compression strength, the three truss-like lattice biopolymer non-stochastic structures examined appear to be well suited for usage in possible impact applications [18]. have investigated various strut-based structures, and triply periodic minimal surfaces that have been derived mathematically, it has been observed that the octet truss structures have shown stretching dominated behaviors [19].

Several authors have studied biodegradable polymers such as PGS, PGA, and PLA and their applications for the fabrication of screws, plates, and engineering scaffolds. Authors have also examined the effect of porosity on the degradation, Structure, Strength, Creep, and Impact resistance. Efforts have been put in this paper to tailor the elastic modulus of the specimen to that of the bone, as the mismatch of the elastic modulus can cause a stress shielding effect. To reduce the stress shielding effect, the specimen has been made porous. The porosity applied across the screw helps inflow of nutrients, cell ingrowth and faster degradation as per the application. In general, screws require compressive strength and torsional stiffness, Structures must be developed to preserve mechanical qualities and maintain degradation. Inducing porosity in a screw diminishes its strength and stiffness, thus it's important to keep the required qualities and keep deterioration under control. Lattice structures based on octet truss have been created. In general, octet truss-based lattice structures have a linear strength-to-stiffness ratio and a spring rebound effect that makes them acceptable for load-bearing applications. It's also lightweight and has strong stress-resistance properties. As a result, it is appropriate for the manufacturing of screws.

2. Materials and methods

2.1 Materials

PLA is the material of choice for compressive specimens. Polyester is a naturally generated substance made from a carbohydrate source such as maize starch or sugarcane fermented under regulated circumstances. Lactic acid or lactide monomers can be used as building blocks. They'll be polymerized into PLA eventually. Wet milling is the first step in the maize processing process. This is where the starch is separated from the water. After that, the starch is cooked with acid or enzymes. This procedure converts starch to dextrose (D-glucose), sometimes known as maize sugar. Finally, glucose fermentation yields L-Lactic acid, which is the primary component of PLA. There are two techniques for making PLA plastic from lactic acid. The first employs lactide as a transition state, resulting in a higher molecular weight. The direct polymerization of lactic acid is the second approach. PLA is rigid, robust, and biodegradable. It emphasizes appearance and strength above toughness. PLA is a biodegradable thermoplastic derived from renewable resources. Cornstarch, sugar stick, tapioca roots, and even potato starch are examples. This makes PLA the most environmentally friendly 3D printing material, as opposed to petrochemical-based polymers like acrylonitrile butadiene styrene (ABS) or polyvinyl alcohol (PVA) Those who print for display screens or tiny domestic uses are generally drawn to the large range of possible colours and translucencies, as well as the glossy-shiny feel.

2.2 Methodology

Internally, bones usually have a stochastic structure. However, because these structures are hard to produce in the actual world, we must rely on non-stochastic structures. The categorization of structures is shown in Figure 3.

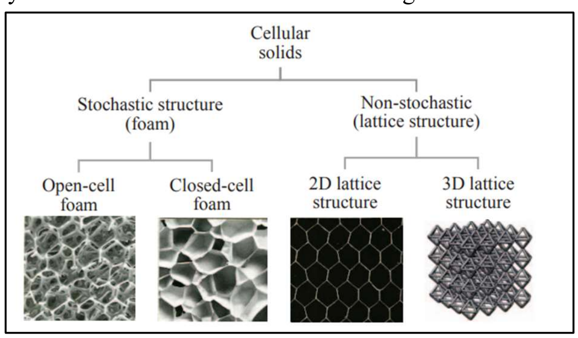

Figure 3 Classification of cellular solids [20].

Because of the free form created structure, the test samples fabricated utilizing the Fused Deposition Modeling technique have porosity. Porous samples vary from dense samples in that they have pores; numerous forms of porous structural designs are shown in Figure 4 [22].

