IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)
e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 19, Issue 11 Ser.1 (November. 2020), PP 54-58
www.iosrjournals.org

Accuracy of 3d Printing in Hard Tissue Engineering

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Abstract
Accuracy in 3D printing is refining by the advancement in 3Dprinting technology and among many 3D printing facilities, Fused Deposition Modelling (FDM) printer is beneficial. As it is simple to modify printing extruder units and easy to calibrate printing parameters according to the design of scaffold and biomaterials used for printing scaffold. These modifications can be used to print porous scaffold precisely to meet the requirement for tissue engineering applications. Based on the information from digital design, printing tip present in the Extruder unit of FDM Printer,which prints scaffold with perfect geometry by layering melted biomaterial. The printing tips used in the extruder varies with dimension and size and depending on the values of printing parameters these printing tips can be changed to print scaffold precisely. Following the values of printing parameters, extruder unit moves 3 dimensionally and fabricates exact structure based on the virtual software design of 3D scaffold with equal and repeatable pore size which is required for the specific application in the hard tissue regeneration. Additionally, recalibrating printing machine could also help in maintaining printing speed and extrusion pressure, temperature is necessary to produce scaffold with ideal structure and physical and mechanical properties for bone engineering. The common biomaterial used in the FDM 3D printers are polymers. Polymers are melted at various temperatures; therefore, modification of melting temperature in the 3D printer is required before printing different polymers to precisely layer melted material that bond well. Modification and maintenance and usability of biopolymer in FDM is crucial for the production of well-structured, well bonded, repeated same sized micro structured scaffold for the applications in hard tissue engineering to accelerate repair process by exposure of body fluids in the scaffold at the implanted site to enhance mineralization and bioactivity

Keywords *FDM printer, calibration, scaffold, precision of printing, printing tip, biomaterial, regeneration, bone engineering.*

Date of Submission: 26-10-2020 Date of Acceptance: 05-11-2020

I. Introduction

To move, to support, to protect, are the characteristic features of bone which plays a major role in human life. To do these as a life time process bone has to remodel in order to help, to move around, to hold body together, and to protect organs present in human body. When there is bone destructionwhich can be due to physiologic, pathologic or traumatic, bone has the self-healing property to restore the lost function. But when the defect is larger, it does not have ability to repair itself. Therefore, the usage of biomaterial is necessary to repair large bone defects. However, treating those large bone defects remains as challenging [1]. To compact this challenge biodegradable, economical, 3D structured scaffold can be fabricated with microarchitecture of interconnected pores to facilitate the exposure of body fluid, mineralization, cell attachment and bone formation [2].

In the fabrication of three-dimensional scaffolds, 3D printing machine plays a major role. In bone tissue engineering with modern facilities the possibilities of printing any missing part of the hard tissue has accomplished great achievements in personalised tissue engineering [2].One of such modern technology is 3D printer which is used to make 3D printed scaffold with perfect structure and optimalstrength which is used for the specific application in bone tissue regeneration. The quality of the 3D printed scaffoldis based on the type of biomaterialused in the 3D printer for the fabrication of scaffold. Polymers have been used commonly because of the printability of any possible size, shape and structure of missing hard tissue for the application in bone regeneration[3].

Modification of components of the FDM printerand changing printing parameters to use various biopolymers to print scaffold with precision isvital for customised application.According to the biomaterial used for printing scaffold, FDM printer is calibrated to manage melting temperature and printing speed that enables accurate layering in the required shape and structure with quality. The printing head of the FDM printers moves in X, Y, Z axial direction, which guides the construction of scaffold with the specific dimensions[4]. Understanding of the working methods of fused deposition modelling printer is essential in order to construct scaffold for bone regeneration with precise repeated pore size with 3Dstructure which plays major role in the tissue engineering such as exposure of body fluid, biodegradation, cell attachment, migration, mineralisation during repair process.

Scaffold for bone regeneration

Scaffolds used in hard tissue engineeringare three-dimensional porous solid material fabricated with the aim of promoting biological activity in the implanted site where healing processes is delayed due to large amount of tissue is damaged because of trauma, tumour, infection, and inborn abnormalities. The aim of making scaffold is to fabricate it with no immunological reaction or toxicity to the host tissue and the rate of biodegradability is to be comparable with tissue to be regenerated and to promote activities such as transporting molecules, nutrients, and gases for the cell survival at the healing site[5]. To regenerate tissue or organ, scaffold is incorporated into human body where large tissue damage needs to be repaired. To repair the large amount of tissue defect,design and fabrication methods of scaffold is important as it plays major role in the interaction with body fluid and cell attachment, migration, proliferation and differentiation of a particular lineage to form hard tissue in large defect area. The significance of scaffold fabrication with particular size and shape of porous structure is to improve physical and mechanical properties of the scaffold that is similar to the organ or tissue that need to be repaired or regenerated. There are many scaffold manufacturing methods available in the market among themcommonly used methods are electrospinning, freeze and drying, casting, moulding and 3d printing. Between them 3D printing is popular because of its accuracy in the scaffold printing for organ regeneration which enablesbioactivities for regeneration [6].

