Applications of Additive Manufacturing to Tissue Engineering

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Abstract: Due to rapid-fabrication of the different models, high-precision of produced parts and customized production rate of the manufacturing methodology, Additive Manufacturing (AM), 3-dimensional (3D) printing technologies and rapid prototyping (RP) systems are recently developed in many applications. Complex geometries, porosities, co-culture of multiple cells, can be created and incorporate growth factors can also be considered by using 3D printing systems. In the research work, applications of additive manufacturing systems in bioengineering products are presented. Finite element analysis can be used to analyze the mechanical properties of the structure in order to detect weak components of the entire scaffold and prevent structural failure in real working conditions. Furthermore, topology optimization methods are developed in order to obtain the best use of material for designed scaffolds which are subjected to either a single load or a multiple load distribution. As a result, “shape” optimization as well as “layout” optimization can be obtained in order to increase efficiency of part manufacturing.

Keywords: Additive manufacturing, 3D Bioprinting, Finite Element Analysis, Topology Optimization, Bone Scaffold, Tissue Engineering

1. Introduction

In the recent years, advanced additive manufacturing technologies are developed to fabricate complex 3D structures with highly porosity architecture in tissue engineering applications. These technologies combined with computer-aided design (CAD) enable to produce three-dimensional structures layer-by-layer in a multitude of

materials. 3D structures with a predefined geometry and size and porous architecture characterized by a fully interconnected network of pores as well as customizable size, shape and distribution can be produced using the additive manufacturing technologies.

The conception of 3-dimensional (3D) printing technologies was first introduced in 1986 by Charles W. Hull [1]. It referred to as additive manufacturing (AM) or rapid prototyping (RP) has acquired reputation over the past few decades [2–4]. 3D printing is one of the additive manufacturing processes [5, 6]. 3D printing is a proper name to describe the technologies that create 3D structures by adding layer-by-layer of material, whether the material is ceramic, metal, plastic, and polymers (synthetic or natural polymers) [7].

Recently, the aim of tissue engineering is regeneration, restoration, or replacement of defective or injured functional living organs and tissues [8–10]. In order to achieve this aim, biomedical scaffolds made of natural or synthetic polymers have been commonly used in biomedical and tissue engineering applications [11, 12]. The major focus of these scaffolds is to replace or regenerate the native tissues functionally and structurally. In general, the scaffolds for use as tissues and organs have a several mandatory functions: it should provide internal pathways for the cell attachment and migration, it must transfer various growth factors and waste products, and it should keep its shape while the cells are growing, and have adequate mechanical properties. [13]. To achieve these functions, biomedical scaffolds for tissue engineering require a highly porous 3D structure that allows cell affinity such as proliferation, migration, attachment, and differentiation, even enables nutrients and oxygen transport [14, 15]. Therefore, 3D bioprinting technology is one of the most appropriate methods for producing a 3D structure for use as biomedical scaffolds, tissues, and organs.

In the present research work, applications of additive manufacturing technologies in bioengineering are presented in section 2. Methodology of additive manufacturing in the tissue engineering is presented in section 3.

2. Additive Manufacturing Technologies in Bioengineering

3D Bioprinting form biomaterials are an emerging technology which aims to develop new organs and tissues. 3D bioprinting is a process for controlling the cell proliferation, attachment, and migration within 3D structures [16, 17]. As a result, various 3D bioprinting methods are presented for a variety of tissue engineering applications. In the study, the four types of 3D bioprinting methods that are most commonly used such as SLA and DLP in vat photopolymerization, FFF in material extrusion, SLS in powder bed fusion, and inkjet 3D printing in binder jetting methods will be introduced. So, depending on the fabrication principle, four categories are presented for the most extensively applied AM techniques for tissue engineering scaffold fabrication. The categories are (a) stereolithography (SLA); (b) selective laser sintering (SLS); (c) three-dimensional printing (3DP); and (d) fused deposition modelling (FDM) [18]. Schematic of various 3D bioprinting for tissue engineering applications is shown in the Fig. 1 [19] https://biomaterialsres.biomedcentral.com/articles/10.1186/s40824-016-0058-2 Accessed 7 April 2019.