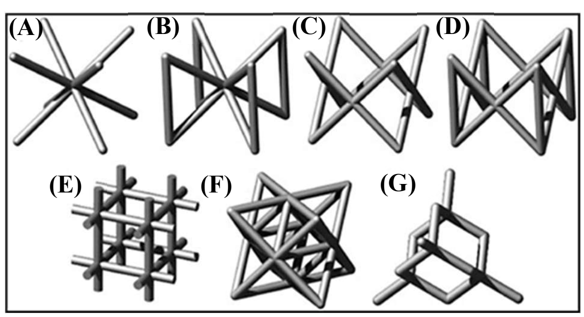

Figure 4 Different kinds of lattice structures (A) BCC, (B) BCCZ, (C) F2CC, (D) F2CCZ, (E) Cubic, (F) Octet Truss, (G) Diamond.

Different types of lattice structures with differing Porosity levels have been created, and these structures are employed in the characterization of load-bearing implants. Gyroid, diamond, Gibson Ashby, and others are examples of structures. These are seen in Figure 5.

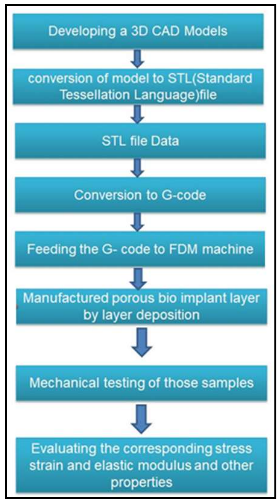

Figure 5 Key steps involved in this methodology.

Fused Deposition modelling has opted for the manufacturing of the specimens that are to be tested mechanically. The FDM process is a type of additive manufacturing process by which 3D objects, prototypes and products can be easily manufactured through a computer-aided process. The machine has an extrusion head attached which can move along the 3 axes to get the desired shape. Polymers such as PLA, ABS, Polyethylene terephthalate glycol modified (PETG) and Polyetherimide (PEI) can be used as input materials as they have lower melting points, and the extruder head can melt the material and form the required shape. The printer builds the required object in a layer-by-layer fashion, once the material is deposited, it cools down and solidifies forming a firm shape. As the material of interest in the paper is PLA, it can easily be manufactured using FDM and can be manufactured with high precision and tolerance.

2.2.1 Design of porous PLA specimens

The PLA has been put through compression and tensile tests in this chapter. Compressive specimens are tested for compressive strength over a wide range of porosity. As previously stated, the specimens are developed by American Society for Testing Materials' guidelines (ASTM) requirements. The complete process is broken into two sections, The complete process is simulated in the first section and validation of it is done experimental they in the second section The findings of the experiment and simulation are compared, and graphs are plotted accordingly.

Strut sizes have been altered in the Lattice Structures, resulting in a variation in the volume of the specimen formed. The general volume of the specimen is taken into account according to ASTM rules. ASTM D695-Standard test method for testing stiff plastics. The dimensions of the specimens according to the ASTM standard are as follows. The specimen has a 30 mm diameter and a 25 mm length. It’s a design with a short column structure. The volume of the solid compressive specimen is computed using the parameters supplied, and the volume is 1.77×10^4 mm^3.

In Cura slicing software, the designed sample is sliced to prepare for 3D printing; the specimen with an adhesion plate is shown in Figure 6. The specimen is translated to G-code by the machine's needs, and additional build attributes are applied to improve the specimen's finish.

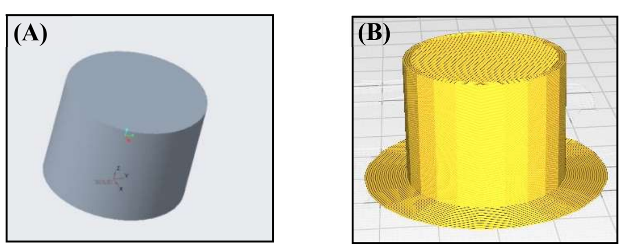

Figure 6 (A) 3D CAD model, (B) layered model with adhesion plate.

There are two ways to modify the volume of the specimen and regulate it accordingly: 1) changing the strut size about volume and 2changing the number of lattice structures included in the volume. The porosity levels and related strut sizes for compressive specimens are listed in Table 1.

Table 1 Porosity variation with strut size.

