Fabrication of Bio-Scaffold by Additive manufacturing technique for Bone Synthesis using Generative Design

Renold Elsen S1, Mahendran T2, Piyush P Atkare3, Amit A Bhosale4

1 Associate Professor, School of Mechanical Engineering (SMEC), VIT University, Vellore.
2 Research Scholar, School of Mechanical Engineering (SMEC), VIT University, Vellore.
3 Masters Student, Master of Engineering, School of Mechanical Engineering (SMEC), VIT University, Vellore.
4 Masters Student, Master of Engineering, School of Mechanical Engineering (SMEC), VIT University, Vellore.
E-mail: renoldelsen.s@vit.ac.in

Abstract. In tissue engineering, the main objective is the regeneration of damaged tissues and organs which is caused due to diseases, injuries, and inherited medical conditions. Today with the help of tissue engineering, damaged tissues and organs can be repaired with the use of artificially made structures for support, often called the scaffold. To construct a 3D structure, materials with special properties such as bioresorbability and biocompatibility are used. This 3D structure is similar to the area of tissue implant to recover injury and regeneration of tissue. The porosity of the matrix should be maintained to promote the differentiation of cells at the control rate. Generative design is an iterative process that includes a Machine-learning program that creates a definite number of design outputs for the given loads and boundary conditions by the designer. The main objective of generative design is to increase the stiffness and to reduce the mass keeping the strength constant. In this Generative design has been used to get the optimum results using Polylactic Acid (PLA). The model of scaffold is based on the injury in femur bone thus the forces acting on the Juvenile hip joint in a one-legged stance are considered. In the last decade, intensive research had been carried out for the development of scaffold with the use of bio fabrication methods to reduce the time of manufacturing, increase precision, and decrease the cost of production.

Keywords: - Tissue Engineering, Bone Scaffold, Generative Design, and 3D printing

1. Introduction

Tissue engineering is using a mixture of cells, engineering, and substances with methods, as well as appropriate biochemical factors to improve or update organic tissues [1]. For the generation of new tissue in diseased/removed tissue scaffolds are used. The three key elements to be considered for the success of tissue improvement are (i) tissue creating cells (ii) supporting structure (scaffold) and (iii) cellular-matrix (scaffold) interactions that direct the tissue growth [2]. For the proper formation of bone Mesenchymal stem cells are used and it gets differentiated in the bone and starts to multiply. Generally, for the proper growth of a human cell, strong support is required which is provided by scaffold. These scaffolds are a 3D structure made of biomaterial that gives a certain surrounding for the cells in order to regenerate tissues and organs. Scaffolds provide proper directions to tissue growth and to maintain structural strength. Until the tissues are regenerated, the scaffold should be structurally stable. Scaffold biomaterials for tissue engineering may be produced in plenty of distinct strategies relying on the applications and the substances used [1]. Moreover, they are implanted in the body to regenerate different tissues and organs in the body. Irrespective of the tissue form, Physical properties, Mechanical properties, and Biological properties are imperative while designing a scaffold to be used in tissue engineering. For a 3D generation of tissue, differentiation, cell growth, proliferation and the degradation product of scaffold huge porosity and connectivity of pores are essential. The porosity is the measure of the availability of void spaces present in
the scaffolds. Highly porous scaffolds are needed for the body cells to infiltrate and attach to it. Hence, the porosity of the scaffold plays a very important role in bone formation and the diffusion of nutrients within the construct. The ideal porosity of the three-dimensional structure should be more than 90%, to successfully promote cell adhesion and growth. For the better supply of oxygen and nutrients pore dimensions should be more. Generally, the scaffold pore size is required to be in the range of 300 to 500 μm [4]. Numerous scaffolds were manufactured with different biomaterials using quite a few fabrication techniques. The biomaterials used to develop scaffolds acts as a template for tissue regeneration and to support the growth of new tissue development in the affected part [5]. Among all the materials available, the most appropriate materials for generating scaffolds are the ceramics, natural and synthetic polymers. These materials are fully degradable into the body’s natural metabolites providing the apt background for the rejuvenation of tissues and organs [6]. Normally replacement of bone tissues will be done by autograft or allograft. Nevertheless, harvesting autograft is costly, traumatic, and restricted with anatomical restrictions. Similarly, allografts also have some limitations in accessing the required limit of tissues for all of the patients and the transplant rejections due to the fact that the recipient’s immune system does not comply with donor tissue [7]. This may also lead to the likelihood of introducing infection or disease to the patient from the donor. To overcome issues like this, highly porous biomaterials can be utilized as a permanent implant that can be synthesized according to various patients’ requirements. These bio-material based scaffolds are biocompatible with the body cells and should guide the cell growth [8]. The scaffold's effectiveness is assessed by how well the cells adhere to the scaffold and the manner it interacts with proteins and other bioactive agents embedded with the scaffold. The most important consideration to be adhered to in selecting any scaffold for tissue engineering is biocompatibility. The biocompatibility is the ability of the biomaterial to adhere naturally to body cells, supports successive cellular activity, and migrate onto the surface forming a new matrix. Post implantation of the scaffold, it must act in accordance immune response to avert from developing adverse inflammatory response which might affect healing negatively or effects rejection by the body [9]. The biodegradability of bio-scaffolds is another important consideration in tissue engineering. Biodegradability is the ability of the bone substrate scaffolds to facilitate the body’s tissues, over time gradually substitute the implanted scaffolds, which allows the cells to develop extracellular matrix. Also, scaffolds materials must be non-toxic and able to exit the body without being toxic or affecting the function of other organs [10]. 3D scaffold fabrication can be done with the use of 3D printing technique namely fused deposition modelling & ink-jet printing with the scaffold design developed in Computer Aided Design (CAD) software. In the above-mentioned method, a Polymeric material is melted and parallely forced out through a moving nozzle in the X and Y direction and stacked layer by layer in the Z direction. 3D printing provides the freedom to incorporate biological agents in the scaffold. Based on the above discussions the scaffold design and materials are an important factor for the regeneration of cells.

