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Current 3 Dimensional Printing Technologies Used in Scaffold Design in Tissue Engineering

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ABSTRACT

In this study, current 3 dimensional printing technologies have been critically reviewed and most suitable printer technologies for tissue scaffold building used in bioprinting has been identified. The specifications of 3 dimensional printers appropriate for biomaterials used in building artificial tissue structures include the bioplotter's ability to provide porosity, structural strength, smoothness and biodegradability conditions required for tissue engineering. The findings outlined in this paper can be useful to both academic and industry followers interested in bioprinting applications such as Scaffold Design and manufacturing by 3 Dimensional Printer Technologies.

Keywords: *3 Dimensional printing, scaffold, tissue engineering*

Doku Mühendisliğinde Doku İskelesi Tasarımında Kullanılan Güncel 3 Boyutlu Yazdırma Teknolojileri

ÖZET

Bu çalışmada günümüzde kullanılan 3 boyutlu yazdırma teknolojileri incelenmiş ve biyoyazdırma için kullanılan yazdırma teknolojileri arasında en uygun doku iskelesi inşa edenler tanımlanmıştır. Araştırma kapsamında üretilmesi hedeflenen doku yapısına göre kullanılacak olan biyomalzemelere uygun 3 boyutlu yazıcıların özellikleri, 3 boyutlu yazıcıların doku mühendisliği için gerekli olan gözeneklilik, yapısal dayanım, pürüzsüzlük, biyoçözünürlük kalitesini sağlama koşullarına yer verilmiştir. Bu araştırmada özetlenmiş olan bulgular, Doku İskelesi Tasarımı ve 3 Boyutlu Yazdırma Teknolojileri gibi biyoyazdırma uygulamaları ile ilgilenen akademik ve sanayi çevre için yararlı olacaktır.

Anahtar Kelimeler: *3 Boyutlu yazdırma, doku iskelesi, doku mühendisliği*

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I. INTRODUCTION

Tissue engineering is regeneration, restoration, or replacement of defective or injured functional living organs, bones or tissues. The need for tissue engineering is emerged from long waiting list and supply shortage of organ transplant [1], long recovery times of surgery patients, unhealed parts, compulsory removal of organs due to cancer, accident or birth defects [2].

Tissue engineering starts with data acquisition, namely collecting cells by biopsy from the patient, imaging of body parts and defining which material composition to be used. Imaging methods include X-ray computed tomography, magnetic resonance imaging, ultrasound echoscopy, single-photon gamma rays (SPECT) and bioluminescence imaging [3]. Although a patient's organ can successfully be scanned using one of these methods, scan results usually lacks sufficient information about the organ layers. Each one of these layers can have different density and porosity, thus making the design of tissues more complex than traditional scan-and-print process of industrial production methods.

The complex cellular behaviours of organs and heterogeneous nature of native cellular environments impede 2-dimensional fabrication techniques since they cannot precisely tailor 3D culture systems [1]. In order to form complex 3D organ structures, a combination of biomaterial scaffolds, cells and growth factors (bioreactors) are required (
Figure 1. Osteochondral tissue [3])

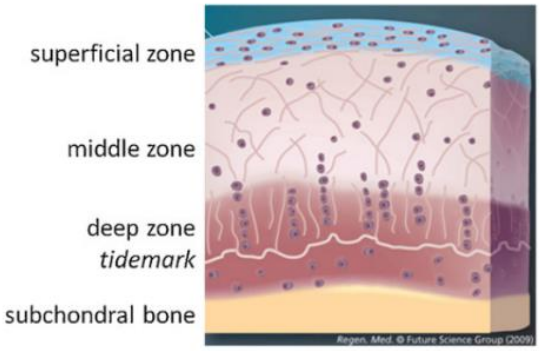

Figure 1. *Osteochondral tissue [3]*

Tissue engineering field relies extensively on the use of these porous 3D scaffolds which essentially act as a template for tissue formation [4]. Scaffolds can be produced either growing or fabricating biomaterial scaffolds and a person's own cells [1]. Two types of strategy are utilized in developing scaffolds. In the first strategy, the scaffold has to provide support in vivo. It is directly implanted into the injured site, using the body's own systems, meaning regeneration of tissues or organs is induced in living environment. In the second strategy cell-seeded scaffolds are cultured in vitro (test tube, culture dish etc.) to synthesize tissues which can then be implanted into an injured site [4]. The scaffold only provides support in vitro until the cells are strong enough to support themselves in vivo [5].