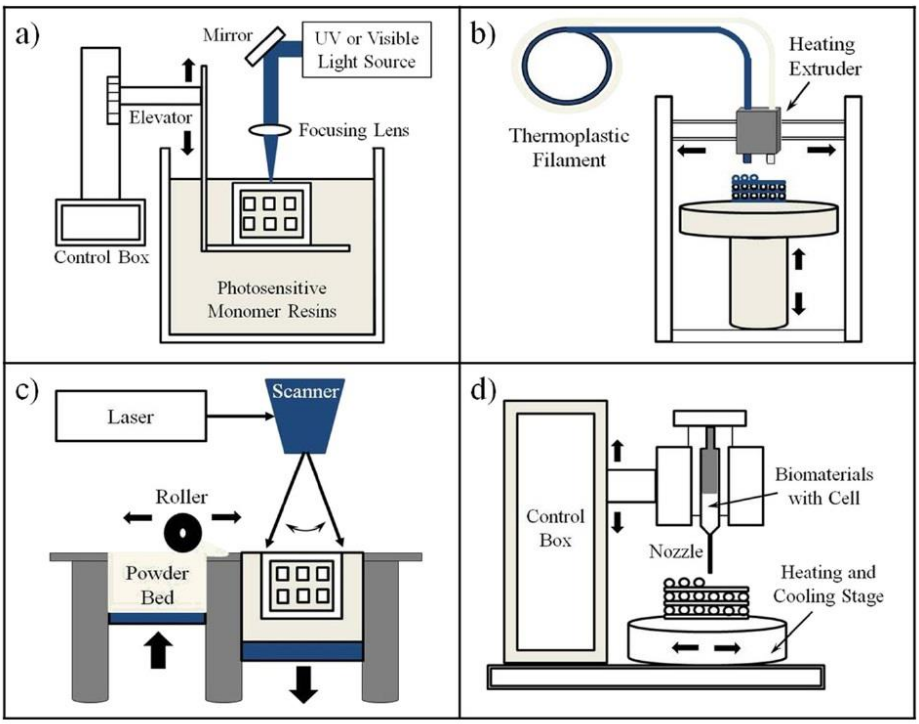

Fig. 1. Schematic of various 3D bioprinting for tissue engineering applications, a) Vaphotopolymerization b) Fused filament fabrication C) Selective laser sintering d) Inkjet 3D printing [19].
https://biomaterialsres.biomedcentral.com/articles/10.1186/s40824-016-0058-2 Accessed 7 April 2019.

2.1. Stereolithography-Based Bioprinting

SLA method using the UV light is one of the various methods used to create the 3D structures. This method has been the oldest and still widely used. This process has obtained the patent in 1986 by Charles Hull [1]. These machines produce parts by using a light source UV laser or projector to cure liquid resin into hardened plastic. It is a multi-layer procedure through the selective photoinitiated curing reaction of a low-molecular weight prepolymer, additives and photo-initiators. Either a focused ultraviolet beam light or a mask-based irradiation can be used to selectively solidify the liquid photopolymer.
The advantage of stereolithography based bioprinting in organ 3D bioprinting is the high building velocity and accuracy. The disadvantages of stereolithography-based 3D bioprinting in organ 3D bioprinting is the high cost of the devices and the cytotoxicity of the lights and photo-initiators [20–22].
Schematic diagram of Stereolithography-based bioprinting is shown in the Fig. 2. [23]
https://formlabs.com/blog/ultimate-guide-to-stereolithography-sla-3d-printing/ Accessed 7 April 2019.

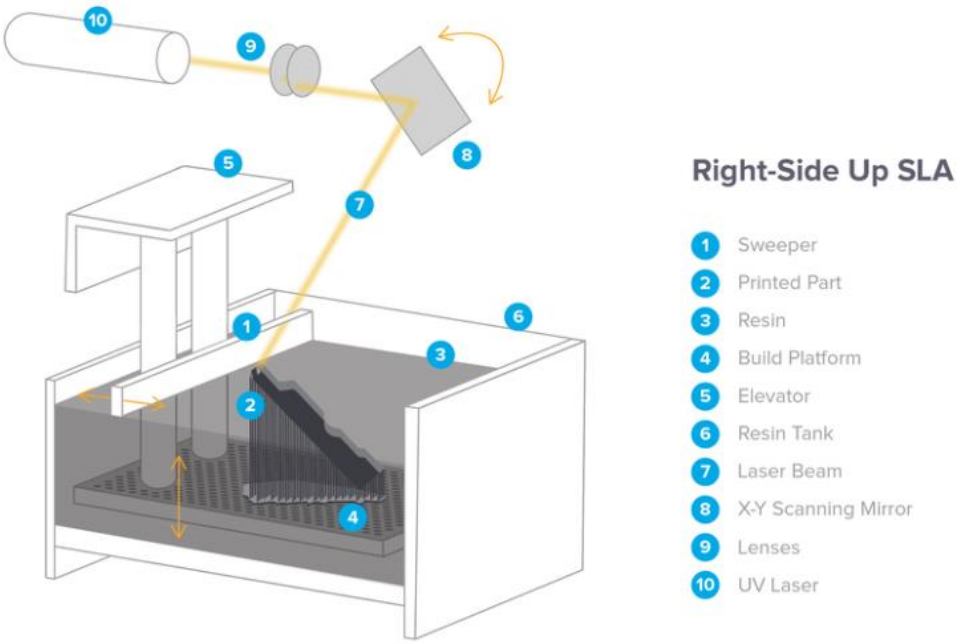

Fig. 2. Schematic diagram of Stereolithography-based bioprinting [23].
https://formlabs.com/blog/ultimate-guide-to-stereolithography-sla-3d-printing/ Accessed 7 April 2019.

2.2. Extrusion-Based 3D Bioprinting

FFF printers in material extrusion method use a thermoplastic filament to produce parts. This filament is heated to the melting point and then extruded to prepare a 3D structure. These thermoplastic filaments are deposited through an extrusion nozzle during printing. The nozzle melts the filaments and then extrudes onto the substrate for fabricating 3D structure (FFF method). The nozzle and substrate are controlled by a computer that translates the dimensions of a structure into X, Y and Z coordinates during printing. FFF method is a thermalheating technique for use 3D scaffolds fabrication in tissue engineering applications. Some specific plastics, such as acrylonitrile-butadiene-styrene (ABS) and polylactice acid (PLA) that melting about 200 C are the most suitable printing materials in order to produce nonbiodegadable hard tissue and organ engineering scaffolds.
The advantages of extrusion-based bioprinting in organ 3D bioprinting include high cell densities, large 3D constructs and fast printing speeds. Beside polymeric solutions or hydrogels, extracellular matrices (ECMs) and cell aggregates can also be used as bioinks. Currently, it is one of the least expensive methods to create solid 3D scaffolds with go-through channels.
The disadvantage of extrusion-based bioprinting in organ 3D bioprinting is that there are limited polymeric solutions or hydrogels that have good biocompatibilities and can be printed into large constructs in layers [24–27].
Schematic diagram of extrusion-based bioprinting is shown in the Fig. 3 [28] http://ijb.whioce.com/index.php/int-j-bioprinting/article/view/128 Accessed 7 April 2019.