Porosity (%)	Lattice size (mm)	Strut size (mm)	Solid volume (mm^3)	Porous volume (mm^3)
20	13 x 13 x 13	5	1.77×10^4	1.40×10^4
30	13 x 13 x 13	4.4	1.77×10^4	1.26×10^4
40	13 x 13 x 13	3.8	1.77×10^4	1.07×10^4
50	13 x 13 x 13	3	1.77×10^4	8.86×10^3
60	13 x 13 x 13	2.5	1.77×10^4	6.87×10^3

The compressive test samples are created by the ASTM Compressive Testing of Specimens is described in ASTM D695. Important mechanical parameters like yield strength, ultimate compressive strength, and reduction of area are determined in this test. Material development, design, quality control, and comparison of different sets of Plastics all benefit from this knowledge. These are the dimensions which are taken in mm, which are followed in doing the tensile samples, and the 3D CAD model is shown in Figure 6, which is designed in Creo Parametric 6.0.

A lattice structure is a two-or three-dimensional periodic arrangement of unit cells that are connected to cellular solids. Based on the spatial arrangement of their unit cells, cellular solids may be classified into two types: stochastic and non-stochastic structures. [2-3]. Materials with stochastic structures have a random distribution of their unit cells, whereas materials with non-stochastic structures have an ordered distribution. With the addition of a shell to the specimen, the porous structure is designed and replaced with a solid. Because of its spring-back action, the lattice structure employed is the Octet truss, as seen in Figure 7.

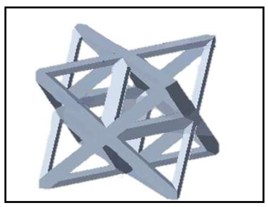

Figure 7 Octet truss lattice structure.

2.2.2 Fem analysis and simulation of porous structures

Diverse porosity levels in 3D CAD models are used to prepare various constructions. In the ANSYS program, these models are turned into analysis files and combined with the PLA material. The analysis is carried out by supplying suitable boundary conditions to the models. The structures have been given a compression load of 300 MPa due to the load-bearing applications by retaining the same amount of stress and strain (von misses). Several authors use finite element method (FEM) to analyze the lattice structures of gyroid, diamond, and Gibson-Ashby analyses. By comparing the other structures, we may obtain different Stress and Strain plots for different structures. The right and appropriate structure must be solved. By including additive manufactured features in the program, the simulation work approximates the theoretical work and exhibits the same behaviour as the real work. The Designed file is converted to International Graphics Exchange Standard (IGES) format, which is accepted by the analysis program, to complete the analysis. The structure is imported into Ansys Workbench 19.1 and the necessary alterations are made, such as changing the units to match the structure's planned dimensions. The structure is meshing and being simulated under the given conditions.

2.2.3 Fabrication and characterization

Feeding a thermoplastic filament into a 3D printer, along with any supports or additional materials if it's a dual extruder 3D printer, is considered fused deposition modelling. The filament is heated to its melting temperature, which varies according to the material, and then extruded onto the build platform, tracing the part’s dimensions given by the Standard Triangle Language (STL) file.

The print head advances up by one layer height after the first full layer is completed before tracing the following layer. This process is repeated layer by layer until the component is finished. The filament hardens to produce a solid object once it has been placed, with each layer cooling before being heated briefly again when the layer above it is deposited. The layer sizes vary according to your taste, but they are approximately a sixteenth of an inch, typically. The compressive specimens were produced using a 3D FDM machine with the process settings listed below. The ideal process parameters for the construction are determined and applied concerning the specimen build, according to one of the authors, Jordan E. Trachtenberg., et al., Process variable characteristics such as layer thickness, raster angle, part orientation, raster width, and air gap have a significant impact on the component quality and mechanical attributes of FDM manufactured parts [21]. As a result, selecting and optimizing FDM process parameters is critical. Printing temperature of 210°C, build platform temperature of 60°C and Room temperature of 32°C are to be maintained. Layer height of 0.12 mm. nozzle diameter of 0.4 mm. Printing speed of 40 mm/s and Infill density of 100% are to be considered.