Apart from the material and functional properties of tissue scaffolds, successful production of organs also requires the correct 3D printing mechanism to be chosen. The vulnerability of tissue materials

makes it hard to choose any 3D printing device normally used in custom made non-living products. Building speed, nozzle temperature, porosity requirements, provision of different material options are among many of the factors affecting cell viability and structure. Thus, in order to engineer a tissue, 3 factors must be taken into consideration: 3D bioprinting device specifications, types of materials and materials' design structure.

In general, 3D printing technology advanced mostly on solid – non-living structures, nonetheless bioprinting using this technology is emerging as new application. Proprietary materials developed for 3D printing devices available for various applications. These materials are the major part of what makes this technology works and made available after they have been tested again and again to find the correct settings of device, material composition, environment and structure. For the tissue engineering the problem is further complicated as the limitations on tissue engineering research is that the subject materials are not easy to find, reproduce and to test. To overcome the difficulties of material consumption and real life testing impossibilities, artificial environments like 3D simulation analysis software can be used to design and test the final products. The success of this analysis depends mostly on the previously collected bioinformatics data.

II. 3D Bio-Production Methods

Production of artificial tissues started for cells, peptides and biomaterials around a half century back. However manufacturing of biological constructs including living cells (
Figure 2. History of 3D Bioprinting Technologies [3]) dates back to 1990s [3] . In year 2000, bioengineer Thomas Boland get inspired by scientists who modified inkjet printers to print fragments of DNA to study the gene expression. Boland discovered that the same hardware could print other biomaterials since the smallest human cells are 10 μm, roughly the dimension of standard ink droplets. Thus he printed proteins with Lexmark's ink cartridge, and after 90% successful trials with other materials, Boland has patented the bioprinting device used for the tests in 2003 [6]. After this major breakthrough, several patterns, viable cells and biomaterials have been developed, and the structures turned into a 3D format. Printing technologies like biolaserprinting, stereolithography and robotic dispensing (fused deposition modeling, bioplotting) have been developed or modified to include cells in the fabrication process. In vivo printing is realized by biolaserprinting recently [3].

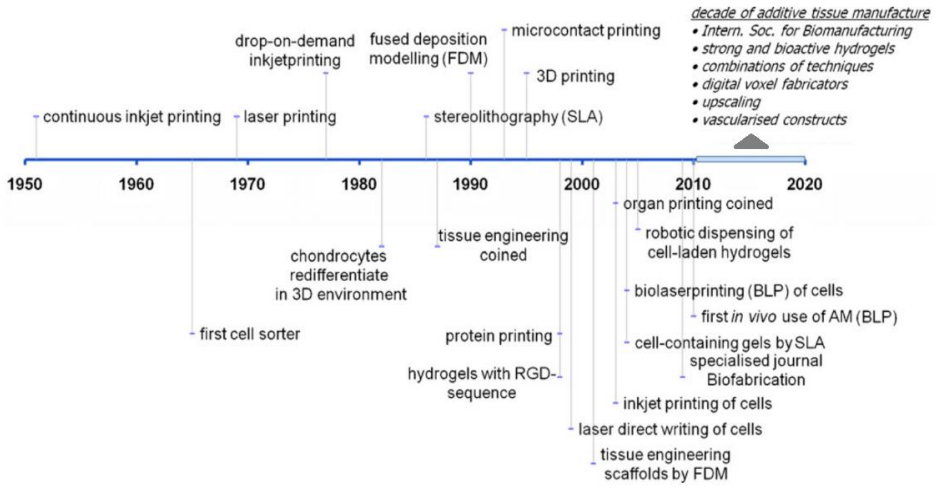

Figure 2. *History of 3D Bioprinting Technologies [3]*

In a recent study [2], Xiaohong and colleagues classified the 3D printing technologies under 4 different categories, namely: the working principles; starting material states; energy sources; and the biological functions.

