Various methods of 3D and Bio-printing

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SUMMARY
There is growing need for synthetic tissue replacement materials designed in a way that mimic complex structure of tissues and organs. Among various methods for fabrication of implants (scaffolds), 3D printing is very powerful technique because it enables creation of scaffolds with complex internal structures and high resolution, based on medical data sets. This method allows fabrication of scaffolds with desired macro- and micro-porosity and fully interconnected pore network. Rapid development of 3D printing technologies has enabled various applications from the creation of anatomical training models for complex surgical procedures to the printing of tissue engineering constructs. The aim of current investigations was to develop compatible printers and materials (bioinks) to obtain biomimetic scaffolds, which allow printing of living cells without significant loss of cell viability. The advanced level of such printing assumes “in situ” printing, i.e. printing cells and biomaterials directly onto or in a patient that will reduce recovery time.

Keywords: 3D printing; bio printing; scaffolds, biomaterials

INTRODUCTION
Tissue engineering strategy typically involves combination of cells and bioactive factors with a 3D scaffold to form useful construct for implantation [1, 2]. Ideally, scaffold should be completely resorbed during tissue integration. Biomaterial scaffolds should mimic important aspects of targeted tissue, restoring their function and providing an environment suitable for cell differentiation and proliferation [3, 4]. Traditional techniques used for production of such scaffolds are gas foaming, solvent casting, fiber bonding, phase separation, particulate leaching, and freeze drying techniques that provide macroscale scaffold features but often lack the complexity of native tissue [5].

Fabrication methods that enable production of complex geometries have significant advantages since they provide production of scaffolds of irregular shape that can perfectly fit the defect geometry. Besides, they can also mimic tissue complexity through precise positioning of multiple materials and cell types. As it is known, scaffolds should provide not only macroscale structural design, but also microscale features necessary for cellular sophisticated control over fabrication of a new tissue. Recently, 3D fabrication or rapid-prototyping technology has become popular and accessible, allowing everyday investigations of potential new fabrication techniques with better geometric accuracy on the macro and micro scale level [6, 7]. Those investigations have opened door to innumerable approaches of scaffolds engineering such as high-resolution imaging and 3D printing technology known as laser sintering which was successfully used to create functional jawbone replacement [8]. This method has enabled creation of articulated joints, cavities that promote muscle attachment, and grooves to guide nerve and vein regrowth, and also reduced surgical preparation and accelerated recovery. In addition, designed vasculature may enable creation of larger constructs useful for nutrient transport for tissue growth. Functional tissue constructs could also be applied as a diagnostic tool for drug testing or other therapeutic procedures.

Currently used 3D biofabrication printing methods can be divided into acellular techniques which include stereolithography (SLA), powder-fusion printing (PFP) and fused deposition modeling (FDM)), and bioprinting of cellularized constructs that can be inkjet-based, extrusion-based, or laser assisted (LAB)) [8].

ACELLULAR SCAFFOLD FABRICATION
Rapid prototyping techniques use multi-axis positioning systems and one of various methods to generate a 3D construct through subsequent layer fabrication (extrusion, deposition, solidification, polymerization, sintering or binding using many other methods) [8, 9]. First step is creating a model in a computer-aided design (CAD) program and export it into the file format that describes the volume or surface mesh in 3D space such as *.stl (stereolithography), *.obj (object), or *.amf (additive manufacturing file). Second step is translation of the 3D data into slices to be patterned by the printer program using the
Powder-fusion printing (PFP) program generally known as a ‘slicer’. These techniques enable user to configure algorithm that determines pattern used to fill the layers and then the program calculates necessary parameters such as extrusion speed, cure time, or laser speed to accurately fill the pattern.

Previously, these techniques were adapted to mold casting, but recent rapid development increased their versatility and precision. Nowadays techniques are able to create scaffolds that fully mimic macroscale organs geometry and print layers with thickness less than 20 μm allowing complete reproduction of the tissue microarchitecture. Techniques with higher precision are currently under investigation to enable reproduction of smaller tissue features such as hepatic lobules and kidney nephrons.

**Stereolithography (SLA)**

SLA techniques use deflected laser beam or projected light source to cure and harden given areas of photopolymer at the surface of some material (Figure 1) [8, 10]. Various photopolymers with suitable viscosity and ability to harden can be used in construct creation with SLA. Cooke used SLA to fabricate 3D scaffolds for bone tissue engineering using biodegradable polymers, like diethyl fumarate and polypropylene fumarate [10]. Also, photo-curable ceramic acrylate suspension was used to form a construct of cancellous bone and bone scaffolds using hydroxyapatite [7].