A variety of optimization strategies and experimental designs for determining the optimum process parameter have been investigated, with the best parameters being used for the construction. higher layer thicknesses may not generate a good specimen surface, resulting in incorrect geometric shape, and to prevent warping of the specimen, the nozzle and bed temperatures should be adjusted to roughly 210°C and 60°C for the PLA material, respectively. The specimens created by the FDM procedure are depicted in the diagram below (Figure 8).

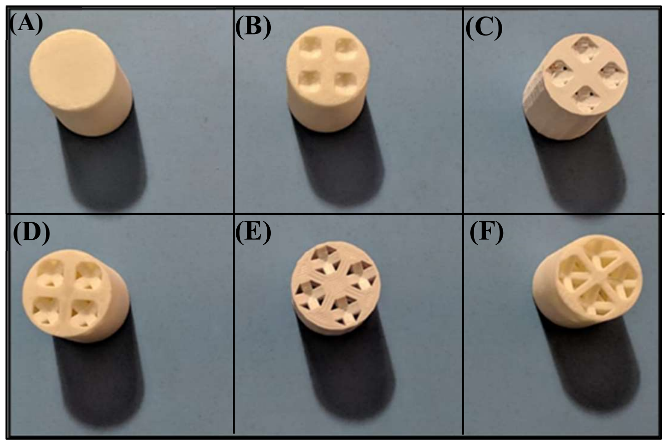

Figure 8 Photographs of Compressive test specimens with varied porosity (A) 0% (B) 20% (C) 30% (D) 40% (E) 50% (F) 60% porosities.

3. Results and discussion

According to the approach, the specimens are developed with varied porosity ranges as shown in Figure 9 ranging from 0% to 60% porosity. The created specimens are then transformed to the analysis software's format, which is IGES, and input into the ANSYS 19.1 application. To match real-world material qualities, the material is allocated to the specimen with poisons ratio, plasticity, and elasticity properties. The specimen's discretion is very significant in finite element analysis. The burden on the specimen is distributed according to the number of nodes and elements given to the specimen, and the correct kind and size of elements are significant. Because porous structures are difficult to mesh with larger size elements, the size of the element in the FEM of porous PLA structure is selected to be 0.01mm. This is because meshing with bigger size elements becomes challenging at the narrow pore edges. The time of iteration is also taken into account, as the time of iteration determines the exact amount of loading time, as well as the corresponding deformation, noticed when the load is applied. The specimen is simulated in the identical environment as the experimental settings, and the values and graphs for the same are presented. In ANSYS 19.2, the simulation is run with the same material characteristics as the real specimen. To get the closest values to the actual circumstances, the specimen meshes into a fine mesh. Tetrahedron Elements are woven into the Specimen. The total number of nodes produced is 117647, while the total number of elements is 71263. For the simulation, a total of 30 steps were taken into account. The sample is secured on one end and loaded with a force of 3000 MPa on the other end.

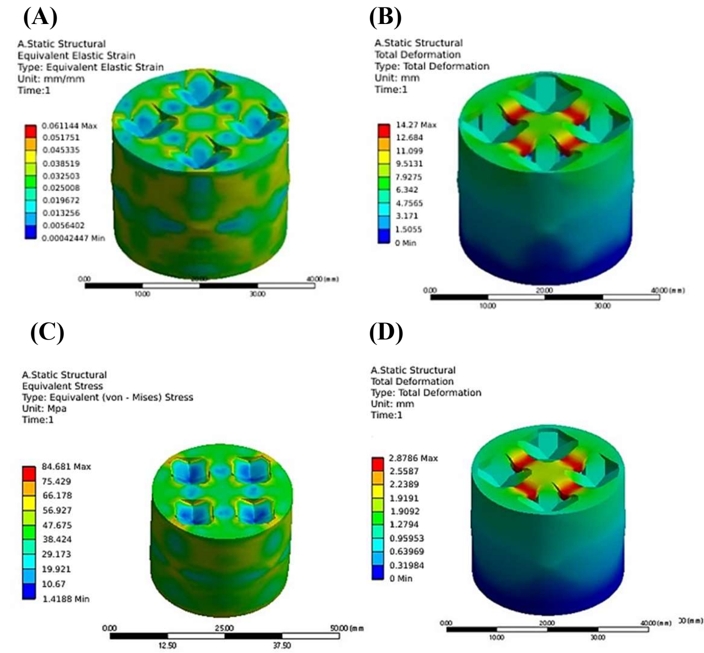

Figure 9 FEM images of porous structures: (A) Equivalent Strain of specimen with 30% porosity, (B) Deformation of the specimen with 60% porosity, (C) Equivalent Stress of specimen with 20% porosity, and (D) Deformation of the specimen with 50% porosity.