A. 3D BIO-PLOTTING TECHNOLOGIES

A.1. BINDER JETTING

This is one of the earliest rapid prototyping technologies and also known as powder bed and inkjet head 3D printing. Broadly, a liquid bonding agent (such as a polymer solution) is selectively deposited in conjunction with the powder materials. 'Adhesive Mediated Fabrication' is the bioplotting process based on this well-known binder jet printing technology. A natural biopolymer powder is binded with adhesives or solvents, by using binder solution as a cartridge in such inkjet printers. Multiple layers can be fabricated on the scale of polymer particle size (approximately 200–300 μm).

Scaffolds composed of natural biopolymers such as starch, dextran, and gelatin can be formed using aqueous solvents, and micropores can be formed by particle leaching. Griffith at al. used PLGA for liver tissue engineering and demonstrated rat hepatocyte attachment [7].

A.2. MATERİAL EXTRUSİON

Also known as Fused deposition modeling (FDM) / fused filament fabrication (FFF) and stick deposition molding (SDM), is a process in which material is selectively dispensed through a nozzle or orifice. FDM creates models using thermoplastic materials melted and extruded through a nozzle over a computer-controlled table. The thermoplastic material is usually needed in filament form as the printing material. 3D structures of any complexity can be formed by adding 2D layers on top of one another. In the emerging field of so-called computer aided tissue engineering (CATE) several groups are reporting the use of FDM for the fabrication of 3D constructs. The FDM is the most common and simplest 3D printing technology [8].

A.3. 3D BIO-PLOTTİNG

This process is similar to binder jetting with the difference is that the actual model/part material is deposited through microsyringe/micronozzle rather than depositing a liquid bonding agent on the powder material to hold them together. In bioplotting, also known as Precise Extrusion Manufacturing (PEM), a micro needle (outer diameter around 10-20μm [7]) is employed as the extrusion nozzle where liquids, reactive oligomers or dispersions are stored in a heated cartridge [9]. The syringe / cartridge setup makes it easier to change between materials which allows ease of use and versatility [10]. These materials are then extruded into a temperature controlled liquid dispensing medium which induces solidification of the deposited material by cooling, heating or through chemical reaction [10]. The polymer stream thickness can be modified by varying the solution viscosity, syringe-tip diameter, syringe pressure, and stage motor speed which enables creation of structures from 5 μm to 600 μm [7].

The use of dispensing solution which has similar density as the building material, is that the buoyancy exerted by the medium on the build can prevent the collapse of complex structures thus eliminating the need for sacrificial support structures [9] and allows the seeding of living cells into the scaffold [10].

This technology has been used by Mulhaupt and coworkers to deposit agar and gelatin solutions heated to 90 ^{0} C into a cooled plotting medium, resulting in a 3-D hydrogel scaffold [7], and also used by Ang and colleagues in forming chitosan and chitosanhydroxyapatite scaffolds [7]. A recent work by Hee et al. an oscillating nozzle system was used in order to increase elastic modulus and yield strength of the strand in the scaffold [9]. Various materials were studied including PLGA Scaffold with controlled pore structures by Daoud et al. [9], non-stoichiometric apatite (ns-AP) and poly(ε-caprolactone) (PCL) scaffolds with well interconnected 400–500 μm pores by Ye et al. [9] and starch/polycaprolactone SPCL scaffolds with a 0°/90°strut structure by Oliveira et al. [9].

A.4. DİRECTED ENERGY DEPOSİTİON

This process uses focused thermal energy (e.g., laser, ultraviolet (UV), electron beam and plasma arc) to fuse or melt the materials being deposited. Electron beam manufacturing (EBM) and laser powder forming (LPF) are some well-known individual processes using this technology.
EBM comprises of an electron gun (similar to one in a scanning electron microscope) which operates at a power of 60 kW to generate a focused beam of energy density above 100 kW/cm2. The beam focus is controlled by the electromagnetic lenses and the beam movement is controlled by deflection coils. For building a part, a powder layer of ~100 μ thickness is spread over the table. The powder is supplied from two hoppers (cassettes) kept inside the build chamber. A moving rake spread the powder over the table. The electron beam first pre-heats the powder layer with a higher scan speed, followed by melting the powder layer based on the geometry defined by the CAD file. In EBM, every layer of a part is built in two steps. The contour part is produced first and then the powder inside the contour is melted and together they form the first layer [11]. EBM is used for production of orthopedic components such as knee, hip replacements, and maxillofacial plates [11].