2. Materials and Methods

2.1 Scaffold Material and Design

Polylactic Acid (PLA) is used as a material for a scaffold in this work, due to its biocompatibility and biodegradable assets, which can be similar to ideal bio-scaffold properties. It is the most frequently used homogenous, isotropic, and linear elastic fabric used for 3D Printing [11]. PLA 3D-scaffolds can bear up to three compression-heating-compression cycles without delamination [12]. Lactic acid is formed when PLA is degraded which is the main reason to select PLA [13]. For Scaffold design, requirements on a macroscopic scale must duplicate human anatomy, whereas, on a microscopic level, it must accomplish short-term tissue function and in turn improves tissue regeneration. They must be balanced perfectly to have achieved impeccable scaffold design. The scaffold must be designed to have the mechanical properties consistent with the structure of the human part. It must be resilient enough for surgical handling in the course of the implantation procedure. The mechanical property needed in a scaffold will be varying in accordance with the part in which the implantation has to be done. A recent tactic to scaffold design is
established on the usage of hierarchical structures formed by the replication of a unit cell of known properties and geometry [11]. This offers the opportunity to forecast the properties of the scaffolds with the known properties of the unit cell. However, the unit cell approach does not mimic the structure of the target tissue [14,15]. To overcome this limitation, generative design approach can be used to create scaffolds that can mimic the target tissue structure. A porous and interconnected microstructure, as seen in bone, can be achieved using this method.

2.2. Generative Design

The Generative Design (GD) uses the Machine learning approach by exploring the design space autonomously based on the load case provided, and then reporting again to the designer with options it considers promising for further assessment. Because a computer can process data a lot faster than a human, this sort of software permits a far deeper exploration of complex design areas. Traditionally, such an approach has been used to optimize a given model to attain maximum viable overall performance primarily based on a concrete goal. Besides exploring many format options, some other gain of the generative design technique is that we will evaluate designs at a higher degree of detail than possible with traditional processes. Once a set of interesting designs is chosen, they can be further analyzed by way of the human designer, discussed with the stakeholders, and advanced right into a final design. It is vital to note that as it follows a stochastic method based totally on sampling a restrained huge kind of designs from the design space, the overall optimal design will no longer be available through the search process [16]. In this work Porosity, Pore Diameter, Pore interconnections, and Pore Structure are the parameters considered for designing the bone scaffold. For different injuries, different scaffolds have to be modeled. The pore diameter depends on the normal bone unit (223 μm) but pore diameter ranges from 300 and 500 μm.