3.1 Effect of elastic modulus and deformation concerning porosity

The values of the Strain about the porosity levels are as follows, based on the simulated values of the designs. There is a substantial shift in the deformation of the body about the porosity %, as seen in the graph below. As can be seen from the graph, when the porosity percentage is at 40% or more, there is a significant shift in the deformation of the body. As a result, design considerations must be less than 40% of the body's real porosity.

A finite element analysis of the compression specimens has been carried out and compared to the actual values. The analysis is carried out in Ansys 19, and the corresponding legend values are tabulated in Table 2 and 3.

Table 2 Finite element analysis results showing the variation of elastic modulus and deformation with change in percentage of porosity.

Porosity (%)	Elastic modulus (GPa)	Deformation (mm)
0	9.89	3.49
20	7.75	4.89
30	5.32	6.38
40	3.21	8.36
50	1.87	9.20
60	2.89	11.32

Table 3 Process parameters for the print.

Property	Value
Printing temperature	210°C
Build platform temperature	60° C
Infill density	100%
Layer height	0.12 mm
Build plate adhesion	Skirt
Printing speed	40 mm/s
Nozzle diameter	0.4 mm
Print cooling	Enabled
Room temperature	32°C

3.2 Experimental investigation of compressive stress

The compressive samples were generated by 3D printing with the appropriate parameters described before conducting the experimental inquiry. The computerized universal testing machine is used to test the produced specimens.

The Universal Testing machine (UTM) is secured and locked with compressive jaws. The specimen is place once the gauge length has been adjusted correctly. The specimen is precisely centered in the jaws, resulting in a homogeneous load on the specimen. The specimen is shown in the Figure 10, sandwiched between the UTM's compressive jaws. The specimen is put between the UTM's jaws, and a load is applied to it; the appropriate load is approximately 3000 kg, and the actuation speed is around 1 mm/min. The jaw is just touching the workpiece surface after the gauge length has been adjusted. The specimen's standard, as well as the dimensions data, are entered into the testing machine.

Figure 10 Compression of the 40% porosity specimen in Universal testing machine.

3.3 Effect of elastic modulus and deformation with respect to porosity

Buckling is seen to be more common in structures with higher porosity than in structures with less porosity as seen in Figure11. The structures are entirely crushed, and the associated stress levels are calculated and graphed as shown in Figure 12 and 13. When compared to comparable porous constructions, the specimen's buckling at a porosity level of 40% is extremely noticeable. In terms of application, the porous structure with a porosity range of around 40% is advantageous since the buckling and stress caused are modest when compared to other structures with varying porosity. The specimens' stress and strains are shown in the table below for various porosity ranges.

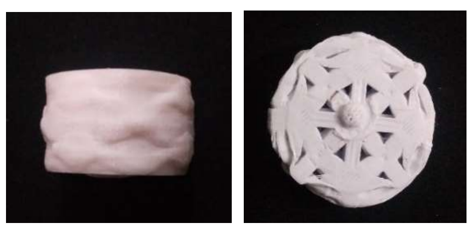

Figure 11 Buckled compressive sample.