A.5. POWDER BED FUSİON

Similarly to the directed energy deposition, powder bed fusion uses thermal energy to fuse regions of a powder bed. Selective laser sintering (SLS), selective laser melting (SLM), selective heat sintering (SHS), and electron beam melting (EBM) are all exemplary processes named after the source of thermal energy. Of these processes, SLS is the most widely used technology in the powder bed fusion process [8]. SLS is a heat based technique, where a thin layer (~100 – 200 μm) of powdered polymeric material is spread on a surface using a cylindrical roller. A laser is then scanned over the powder bed, which heats the powder locally and sinter-bonds the adjacent particles to form layer of patterned structures. High porosity and surface area obtained by removing the non-sintered particles released from the part without loss of mechanical integrity makes this technology especially suitable for scaffold manufacturing [7]. SLS parameters, notably scan direction and spacing can significantly effect on the dimensional accuracy and mechanical properties of scaffolds. The effect was shown for HA (hydroxyapatite) and PCL (poly(ε-caprolactone)) scaffolds. Cellulose based scaffolds like starch cellulose and cellulose acetate can be produced at satisfactory level of porosity and mechanical properties for the design and fabrication of scaffolds with potential use in tissue engineering and drug

delivery by optimizing the laser power and scan speed. SLS is also a successful method in fabricating nanocomposite scaffolds for bone tissue engineering using Calcium Phosphate (Ca–P) / poly (hydroxybutyratecohydroxyvalerate) (PHBV), and carbonated hydroxyapatite (CHAp)/poly(L-lactic acid) (PLLA) nanocomposites [9].

Selective Laser Melting (SLM) has a similar principle to SLS, except that a fibre laser source is used to melt very fine metal powders in an inert gas filled chamber which ensures higher purity by minimizing the oxygen in the environment and reduces the risk of hydrogen pick up [11]. In order to build a part, a powder layer of 20–100 μm thickness is spread over the table which can be preheated up to 200˚C. The powder is carried and spread by the powder re-coater across the table [11]. Advantage of full melting is that it eliminates the need for post process furnace operations which is done in SLS, and it doesn't require any binders or laminating operation [9]. Different types of metals are used in SLM (such as stainless steels, cobalt-chrome and titanium), mostly for production of custom fitting implants and prostheses. Research conducted by Lindner et.al.(2011) [9] to assess the SLM production of scaffolds using β-tricalcium phosphate (β-TCP) and poly (D, L)- lactide (PDLLA). Results showed that biodegradable composite materials can have completely interconnected porous structure offering a regular and reproducible morphology of the pores [9].

A.6. MATERİAL JETTİNG

Processes such as multi-jet printing (MJP) / multiJet modeling (MJM), polyJet printing, and contour crafting (CC) can all be considered under material jetting process in which droplets of build material are selectively deposited.

When this technology is used for biological materials, the process is called Inkjet Bioprinting. Inkjet bioprinters are one of the most widely used for bioprinting applications. The ink cartridge found in the material jetting machine is filled with a biological material. Thermal or acoustic forces are then used to eject drops of liquid from a nozzle onto a substrate. Thermal inkjet printers function by electrically heating print head to produce pulses of pressure that force droplets from the nozzle [12].
Several studies have shown that this localized heating (from 200 °C to 300 °C) does not have a substantial impact either on the stability of biological molecules or on the viability or post-printing function of mammalian cells. It has been demonstrated that the short duration of the heating (~2 μs) results in an overall temperature rise of only 4–10 °C in the printer head. The advantages of thermal inkjet printers include high print speed, low cost and wide availability. However, the risk of exposing cells and materials to thermal and mechanical stress, low droplet directionality, nonuniform droplet size, frequent clogging of the nozzle and unreliable cell encapsulation pose considerable disadvantages for the use of these printers in 3D bioprinting [12].