Biomaterials for bone tissue engineering

Biomaterials used in tissue engineering includes metal, polymer, ceramic and composite materials. Each of these materials has advantage over one another based on their physical and mechanical properties. Therefore, biomaterials have been chosen based on the application in a particular tissue or organ regeneration.Among all material polymer usage in bone tissue engineering is advantages as the physical and mechanical properties are comparable with hard tissue that need to be repaired.

Biopolymer can be divided in to following types natural, synthetic, hybrid. Natural polymers are starch, alginate, chitosan, and hyaluronic acid, protein such as soy, silk, fibrin gel, and collagen. The applicability of natural polymer is limited because of limited availability, difficulty in processing in particular shape and also expensive. Synthetic polymers are beneficial because of its usability in the 3D form for biomedical application and less expensive, easy of processability. Most commonly used synthetic polymers are PLA [poly lactic acid], poly glycolic acid [PGA], poly co glycolide [PLGA], and poly e-caprolactone [PCL]. Composite material made of by combining both natural and synthetic polymers to improve properties are called hybrid polymers[7] (Table 1).

PLA physical properties	Values
Melting temperature	173-178
Glass transition temperature	60 degree C
Density	1.3 g/cm3
Size of filament	1.75 mm
Chemical formula	(C3H4O2)n

Table 1: Determination of FDM printer settings with regard to geometrical accuracy [8]

Fused deposition modelling[FDM] printer

Fused deposition modelling [FDM] printing method are also called fused filament fabrication [FFF], rapid prototyping, solid free form technology, additive manufacturingand also one of the most common 3D printing methods used in commercial and biomedical and industrial applications[9]. During printing samplebased on the computer aided design, FDM printer prints biomaterial in a thin filament form and letting it to cool and bond together on the printing preheated table to adhesive, the first layer to produce stability while printing scaffold. Optimal structure of the scaffold for regeneration is specifically designed using software which is used in the FDMprinter where feeding material is used in the form of filament that is melted at high temperature followed by printing it in layer by layer without moulds on the printing table with perfect shape and size that meets the requirement of hard tissue engineering [10].While printing scaffold, printing tip of the FDM printer moves in a controlled manner in the X and Y and Z axis to print scaffold vertically and horizontally to fabricate structure that is suitable for regeneration of hard tissue [11]. Testing sample with trial printing in the FDM is useful in adjustment and calibration of parameters to improve quality and achieve the precisionbefore printing the master sample.

Other trade names of identical technology of printing by extruding polymers are[8];

FDM- fused deposition.
PJP- plastic jet printing.
FFM- fused filament modelling.
MEM- melted and extruded modelling.
FFF- fused filament fabrication.
FLM- fused layer modelling

FDM printing principle[12]

Printer type	Form of Material	Materials used	Technique used	benefits	problems
FDM printer	Material used in the form of filament. filament size is 1.75mm	Thermoplastics Which is PLA, PCL, PLGA, and PEEK	Melting, extruding, depositing	Economical, improved properties, multi material usability	Anisotropy, nozzle blocking

FDM Data preparation for 3D printing

The first step to prepare data for 3D printing for tissue engineering is to check the reliance amidst geometry model of the 3d structure and the printing parameter such as printing speed, temperature, and height of the printed filament layer. To do this, scaffold sample design printed by FDM can be used to substantiate the geometry with the various printer settings. In the printer settings two vital parameters need to be validated in the drawing of the 3D scaffold. Those are chordal tolerance and angular tolerance for exampleit can be fixed around 0.05mm and 5 degree respectively to match geometry of design and parameters of printer. In the market,varioustype of software's are available for designing samples to print the scaffold.Most of the software allows the developed design to be converted into file format. Laterthis file isconverted into STL [standard triangle language] which is sent for the slicing which is ready to be printed. Slicing is called machine language or G-code. In the slicer software Parameters are commonly fixed and some of them are modified based on the need such as temperature, speed, layer thickness. Thickness of the printing model is based on the printing tip diameter; therefore, printing tip size must match the printing design.