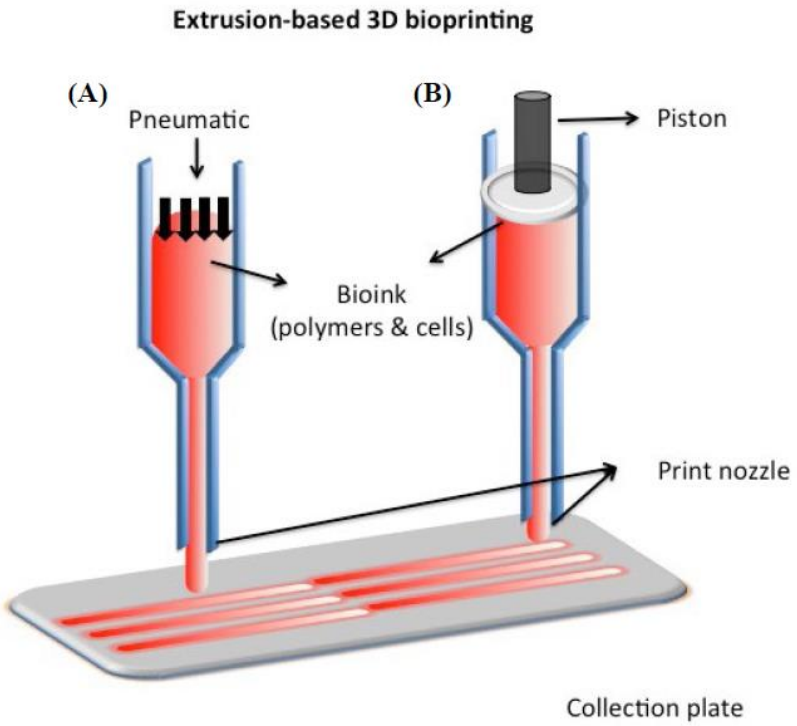

Fig. 3. Schematic diagram of extrusion-based bioprinting (A: Pneumatic; B: Piston) [28]. http://ijb.whioce.com/index.php/int-j-bioprinting/article/view/128 Accessed 7 April 2019.

2.3. Laser-Based 3D Bioprinting

Laser-based 3D bioprinting technologies are a group of printing methods that use laser energy to transfer or coordinate starting biomaterials. The production method is based on the laser pulse to generate a high-pressure bubble between a solution and a piece of glass containing cells towards the collective substrate [29, 30].
The advantage of laser-assisted bioprinting in organ 3D bioprinting includes avoiding the problems of nozzle clogging with cells and/or polymeric biomaterials.
The disadvantage of laser-assisted bioprinting in organ 3D bioprinting is the high cost of the laser-assisted 3D bioprinters.
Schematic diagram of laser-assisted bioprinting is shown in the Fig. 4 [28] http://ijb.whioce.com/index.php/int-j-bioprinting/article/view/128 Accessed 7 April 2019.