Piezoelectric crystal is another method used to break the liquid into droplets at regular intervals by acoustic wave inside the print head [12].

Thermal and piezoelectric ink-jet printing model can be seen in Figure 3. Drop-on Demand Printers [13].

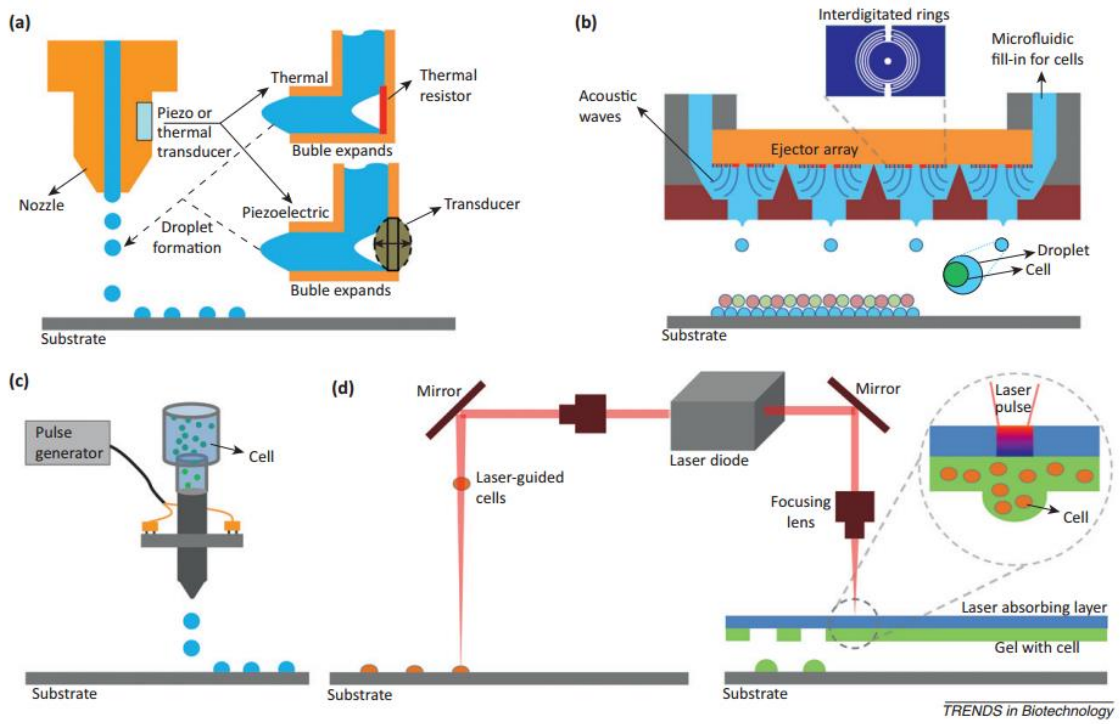

Figure 3. *Drop-on Demand Printers [13]*
(a) Thermal and piezoelectric ink-jet printing; (b) Acoustic droplet generation; (c) Valve based printing; (d) Laser induced printing

Inkjet bioprinters have limitations on material viscosity. Higher viscosity materials requires excessive force and can cause clogging of the nozzle. In piezoelectric inkjet printing systems, ideal viscosity is below 10 cps for PLGA (poly(lactic-co-glycolic acid) and ideal surface tension of bio-ink should be in the range of 30–70 mN/m, high enough to prevent dripping of the ink from the nozzle and low enough to allow spreading over the substrate and prevent clogging at the nozzle [14]. Acoustic Droplet Ejection uses focused acoustic radiation pressure to eject discrete droplets of uniform size from the surface of a dextran solution. The pulse duration, and amplitude size of droplets is controlled by adjusting ultrasound parameters. The ejected dextran droplets are captured on a cell culture substrate that can be positioned by 3D positioning system [15].