The disadvantage of SLA methods is limited resolution by the diameter of laser beam (about 250 μm), although small-spot laser systems and digital light processing projection produced features of about 70 μm. These techniques can also be used to design hydrogel scaffolds from natural and synthetic polymers that expand in water and are significantly less rigid than traditional SLA constructs. Hydrogels have become popular as tissue engineering biomaterials due to their high water content and mechanics similar to soft tissue. Some researchers use this technique for creation of 2- hydroxyethyl methacrylate scaffolds using photolithography for formation of patterns from non-swollen prepolymer, which were then hydrated and seeded with cells [11]. SLA has also been used to make molds that are used to cast negative replicas of the printed molds. Chu et al. made printed mold of a mandible generated using CAD program and data from computed tomography imaging. The mold was filled with a hydroxyapatite/acylate mixture and heated to cure the scaffold [12].

Accordingly, SLA seems to be a versatile and attractive technique for creating tissue-engineering scaffolds because of its precision and increasing availability of biologically relevant photopolymers.

**Powder-fusion printing (PFP)**

PFP uses granular materials (plastic, resin, or metal) for printing that are selectively bound together (Figure 2) [8, 13]. In selective laser sintering-melting (SLS/SLM), plastic or metal granules are sintered together by a laser beam that is directed across the powder bed, to increase local temperature influencing particle fusion in the heated area along the laser path. 3D scaffolds are generated by layer-by-layer deposition of the powder. After fabrication, unfused powder is removed and the resulting part is mechanically strong construct with carefully designed geometry and porosity. As in SLA, the resolution of SLS printing depends on the spot size of the laser beam and the size of powder particles. Typical laser-based systems have minimum features of about 400 μm, with minimum void size of about 50 μm. SLS techniques have also been developed to fabricate constructs with various biopolymers used in a wide variety of medical implants.

Scaffolds can also be made from granular material by binding the particles with solvents or adhesives whereby
they are built layer-by-layer. Also, scaffolds can be fabricated from natural biopolymers and polysaccharides like gelatin, dextran, and starch. Microporous structures can be achieved with the addition of porogens and particulate leaching. For example, Simpson et al. fabricated porous poly (lactic-co-glycolic) acid scaffold using PFP and precisely reproduced the shape of an entire human finger phalanx [14]. These porous structures were also investigated from the aspect of cell attachment, growth, and matrix deposition.

Although PFP is limited to powdered materials, its advantage is capability to fabricate scaffolds from several materials such as titanium and magnesium that are not readily printable with other techniques. PFP is particularly suitable for bone and other rigid tissues scaffolds because bound or fused material creates constructs of superior mechanical properties. In addition, some materials naturally found in bone such as tricalcium phosphate can also be printed using PFP techniques, allowing creation of complex scaffold shapes, including in advance designed interconnected porosity. The resolution and minimum pore size are limited by the powder characteristics, and additional sintering is necessary to solidify parts that contain cracks and other damages. The focus of current research is on developing new materials for PFP and refinement of printing parameters to improve scaffold surface design.

Fused deposition modeling (FDM)

FDM techniques enable useful platform for scaffolds creation by using precise xyz positioning system to direct the position of a nozzle during material deposition [8, 15]. The material is deposited in layers and solidified into a previously defined shape. Traditional SFF printers are frequently used for rapid prototyping by using a small diameter polymer feedstock of acrylonitrile butadiene styrene which is forced through the nozzle heated to temperatures higher than 200°C.

Biodegradable polymers used in tissue engineering typically melt at lower temperatures and can be printed at more moderate temperatures (60-100 °C). Using this method it is possible to produce precise lattice structure, if temperature is precisely controlled and optimized with speed parameters during generation of filament with required accuracy. Newer generations of FDM systems use heated reservoir for extrusion of polymer pellets rather than fibers. Scaffolds produced by this technique from alginate and PCL implanted in mice have shown enhanced cartilage and collagen formation over a 4-week implantation [8].

Decreasing nozzle size and layer height increases x–y and z resolution, leading to significantly slower extrusion rates. Theoretical resolution is limited by the precision of the linear motion system (motors, gears, timing belts, and leadscrews) and retention properties of extruded material. Although FDM techniques enable the achievement of high degree of positional accuracy in the xy plane, their substantial limitation is in disability to print overhanging or unsupported parts because there is no supporting material from previous layers. Therefore, hardening during cooling or cross-linking after extrusion is essential for satisfied support of subsequent layers. Also, this drawback can be solved with introducing filament of support material during the process of printing, usually through additional extruder (Figure 3).