Range of parameters for test 3d printing[8]

Printing parameters	Example of sample testing values
Temperature	180, 200, 220 degree C
Speed	20mm/s, 30mm/s, 40mm/s.
Layer height	0.1mm, 0.2mm
Nozzle diameter	0.2, 0.3, 0.4, 0.5 mm
Printing table temperature	40 degree C

Test sample printing

High quality scaffold made of biomaterial for tissue engineering printed using FDM method is tested by printing samples, because it could help to checkprintability of the required designand also help findprinting error in the samples such as visible defects, imperfection, layer delamination, discontinuation of shell and infill and because it could also show changes in sample property [13].The chances of printability of scaffold with any design are starting with the structure planned in the 3D printing software. Trial printing many samples with different design could reveal the chances of printing any structure in the following ways.According to the command from the software the printing tip in the printing head moves in dissimilar axial directions to complete work. In case of printing scaffold in the square shape, the gridstructure of the scaffold is fabricated by the printing tip displacement in three axial movements [X, Y, Z].The printing tip displacement in the X and Y axial directions determines the equal distance between the filaments in the square grid and printing tip displacement in the Z axis determines width of the filaments in the square grid [14].Printing errors can be identified and corrected by printing multiple samples for few times which could help to get final product with high precision. When printing scaffold, the FDM printer fabricates scaffold based on the information from the software design and after printing scaffold can be measured using measuring microscope to check for error which will be corrected by calibrator.To check physical and mechanical properties of printed samples, grids of the printed samples scaffolds with different infill density, infill pattern and thickness can be tested for the changes in the properties. For instance, weight reduction and Cost reduced by hallowing out inside the printed samplesand also modifying parameters such asinfill pattern and infill density using slicer software to print scaffold with properties comparable to the tissue need to be repaired. Like-wise, the outer layer or perimeter is also important parameter for making samples with better properties to match the host tissue. Sample printing and testing sample is required before printing master sample to make sure that quality of the scaffold is applicable in tissue engineering.

Printing parameter

To print the3D printing scaffold accurately certain alteration are needed, according to the biomaterial used in the FDM printer. Printing samples with hallowed out areas with equal distance shell with right thickness of the filament could facilitate perfect environment for the biological activity required for tissue engineering application. To print filament with right thickness, in correct distance, printing parameters needs to be adjusted based on the requirement. Those parameters are fill density, fill pattern, solid fill pattern, extrusion multiplier. These parameters have influence in the density, tensile strength of the printed scaffolds [13].

Fill density parameter has effect on printing work in particularly it reduces cost when fill density is reduced and when fill density is increased it increases pressure in the extruder and also reduces printing speed. Density of the printed samples depends upon the value of the setting slicers of the fill density pattern**.**

Fill patternin the 3d printing process- when making shell of the 3D objects-melted biomaterial printed closely in the form of threads with high density to form outer layer while inner part of the object is printed in a particular way which will prevent from collapsing printed layer to the other side. There are several methods of fill pattern available in the setting slicer and few of them are honeycomb and Hilbert curve. Among many fill patterns honeycomb considered to be the best as it increases tensile strength of the printed object [13].

Extrusion multiplier parameterapplications have effect on the length travelled by the extruder and amount of the biomaterial extruder when printing scaffold samples. In a particular valueextrusion multiplier parameter with optimal extrusion pressure scaffold can be printed with perfect density but when the parameter value is changed it can cause over flow of the material which will affect printing quality of the scaffold. Closely extruded filament threads increase the tensile strength of the 3D printed scaffold[13].

FDM printing tip

Fabrication of 3d printed scaffold with required structure, size, shape, thickness, and pattern accuracy for a particular application is based on the printing tip diameter. There are different tips available with different size and different diameters. Commonly available diameter size of the printing tip is 0.2, 0.3, 0.4 and 0.5.

II. Conclusion

This review pronounces modification in the parameter settings of FDM 3D printer for customised application in regenerative medicine. The idea of recalibrating FDM is to maintain repeatability, mechanical property, and dimensional accuracy of the printing scaffold for bone tissue engineering [15]. Understanding the influence and applicability of modified Printing parameters in 3D printed scaffold made of biopolymers is advantageous in the tissue engineering.Because the specification of the scaffold required for regeneration is demanding; therefore, accuracy in the printing scaffold is essential for the tissue engineering. Accurately printed scaffold enhances biodegradability, bioactivity, and cell growth to repair and replace the missing part of the human body.The application of 3D printed scaffold in tissue engineering and regenerative medicine has made many achievements such as low cost, high strength, improved physical and mechanical properties of scaffold.However, converting 3D printed scaffold perfectly into lost portion of human body is still a challenge. Therefore, further analysing data of different values of FDM printing parameters on scaffold printing with biopolymers is needed for printing porous scaffold with perfect geometry which could improve cell and biological activity to convert biopolymer into natural tissue

Financial support
Nil
Disclosure of interest
The authors report no conflict of interest.

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Dr. V. Sasi Kumar, et. al. "Accuracy of 3d Printing in Hard Tissue Engineering." *IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)*, 19(11), 2020, pp. 54-58.