Laser assisted bioprinting uses a laser beam, trapping photons and guide cells by focusing the laser is into a cell suspension and the force due to the difference in refractive indexes moves the cells onto an acceptor substrate. The cell–hydrogel compound is propelled forward as a jet by the pressure of vapor bubble passing through laser absorbing layer [13]. Thermal transducers or laser power that's used in droplet ejection can increase the cell compounds to temperatures that cell viability is no longer valid, as a solution near-infrared wavelengths (700–1000 nm) have been used [13].

Robocasting is also referred as direct-write assembly is used to build scaffolds using water-based inks with minimal organic content (< 1 wt%). Scaffolds are formed by depositing highly concentrated colloidal suspensions (inks) capable of fully supporting their own weight during assembly owing to carefully tailored composition and viscoelastic properties so they don't need a sacrificial support material or mold [16].

Miranda and colleagues has studied Tricalcium phosphate (TCP, Ca3(PO4)2) for tissue engineering bone and cartilage, since bioceramics exhibit a higher biodegradability (higher resorption rate) than other materials like hydroxyapatite (HA, Ca10(PO4)6(OH)2), which is the main inorganic component of bone. Among the three allotropic forms of TCP, β-TCP is preferred as a bioceramic because of its chemical stability, mechanical strength, and intermediate bioresorption rate. Commercially available β-TCP (Fluka, Buchs, Switzerland) powders were used to prepare inks for robocasting. The resulting samples were dried in air at room temperature for 24 hours and then at 400ºC (1ºC/min heating rate) for 1 hour to evaporate organics, followed by the appropriate sintering treatment at temperatures ranging from 1250ºC to 1550ºC (heating rate 3ºC/min), depending on the starting powders, and sintering times of from 2 to 6 hours [16].

A.7. VAT POLYMERİSATİON

3d printing methods known as stereolithography (SLA or SL), digital light processing (DLP), scan-LED technology (SLT), multiphoton lithography are all vat polymerisation process where liquid photopolymer in a vat is selectively cured by light-activated polymerization. SLA is curing of thin layers of liquid photopolymer, using a laser beam emitting at UV (ultraviolet) or visible spectrum. There are two methods of irradiation; 1st is a mask-based method in which an image is transferred to a liquid polymer by irradiating through a patterned mask; and the 2nd method is a direct writing by using a focused UV beam produces polymer structures [3]. The light is scanned on the photopolymer resin in accordance with designed model in forms of thin layer. Sequentially printed 2D layers then form a 3D structure [12]. Stereolithography is limited in resolution by laser beam diameter to approximately 250 μm, although small-spot laser systems are capable of production of smaller (70 μm or less) feature [7]. Even smaller features can be produced by a process called micro-stereolithography (μSL) where the laser beam is focused more precisely in order to reduce the spot size to a few micrometers of diameter to solidify a thin layer of 1–10 μm in thickness. Cell proliferation on the μSL scaffold was clearly superior [9].

Biodegradable 3-D polymer scaffolds were produced for bony tissue consisting of diethyl fumarate, poly (propylene fumarate) and the photoinitiator bisacylphosphine oxide. Also, cancellous bone and hydroxyapatite bone tissue scaffolds were formed by photocurable ceramic acrylate suspension, with overall dimensions suitable for healing critical-sized (4-mm thickness, 50-mm diameter) bone defects [7].

Multiphoton Lithography is working on two-photon absorption method; the beam of a fast laser is focused into the volume of the transparent photosensitive material, the polymerization process can be initiated by nonlinear absorption within the focal volume three-dimensionally through the material, so that 3D structures can be fabricated. Materials like acrylate and epoxy and several components have been fabricated such as photonic crystal templates. Difference from SLA is that the monomer/oligomers used as materials should be transparent enough to enable the laser to focus inside the volume, and the transparency should be at the absorption wavelength [17].

The disadvantages of the light processing printing techniques are the long fabrication times and the gravitational incline tendency of the scaffold towards the nozzle direction. To overcome the speed limitation, a mirror device was replaced single laser tip to produce whole layer at once [3].

A.8. SHEET LAMİNATİON

Sheet lamination processes include laminated object modeling (LOM), and film transfer imaging (FTI) or selective deposition lamination (SDL), where sheets of material are bonded to form an object. The process also known as heat-mediated 3D fabrication when pre-fabricated polymer layers are combined by heat energy into 3D structures by raising the polymer above its glass transition temperature and fusing the softened layers together with applied pressure. A setback for this method is that it has low void volume and high density for allowing high cellularity [7].