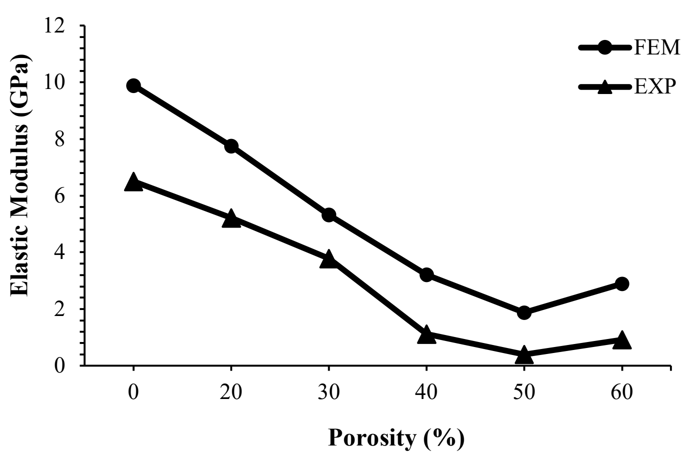

Figure 12 Variation of Elastic Modulus with respect to change in porosity for FEM and experiment in the manuscript (EXP) models.

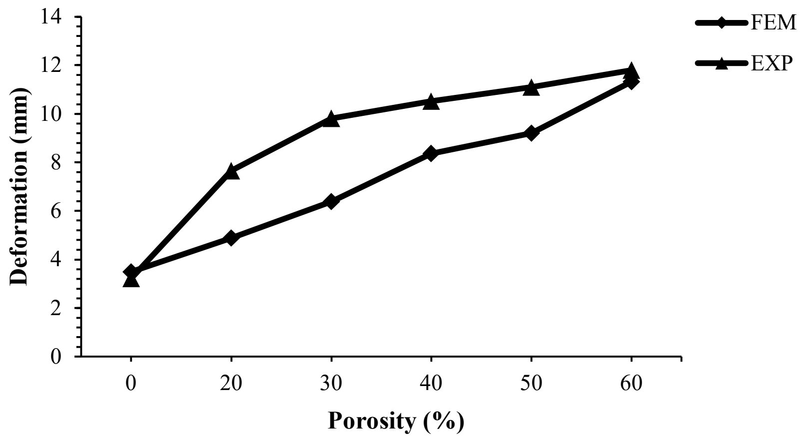

Figure 13 Variation of Deformation with respect to change in porosity for FEM and EXP models.

As investigated by Zhao. et.al. in 2018 the when the porosity range increases from 40% to 90% there is a sharp decrease in the compressive modulus [13], further increase in porosity might reduce the strength below the satisfactory levels, which can be noticed in Figure 12. above 40% there is a sharp decrease in the elastic modulus. In 2019 as studied by Azzuz. et.al., the truss-like non-stochastic lattice structures are well suited for the impact due to their high shear and compressive strength which makes it more suitable in the application of screw as it requires high compressive and shear strength, the structure shows high strength and mechanical rigidity [18], as concluded from the Figure 13 the deformation observed is slow and progressive due to the structural rigidity and the sandwich builds formed from the AM.

4. Conclusion

The compressive stress can be maintained at optimum value when the porosity range is about 40%. The screw is made porous at its center and to maintain its structural integrity the screw is made with an Octet truss lattice structure. It is observed that when the porosity level is maintained at about 40% the screw can be designed with satisfactory values. The compressive tests that are made are to tailor the actual conditions of the build plates and screws. From the graph, it is observed that there is a linear decrease in elastic modulus with respect to an increase in the porosity values but after some significant decrease in elastic modulus there is again a gain in it because of the compression of the specimen, the molecular grains are packed tightly and further compression of the specimen is not appreciated, on further compression, the elastic modulus is gradually increased.

The FEM results and the actual experimental results follow a similar trend line shape; however, it is observed in the elastic modulus that FEM and EXP have differed approximately by 2 GPa, this can be due to the assumptions that have been considered in the FEM such as the contact doesn't change with time, forces applied are slow. Something similar can be observed in the deformation graph plotted for the FEM and the EXP, the FEM method the deformations are slow, and the direction of the force doesn't change with time. These factors have affected the FEM results over the EXP results.

Other Characterization tests are to be performed that tailor the conditions that an actual screw face. Addition of composites and other materials inside the screw to improve its properties. Addition of rheumatic substance in the porous area to improve the healing of the bone. Induction of Nanoparticles in the printed substance and check the wear and surface properties of the specimen. Exploring additional materials that can match the required mechanical properties of the required screw. Osteointegration and Cytocompatibility tests to be performed to check the biocompatibility properties of the material.

5. References

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