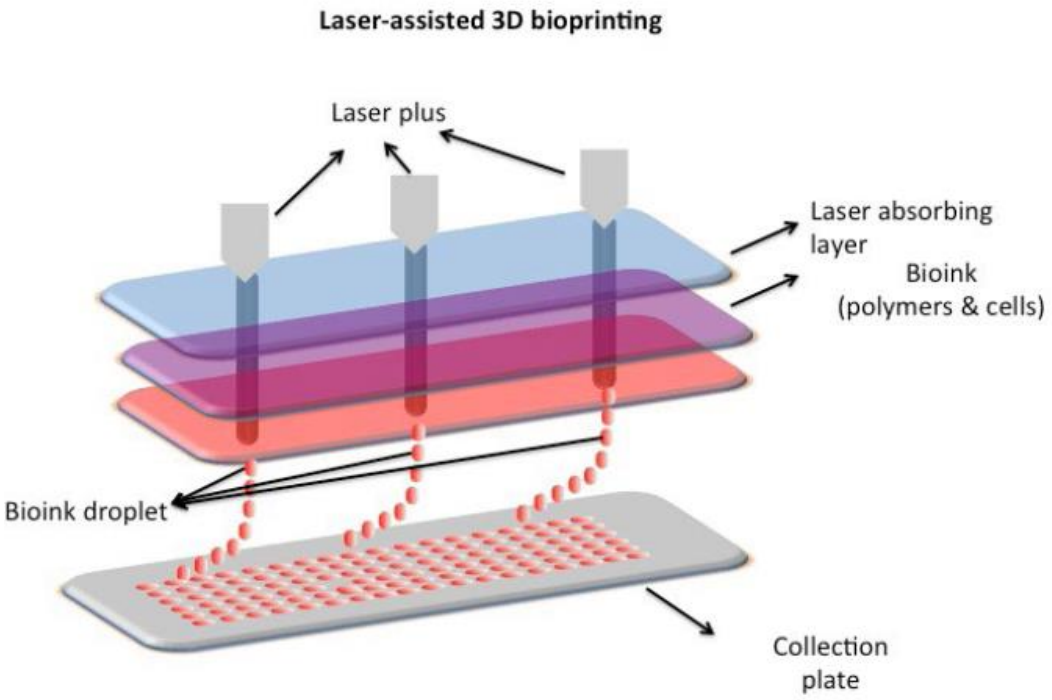

Fig. 4. Schematic diagram of laser-assisted bioprinting [28].
http://ijb.whioce.com/index.php/int-j-bioprinting/article/view/128 Accessed 7 April 2019.

2.4. Inkjet-Based 3D Bioprinting

Inkjet bioprinters, also known as drop-on-demand printers, are the most commonly used type of bioprinter due to its low cost, high resolution, high speed and compatibility with many biological materials. It is a non-contact image reconstruction technology, which includes piezoelectric, thermal and acoustic conductivity nozzles. Inkjet printers use thermal or acoustic forces to eject drops of liquid containing cells onto a scaffold, which can support the final construct. Thermal inkjet printers use electricity to heat the print head producing a pulse of pressure that forces droplets from the nozzle. Before printing, the starting materials need to be liquefied to permit droplets deposition onto a solid platform. During the printing process, a fixed volume of fluid is continually jetted onto the platform through the thermal, acoustic or piezoelectric actuating units and the pre-designed signals reappear on the platform through the ink droplets. The droplets must be solidified into the pre-defined geometry before the next layer of droplets is added.
The advantages of inkjet-based bioprinting in organ3D bioprinting contain the fast response speed, the high formation precision, and the high efficiency.
The disadvantage of inkjet bioprinting in organ 3D bioprinting is that the “bioinks” should be in liquid forms with low viscosities [31]. This has greatly limited the height of the produced constructs by the method. Another obvious drawback of inkjet bioprinting in organ 3D bioprinting is the poor mechanical properties of the 3D constructs. Till now, most of the researchers in this field do their studies by modifying commercial inkjet printing systems to print living cells. This has greatly limited their development in soft and hardware as well as the complexity of printed constructs.
Schematic diagram of inkjet-based bioprinting is shown in the Fig. 5 [28] http://ijb.whioce.com/index.php/int-j-bioprinting/article/view/128 Accessed 7 April 2019.

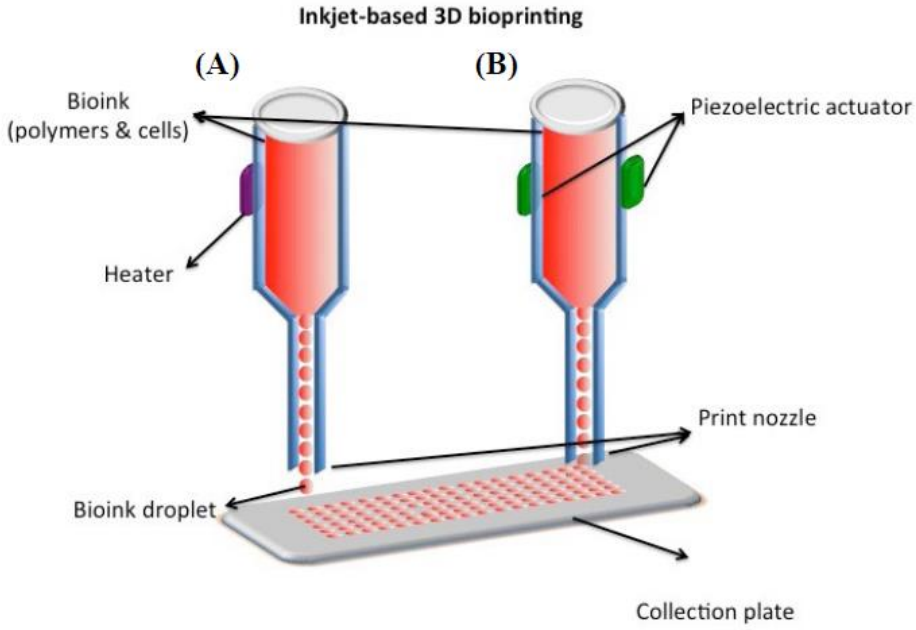

Fig. 5. Schematic diagram of inkjet-based bioprinting (A: Heater; B: Piezoelectric actuator) [28].
http://ijb.whioce.com/index.php/int-j-bioprinting/article/view/128 Accessed 7 April 2019.

Schematic diagram for three most common 3D bioprinting techniques is shown in the Fig. 6 [32]
https://journals.tubitak.gov.tr/chem/abstract.htm?id=22758 Accessed 7 April 2019.