Chen and colleagues found out more intricate scaffolds lamination techniques can be used with biodegradable polyester polymers such as poly(DL-lactic-co-glycolic) acid (PLGA) by micropatterning using various techniques. They included Borenstein and colleagues (2002) laminated thin biodegradable films containing small trenches by casting PLGA onto microfabricated silicon masters and formed a vascular tissue engineering scaffold with 20 μm diameter channels between layers [7]. Bhatia and colleagues (2003) created polymer layers that have similar shape and resolution (20–30 μm) to those on the silicon master and could be fused together [7].

B.OTHER BİO-PLOTTİNG METHODS

B.1. PRODUCTİON BY MOLDİNG

For sensitive biomaterials its inverse production is used by molding. The mold is subjected to the production process, so that the resulting scaffold is not effected by the process environment [7].

B.2. SOLVENT CASTİNG OR PARTİCLE LEACHİNG

Solvent casting or particle leaching is another technique to have least impact on the results. The polymer solution is dissolved in a solvent with uniformly distributed salt particles, after the solvent evaporated the composite is left with uniformly distributed salt particles. After the matrix is immersed in water, salt particles are leached leaving same size of pores (up to 500 μm) and 90% porosity in the resulting polymer [1].

Bajaj and colleagues included the findings of research made for different materials by Park et al.(2007) by using sodium chloride as the porogen and dimethyl sulfoxide as the solvent to form a block polymeric scaffold of PEG and poly(E-caprolactone) (PCL) [1]. Mehrabanian and colleagues (2011) fabricated nanohydroxyapatite (nHA) nylon 6,6 composite scaffolds where the microstructure of the scaffold had pores size ranging from 200 to 500 μm, which was similar to the size of the porogen used [1]. Bajaj referenced another study done by Ford et al. (2006) used a salt-leached PLGA scaffold as a template around which the authors synthesized a hydrogel by cross-linking PEG with poly-L-lysine. This hydrogel was then degraded by sodium hydroxide to create a highly porous hydrogel scaffold that was used for seeding endothelial cells in vitro, and the authors reported efficient microvessel formation [1]. One of the biggest advantages of this technique is that the controllable pore size and hence controlling the size and geometry of the porogen yielding control of the mechanical properties of the scaffold [1]. However, the main disadvantage of the technique is scaffold needs to be repeatedly washed to ensure complete removal of the cytotoxic organic solvents and minimize cell death [1].

B.3. FREEZE-DRYİNG

Freeze-drying, also known as lyophilization, is a process in which a polymer solution is cooled down below its freezing point, so the solvent molecules solidifies, and the polymer to aggregates in the cracks of the scaffold. The solvent is then evaporated via sublimation leaving behind a highly porous polymeric structure with interconnected pores that can be used for seeding the cells [1].

B.4. COMPARİON OF 3D BİO-PRİNTİNG TECHNOLOGİES

In general, techniques that use optics can achieve the highest resolutions. Examples of accurate optical fabrication methods are stereolithography, laser direct writing and biolaser printing. Additionally, photo-initiated polymerization can be used for safe encapsulation of cells and exogenous growth factors into hydrogels. Thermal techniques such as selective laser sintering or fused deposition modeling are not compatible with temperature-sensitive cells, but they can be adapted for processing thermosensitive hydrogels [3]. “Mechanical processes often allow for including cells in the fabrication process, as long as shear stresses induced on cells such as by deposition through a needle or inkjet cartridge orifice are sufficiently low” [3].