2.2.1 Forces in femur

The scaffold design is done by assuming an injury in femur bone for which the forces in the femur during the gait cycle/walking cycle is taken for further analysis. The gait cycle discusses the whole activity from the heel which is initially placed on the ground and the following heel contact with the same feat. Thus, it considers all the static and dynamic forces acting on Femur. A two-dimensional biomechanical model of the juvenile hip joint was developed in the one-legged stance. The one-legged stance is the relevant phase during walking considering the loading of the femur [17].
Figure 1 Forces on the femur

\[ W_b = \text{partial body weight}, \ F_{mh} = \text{force of muscle on hip}, \ R_h = \text{hip joint force}, \ R_t = \text{Trochanter resultant force} \]
\[ F_{mk} = \text{Knee muscle + additional muscle force} \]

Table 1. Forces on femur Vs Inclination to the perpendicular

| Force          | An inclination to the perpendicular |
|----------------|------------------------------------|
| \( W_b \)     | 53,87                              |
| \( F_{mh} \)  | 150,57                             |
| \( R_h \)     | 201,19                             |
| \( F_{mk} \)  | 209,63                             |
| \( R_t \)     | 111,63                             |

\( W_b \) = partial body weight, \( F_{mh} \) = force of muscle on hip, \( R_h \) = hip joint force, \( R_t \) = Trochanter resultant force \( F_{mk} \) = Knee muscle + additional muscle force

This scaffold is modeled in Fusion 360 and will serve as a reference for designing the right parameters as a starting body along with two subcomponents named obstacle and preserve. The above-mentioned model of 20x20x20mm has given the PLA material and loaded with the forces on a Femur bone for the generation of iterative models.

Figure 2 Load case and other constraints in Generative Design
In this study, the Factor of Safety is taken as two, and the weight of the body is taken 85 kg. The load case with the obstacle and preserve geometry is given in Figure 2. The ideal scaffold should have similar features like the extracellular matrix. Thus there is no ideal shape and size of a bone-scaffold and pores, so it is considered with a cube for the scaffold of size 20mm x 20mm x 20mm with various sized circular pores over all the surfaces and the orientation of pores is kept 90°.

2.2.2 Fused deposition modeling (FDM)

The FDM method is one of the frequently used additive manufacturing techniques. The typical process flow of 3D printing begins with a CAD model, converting to an STL file the slicing for CNC code generation, and finally printing. In an FDM process, thermoplastic polymers, like ABS, PC, PA, and PLA, the material filament is constantly delivered and heated inside a moving nozzle at temperatures marginally above its melting for easy extrusion via the nozzle to form layers [18]. Through an FDM, a pore size that can be obtained by printing thermostatic polymer is around 50–1000 μm [14]. The major benefits of 3D printing are high cell density and automation than a traditional scaffold-based method [19].

The developed scaffold is tested for the profile and micro features using a Digital USB microscope and the ImageJ open code is used to assess the geometric features of the scaffolds.

3. Result and Discussion

The Generative design approach gives the various design based on the design constraint provided with considering the manufacturing approach as well is done using AUTODESK FUSION 360. The design provided by the GD can be assessed and the most suitable design is selected and further analysis has been done (Figure 4).
The following are the models' Iteration 1 and 2, which were obtained by Generative design with higher porosity (300 microns and 500 microns) and lower mass than the original model. In Figure 4 the design of scaffold with $20 \times 20 \times 20$ mm$^3$ volume and 300 microns developed by GD with the cross-section views are given to show the interconnected pore structures as well the stress patterns based on the load applied.
The second iteration design of scaffold with 20 x 20 x 20 mm³ volume and 500 microns developed by GD with the cross-section views are given to show the interconnected pore structures as well the stress patterns based on the load applied (Figure 6). The design iteration 1 and 2 had no pore opening at the top and the bottom of the structure so a third iteration was done to have pore opening at the top and bottom. The scaffold design with an opening at the top and bottom with 20 x 20 x 20 mm³ volume and 300 microns is developed. The cross-section views of the final iteration with the interconnected pore structures as well as the stress patterns based on the load applied are given in Figure 7.

To assess the mechanical and biological properties of the generated design Figure 8(c) the other standard rod-type configuration Figure 8(a) and the hole type configuration Figure 8(b) are modeled in AUTODESK FUSION 360.
Figure 8 Design comparison

To develop the actual scaffold prototype using the FDM process a special code is required to covert the Virtual model. To achieve the above task a renowned open-source code name CURA is used and the slicing done for the GD model is given in Figure 9.

Figure 9 Layer Generation in CURA software

The developed scaffold prototypes by the FDM process are shown in Figure 10 and they are used for further analysis. The various samples of scaffolds are analyzed through a USB microscope to have a microscopic view on the surface characteristics. ImageJ software was used to measure pore area, strand diameter, strand area, layer thickness, and distance between the pores both vertically and horizontally from various samples.
The rod type scaffold configuration is given in Figure 11 in which the microscopic view of the side portion is analyzed. The area of the strand is found to be 4.66 mm² and from that value diameter of the strand, found using the area of circle formula is 2.435 mm. Then the distance between the two strands was 0.53 mm. The adjacent layer to the layer of strands measures 1.95 mm.