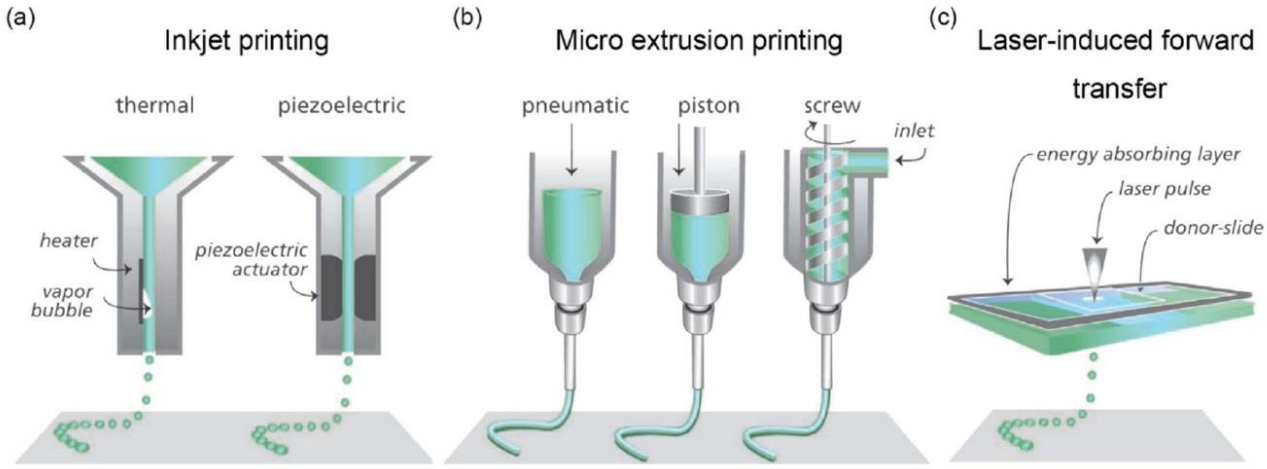

Fig. 6. Schematic diagram for three most common 3D bioprinting techniques (a) Inkjet bioprinting (b) Microextrusion bioprinting (c) Laser assist bioprinting [32]
https://journals.tubitak.gov.tr/chem/abstract.htm?id=22758 Accessed 7 April 2019.

Fig. 7 shows advantages and disadvantages of various 3D bioprinting methods for bioengineering applications [28]
http://ijb.whioce.com/index.php/int-j-bioprinting/article/view/128 Accessed 7 April 2019.

Technique	Advantage	Disadvantage	References
Stereolithography-Based	✓ High resolution ✓ Easy to remove trapped materials	✓ Expensive equipment ✓ Only photopolymer materials	[33-36]
Extrusion-Based	✓ Wide range of material choice ✓ Low cost ✓ Good mechanical properties	✓ Limited materials to thermoplastics ✓ Filament required ✓ Viscosity and temperature of materials	[37-40]
Laser-Based	✓ Wide range of material choice ✓ High resolution	✓ Expensive equipment ✓ Heat effects	[41-44]
Inkjet-Based	✓ Low heat effect ✓ High resolution	✓ limited choice of materials ✓ Limited height ✓ Difficulties in Complex 3D geometries ✓ Poor mechanical properties	[45-48]

Fig. 7. Advantages and disadvantages of various 3D bioprinting methods for tissue engineering applications [28] http://ijb.whioce.com/index.php/int-j-bioprinting/article/view/128 Accessed 7 April 2019.

3. Methodology of Additive Manufacturing in Bioengineering

The 3D model data for scaffold development can be obtained from medical imaging techniques used for diagnostic purposes, such as computer tomography (CT) and magnetic resonance imaging (MRI). Then, the images are generally treated by computer-aided design (CAD) and computer-aided manufacturing (CAM) software. As a result, a simplified 3D model can be directly designed in CAD software or developed by means of mathematical equations or topological optimization. In order to increase efficiency of CAD models in process of part manufacturing, topological optimization techniques are recently developed.

Most CAD software converts the 3Dmodel into a Standard Tessellation language (STL) file containing the information of the 3D object's surface geometry. The STL file is then sliced into layers originating a slice file (SLI) that is loaded digitally into the machine that drives the motions of the build parts.

The procedure of the additive manufacturing process in bone scaffold production is presented in the Fig. 8 [49] https://www.sciencedirect.com/science/article/pii/S2212827113000504 Accessed 7 April 2019.

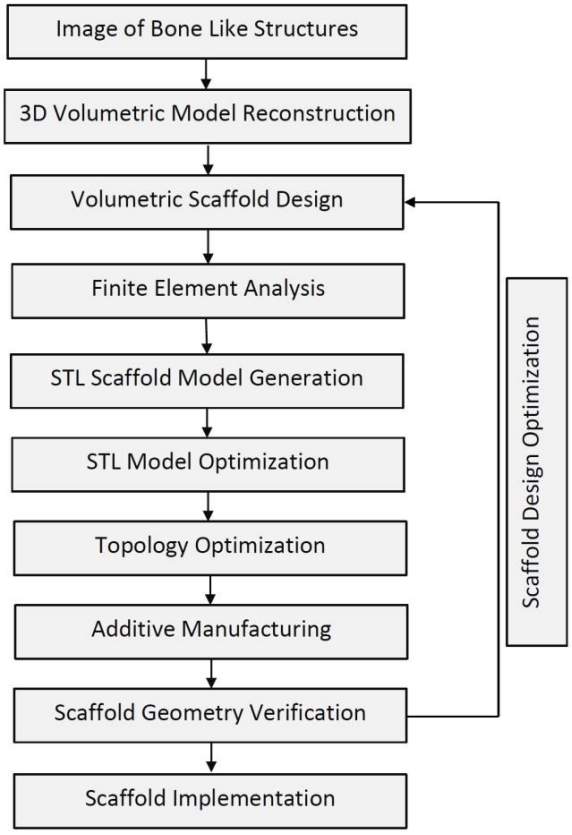

Fig. 8. Procedures of the additive manufacturing process in bone scaffold production [49] https://www.sciencedirect.com/science/article/pii/S2212827113000504 Accessed 7 April 2019.