Table 1. *Comparison of 3D bioprinting technologies [3],[2]*

Technique	**Accuracy (μm)**	**Materials**	**Cells incorp.**	**Advantages**	**Disadvantages**	**Refs**
3D printing	50	Polymers,ceramics	No	Multiple compositions	Requires powder, cell-unfriendly environment	(M)
Binderjetting	200-300	Natural biopolymers (starch, dextran, and gelatin)				
Biolaserprinting	10	Liquids	Yes	High accuracy at high speed	Low viscosity prevents build-up in 3D	(M)
Direct writing	1	Polyelectrolytes, A concentrated colloidal gel (typically 50% HA particles suspended in an aqueous medium)	Not yet	High accuracy, two materials can be printed in a construct	Requires solvents, cell-unfriendly environment, scalability	(M)
Double nozzle extrusion		Natural polymer hydrogels, such as gelatin, gelatin/alginate, and gelatin/alginate/fibrinogen	Yes	A wide range of biomaterials ; cells and bioactive agents can be incorporated	Weak mechanical properties, high concentration of hydrogels, biodegraded in vivo	(X)
Double nozzle low temperature extrusion		Natural and synthetic polymers	Yes	A wide range of biomaterials ; growth factors, cytokines, chemicals, accurate, genes can be	High concentration of hydrogels, organic solvents are needed for synthetic polymer deposition, removal after	(X)

Technique	Accuracy (μm)	Materials	Cells incorp.	Advantages	Disadvantages	Refs
				incorporated, stable, fast, controllable, scalable	printing	
Extrusion based	100	Thermoplastics, composites, Natural or synthetic polymer solutions,	Yes	Technologically simple, wide range of materials, high accuracy, flexible, reproducible, scalable, growth factors, constructs with high mechanical properties	Requires strong filament and high temp, organic solvents are needed for synthetic polymer deposition, cells are difficult to be incorporated; Nozzle easily clogging; harms to cells	(M), (X)
Fused Deposition modeling		Synthetic polymers, hydroxyapatite incorporated polycapralactone		Automated, controllable, fast, accurate, reproducible, scalable	Cells cannot be incorporated directly, limited materials	(X)
Indirect 3D bio-printing		Calcium phosphate modified PCL (PCL-CaP) and treated with fibrinogen	Yes	A wide range of biomaterials can be used; cells and bioactive agents can be incorporated	Low accuracy of the final structures; complex processing procedures	(X)
Inkjet printing	10–100	Liquids, Hyaluronic acid (HA) improved gelatin-methacrylamide	Yes	High mechanical properties; use of	Low viscosity prevents build-up in 3D, low strength, limited	(M), (X)

Technique	Accuracy (µm)	Materials	Cells incorp.	Advantages	Disadvantages	Refs
		(gelMA) hydrogels		existing cheap technology, multiple compositions	biomaterials	
Laser direct writing	20	Cells in media	Yes	Single cell manipulation	No structural support, scalability	(M)
Microstereolithography		Poly-(L-Lactide-co-ε-caprolactone)/gelatin, heparin, transforming growth factor- β1, chondrocytes	No	A wide range of biomaterials can be used; bioactive agents can be incorporated	Low accuracy of the final structures; complex processing procedures; limited mechanical properties	(X)
Robotic assembly	5	Rigid solids	Not yet	No heat,light or reaction required	Expensive machinery	(M)
Robotic dispensing	100	Hydrogels,polymers,ceramic-composites	Yes	Multiple compositions	Relatively low accuracy	(M)
Selective laser sintering	50	Polymers,ceramics	No		Requires powder, cell-unfriendly environment	(M)
Stereolithography (incl.two-photon polymerization)	0,5–50	Hydrogels, polymers, ceramic-composites	Yes	High accuracy	Single composition, requires photo-curable material	(M), (X)

Technique	Accuracy (μm)	Materials	Cells incorp.	Advantages	Disadvantages	Refs
Thermal Inkjet Printing		Collagen solutions	No	The fabrication temperature can be reduced	Low accuracy; low mechanical properties; cells cannot be incorporated	

(M) [3], (X) [2]

II. CONCLUSİON

Considering the limitations of material operation by devices and limitations of material supply, the 3-dimensional printing technology should be chosen upon the material selection. Adjustments can be made to printers; however materials are not in vast amount to be tested. In order to solve this dilemma, using bioinformatics data applied to 3D printing design software would be useful. Simulation of tissue bioproduction tests should be executed with laboratories and 3D printer production companies, not to just enable mass production as most of the 3D printing companies target, but to help the science to advance further.

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