For the hole type configuration as shown in Figure 12 pore area was measured to be 23-micron pore area while the virtual model had 30-micron pore area and the distance between the pores in both vertical and horizontal direction are same and measures 3.914 mm which suggest the Pore distribution is found to be uniform.
The GD model is fabricated and the feature generated by the FDM process is assessed and reported in Figure 13. The distance between the pores along the vertical direction is measured to be 3.543 mm also the angle of inclination of the side surface is 6.072° to the top surface. The distance between the pores along the vertical direction and the layer thickness is measured and the layer thickness is found to be 309 to 330 micron in different regions. The distance between the transverse pores measures 3.84 mm, which is slightly less than the hole type configuration. The strand length measures 2.409 mm and the gap between the two parallel strands is 1.179 mm. The distance between the two pores along the horizontal direction measures 3.716 mm. The distance between the pores in both vertical and horizontal direction are almost the same from 3.716 to 3.56 mm also the area of pore over the undercut surface has a pore area 96 micro.

4. Conclusion

In this work, the Generative Design module available in Autodesk FUSION 360 was used to develop the scaffold design based on the load case and other objectives. To assess the characteristic of the design given two standard configurations namely rode type and hole type is modeled and the geometrical assessment was done using image analysis technique. The physical, mechanical, and biological characteristics are to be done in the future.

Reference

[1] Lacroix D Planell J A and Prendergast PJ 2009 *Phil. Trans. R. Soc. A* (Philos) pp 1993–2009
[2] Murugan R and Ramakrishna S 2007 *Tissue Eng.* vol 13 (New York) pp 1845–1866
[3] Chocholata P Kulda V and Babuska V 2019 *Materials* vol 12 (Basel: MDPI) p 568
[4] Mallick S Tripathi S and Srivastava P 2015 *JOSR J. Pharm. Biol. Sci.* vol 10 (Ghaziabad) pp 37–54
[5] O’Brien FJ 2011 *Mater. Today* vol 14 (Kidlington) pp 88–95
[6] Nikolova MP and Chavali MS 2019 *Bioact. Mater.* vol 4 (Beijing) pp 271–292
[7] Chen FM and Liu X 2016 *Progress in Polym. Sci.* vol 53 (Amsterdam: Elsevier) pp 86–168
[8] Eltom A Zhong G and Muhammad A 2019 *Adv. Mater. Sci. and Eng.* vol 2019 (London: Hindawi) p 1–13
[9] El-Sherbiny IM and Yacoub MH 2013 *Global Cardiology Sci. and Practice* (Doha) pp 316–342
[10] Chen CL Zeng YT Kankala RK Zhang SS and Chen AZ and Wang SB 2018 *Materials* vol 11 (Basel: MDPI) pp 1832–1852
[11] Gómez S Vlad MD López J and Fernández E 2016 *Acta Biomater.* vol 42 (Amsterdam: Elsevier) pp 341–350
[12] Senatov FS Niaza KV Zadorozhnyy MY Maksimkin AV Kaloshkin SD and Estrin YZ *J. Mech. Behav. Biomed. Mater.* vol 57 (Amsterdam: Elsevier) pp 139–148
[13] Gregor A et al 2017 *J. Biol. Eng.* vol 11 (Kentucky: IBE) pp 1–21
[14] Chua CK Leong KF Cheah CM and Chua SW 2003 *Int. J. Adv. Manuf. Technol.* vol 21 (London: Springer-Verlag London Limited) pp 302–321
[15] Johnson J Ghosh A and Lannutti J 2007 *J. App. Polym. Sci.* vol 104 (New York: Wiley–Interscience) pp 2919–2927
[16] Nagy D Lau D Locke J Stoddart J Villaggi L Wang R Zhao D and Benjamin D 2017 *Proc. Symp. Simul. Arch. Urban Design.* vol 49 (Toronto: SimAUD–2017) pp 49–56
[17] Renner S 2007 *Chair of Struc. Anal.* (Munich: Technische Universität München) pp 1–97
[18] Wittbrodt B and Pearce JM 2015 *Addit. Manuf.* vol 8 (Amsterdam: Elsevier) pp 110–116
[19] An J Teoh EM Suntornnond R and Chua CK 2015 *Eng.* vol 1 (Amsterdam: Elsevier) pp 261–268