3.1. Obtaining 3D CAD Model of Tissue Construct Using Mathematical Concepts

The aim is to reconstruct the three-dimensional structure of the tissue regeneration volume from image data. Fig. 9 shows the steps involved in construction of 3D CAD model of tissue construct to be fabricated [50] https://www.sciencedirect.com/science/article/pii/S2212827117305188 Accessed 7 April 2019.
Noninvasive imaging techniques can be used for biological feature recognition. Thin-slice computer tomography (CT) imaging, micro CT imaging, contrast-enhanced MRI, micro MRI and digital photography are some of the techniques that offer a noninvasive quantitative assay for the wound imaging [51]. The image acquisition system must preserve as much information of tissue regeneration area as possible.

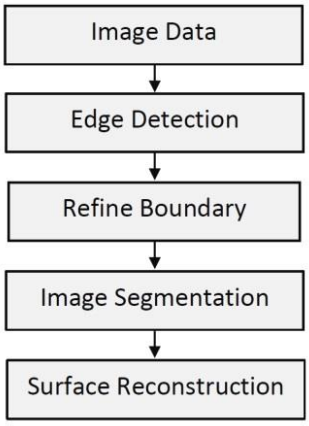

Fig. 9. Overview of entire image construction [50]
https://www.sciencedirect.com/science/article/pii/S2212827117305188 Accessed 7 April 2019.

After the 3D reconstruction of the image is obtained, the outer surface boundaries of the 3D CAD model are used as scaffold boundaries represented with set of scaffold boundaries$\{SB_m\}, m = 1, \dots, M$ and SB_m (u, v) are represented as NURBS surfaces.
This surface model $SB_1(u, v)$ is sliced into layers to decompose it into planar curves or scaffold boundary $Cb(u)$. This information from sliced layered plane is used in an ordered sequence during fabrication process. The slicing thickness parameter Z_h is used to decompose the 3D volume. The set L is introduced to indicate the discrete layers into which surface model is decomposed.

$$L = \{L_h : L_1 < L_2 < \cdots L_{H-1} < L_H \}, h = 1, \dots, H \quad (1)$$

Where L_h represent the h^{th} decomposed layer.

After slicing, in each layer L_h, the scaffold boundary $Cb_{Lh}(u)$, is modeled as p^{th} degree NURBS curve given by:

$$Cb_{Lh}(u) = \frac{\sum_{i=0}^{I} N_{i,p}(u) w_i C_i}{\sum_{i=0}^{I} N_{i,p}(u) w_i} \quad (2)$$

Where $C_i = (x_i, y_i, z_i)$ are control points of curve obtained by curve fitting with weight w_i and $N_{i,p}(u)$ are the B-Spline basis function defined on the knot vectors U.
Once the 3D surface model of tissue was generated, it is imported into CAD software such as Solidworks or Rhinoceros software in order to generate surface refinement as well as non-uniform rational B-spline (NURBS) surfaces. Using the 3D surface model, biological features are obtained and iso-surfaces are also created by volume lofting operation between biological features. Moreover, thin slices can be created and sliced data can also be obtained in the form of lofted NURBS curves. As a result, the iso-composition contours in each slice can be obtained and used for distribution of bioactive particles over the scaffold.

3.2. Volumetric Scaffold Design

After completion of the previous stages, the 3D volumetric model is converted into a surface representation. The surface is checked for the presence of any topological flaws such as small holes and detached parts, and is then repaired accordingly as part of the preprocessing. Then, scaffolds are created in order to be fabricated. These scaffolds are chemically and physically modified during the fabrication process to meet specific needs, such as biodegradability, porosity, size, shape, and bioactivity. These requirements may vary depending on the nature of the biomaterials, the fabrication process and the target tissue. After making the scaffold with the desired properties, the scaffold can be seeded with cells and cultured in vitro to create the desired tissue, or can be placed within the body and have the host cells infiltrate the scaffold and populate. Growth factors, hormones, and

chemical cues are key in both these approaches as they define cell differentiation and functionality of the cultured tissue. This process is shown as a flowchart in the Fig. 10 [52] https://www.mdpi.com/2079-4983/9/1/22/pdf/1 Accessed 7 April 2019.

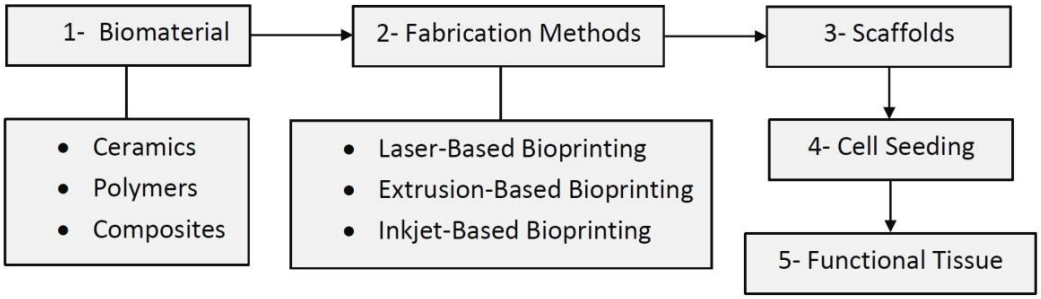

Fig. 10. The flowchart for creating functional tissues from biomaterials [52]. https://www.mdpi.com/2079-4983/9/1/22/pdf/1 Accessed 7 April 2019.

3.3. Finite Element Analysis

Finite element analysis is carried out to study the mechanical properties of the polyamide scaffolds. The analysis is for verifying the mechanical properties of the structure, with the aim of detecting weak components that can lead to structural failure of the entire scaffold. So, the results may help optimize the scaffold by reinforcing load-bearing parts. Also, reliability of produced parts can be increased due to analyzing capacities of designed scaffold in real working conditions.

The finite element analysis of bone scaffold is shown in the Fig. 11 which is presented by Ir. Amelie Sas [53] https://www.materialise.com/en/resources/medical/webinar-recording/automating-key-components-of-finite-element-research Accessed 7 April 2019.

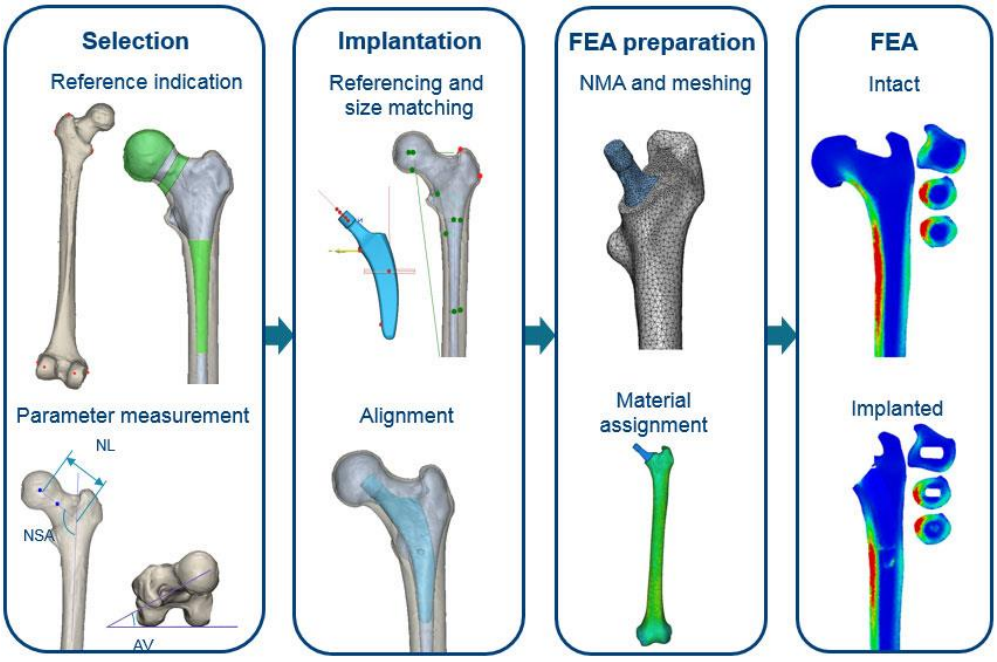

Fig. 11. Finite element analysis of bone scaffold [53] https://www.materialise.com/en/resources/medical/webinar-recording/automating-key-components-of-finite-element-research Accessed 7 April 2019.

Also, application of finite element analysis in designing and additive manufacturing of bone scaffolds is presented by Hendrikson et al. [54] https://www.frontiersin.org/articles/10.3389/fbioe.2017.00030/full Accessed 7 April 2019 in the Fig. 12. In the study, the computational modeling is used to understand the mechanisms behind tissue formation. Then, biomimetic scaffold models based on tissue regeneration strategies are generated in order to be analyzed and optimized by using the finite element methods.

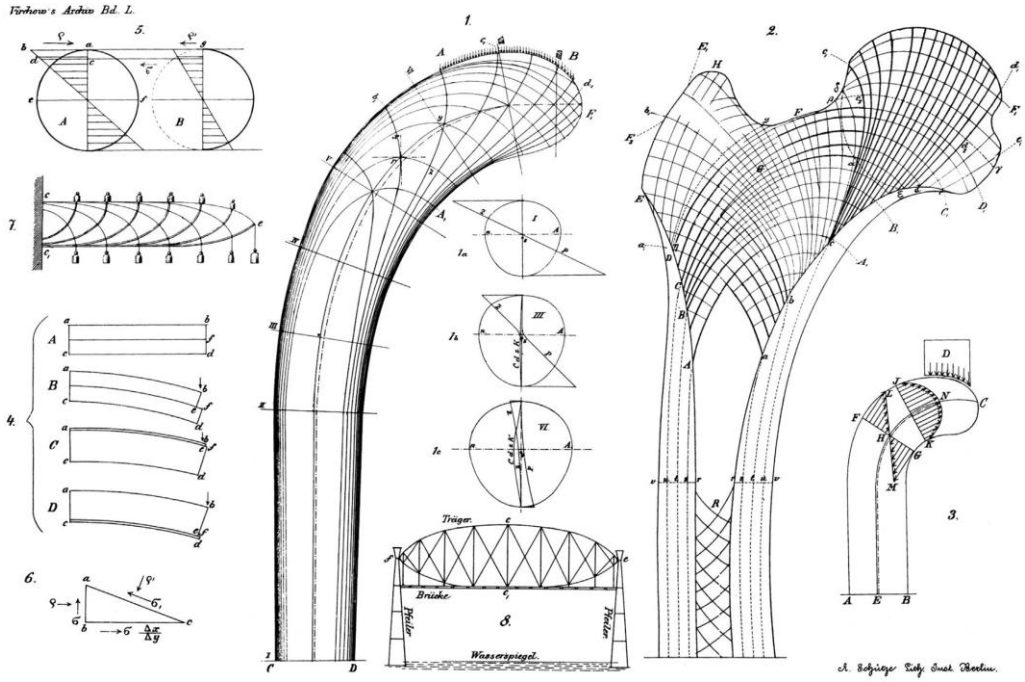

Fig. 12. Finite element analysis of bone scaffold [54] https://www.frontiersin.org/articles/10.3389/fbioe.2017.00030/full Accessed 7 April 2019.

3.4. STL Scaffold Generation

After the mechanical analysis is complete, the designed scaffold is almost ready for manufacturing. So, a surface STL triangular mesh model is constructed from the volumetric 3D model.

3.5. STL Model Optimization

At this point, the surface model includes millions of faces, and due to its size may be impractical for 3D printing machines. So, a mesh decimation algorithm needs to be applied to the STL Scaffold to reduce the number of faces in the model. Then, the surface mesh is optimized for 3D printing by eliminating triangles whose area is below a predefined threshold and by unifying triangles that are located near one another without introducing structural error into the model.

3.6. Topology Optimization

Finite element based topology optimization is a process of finding the optimal distribution of material and voids in a given design space, dependent on loading and boundary conditions,
such that the resulting structure meets prescribed performance targets. Material parameters, processing parameters, scanning parameters, support structures and the printed parts themselves must be optimized to ensure products with smallest environmental impact and highest efficiency and life time.
The process makes it possible to eliminate small details that are not structurally significant and to reinforce

prominent features by changing the thickness of the structure as needed. At the end of this stage, the model is also checked for 3D connectivity by discarding small disconnected pieces.
The Professors Jun Wu, Niels Aage and Ole Sigmund (TU Denmark) and R¨udiger Westermann (TU Munchen) presented a research work in optimization for Additive Manufacturing Process of Bone-like Porous Structures [55]. In the study, a structural optimization method is presented for obtaining stiffness optimized porous structures. These numerically optimized structures visually resemble trabecular bone, which is lightweight and robust with respect to material deficiency and force variations. This makes the optimized interior structures an ideal candidate for application specific infill in additive manufacturing. Cross-section of the optimized porous infill in a 3D bone model is presented in the Fig. 13 [55] https://ieeexplore.ieee.org/abstract/document/7829422/ Accessed 7 April 2019.

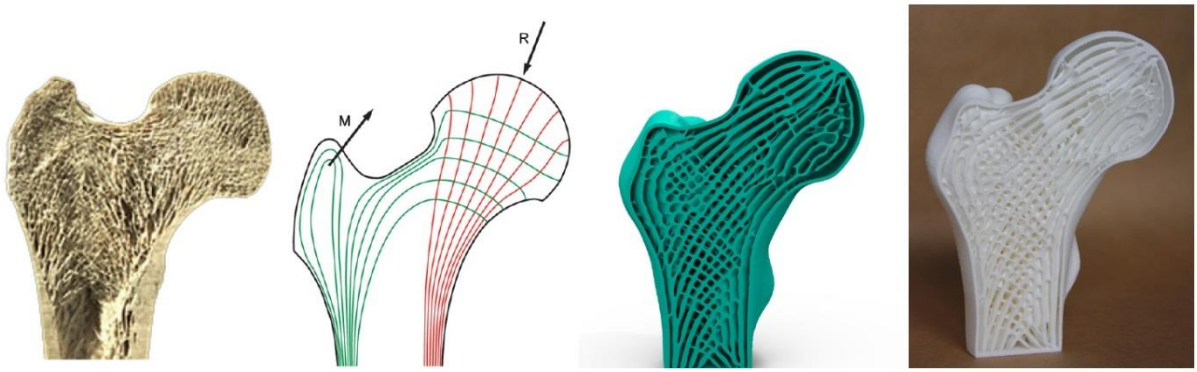

Fig. 13. From left to right: Cross-section of a human femur showing cortical structures on the shell and trabecular structures in the interior [55] https://ieeexplore.ieee.org/abstract/document/7829422/ Accessed 7 April 2019.

4. Conclusion

In this research work, applications of applications of additive manufacturing to tissue engineering are presented. To increase efficiency of part manufacturing, finite element analysis can be applied to the designed scaffolds. The finite element analysis software such as Ansys and Abaqus can be used to analyze the designed scaffolds. Programming languages such as Matlab as well as Visual Basic can also be used for the finite element analysis as well as topology optimization of the designed scaffolds. As a result, accuracy as well as efficiency in manufacturing process of bioengineering products using additive manufacturing can be increased.

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