Recent improvements in hydrogel rheological properties enable printing of these materials using FDM. For example, Hong et al. created printable hydrogel using a network of PEG and alginate with silicate nano-platelets [16]. These gels possessed zero-shear viscosity above 10 kPa·s, enabling shape retention after printing and a shear thinning that facilitated extrusion. The size and accuracy of printed hydrogel construct are dependent on the volume contained in the syringe and rheological properties of the hydrogel. Viscosity plays a key role in construct accuracy, because high-viscosity materials possess structural rigidity that is important for support of extruded successive layers, and secondary cross-linking step is typically used to lock the printed shape and improve mechanical properties of these constructs.

Extrusion-based printers typically use pneumatic pressure or a motor actuated plunger for material deposition. Pneumatic systems simplify control of the applied force to extruded material. The system should be calibrated for each material with adjustments of the nozzle size, nozzle geometry (tapered tip, cylindrical needle, and length), and gas pressure.

FDM seems to be one of the most versatile printing techniques for creation of biomimetic scaffolds due to its ability to make multilayered constructs built from various materials and print soft biomaterials like hydrogels (Figure 4). Scaffolds printed by this technique may exhibit anisotropic mechanical properties that can be useful for creating scaffolds with intended alignment such as ligament or tendon.

BIOPRINTING

Bioprinting belongs to additive manufacturing techniques for creation of the cell-based scaffolds [17]. These techniques are presumably adapted for printing with cells at the same time as material, since they have minimal impact on the cell viability and function. Biological materials used for printing should match natural environment.
of the host tissue to support function of those cells. Additionally, cells should be able to overcome shear stress during the printing process and survive in real non-physiological conditions of the printing regime [8].

Bioprinting techniques are classified into the three categories: microextrusion, laser-assisted bioprinting (LAB) and inkjet-based bioprinting. Among them, inkjet bioprinting is the most promising for the creation of complex architectures, successfully mimicking native tissue and organs. In inkjet-bioprinting, bioink droplets are deposited onto the substrate that gels to form polymeric structures, while microextrusion bioprinting uses mechanical extruder to deposit bioink. Additionally, extrusion-bioprinting is useful for high cell density, due to its easier processing, but it is a slower than drop-based bioprinting. This technique allows accurate deposition of the material and fabrication time to achieve high resolution in complex structures. This method enables successful fabrication of clinically relevant scaffolds for tissue engineering, because it is ideally adjusted for biological materials due to its ability to deposit multiple materials with wide-ranging properties. Extrusion bioprinted scaffolds are typically soft, due to their high water content that makes them limited to soft tissues application.

**Laser-assisted bioprinting (LAB)**

LAB, or biological laser printing, is a group of laser techniques that use laser energy to facilitate densification of scaffold materials (Figure 6) [8, 17]. One type of LAB uses laser pulse (laser based direct writing (LDW)) for local heating a slide with an energy-absorbing layer and solution of cells. The laser pulse induces sublimation or evaporation of material, expelling the solution of cells on the opposite side and precisely depositing them on the substrate. This method includes laser-induced forward transfer and matrix-assisted pulsed laser evaporation, which can be used for deposition of fibroblasts, keratinocytes, human mesenchymal stem cells, various cancer cells and biopolymers.

As lasers technique allows high precision, this method is suitable for bioprinting of the smallest details of native tissues and organs. This technique allows direct printing of cells, but with several limitations, like detrimental effect on cell survival and their long-term behavior.

**Inkjet bioprinting**

Inkjet bioprinting enables precise deposition of cells and biomaterials, using some advances of 2D inkjet printing to create 3D scaffolds [8, 18]. In this method a limited volume of fluid is falling into the precise pattern specified by the corresponding software. One of the most important
The main disadvantage of inkjet printing is request for biological agents to be in a liquid state, to allow deposition. Deposited droplets then solidify into the required geometry, through cross-linking based on physical, chemical, pH, or ultraviolet methods. Due to chemical cross-linking, many natural materials frequently change their chemical properties. In addition, some cross-linking mechanisms induce decrease of cell viability and functionality (Figure 7).

Although inkjet bioprinting enables encapsulation of live cells, their concentration has to be relatively low in order to form cohesive droplets and prevent clogging of the nozzle. Despite numerous disadvantages, this method has a great potential due to its low cost, high resolution, and high compatibility with many biomaterials. Additionally, these printers enable accurate deposition of fine droplets with precise volume to create high-resolution scaffolds with cells intact. Droplet size can be modulated from 1 to 300 pl with deposition rates from 1 to 10,000 droplets per second. Therefore, this method enables scaffolds creation with accuracy within 100 μm, which is very promising for creating complex scaffolds. Although it cannot produce very tall structures, influenced by the typical mechanical properties of the gel inks, due to its ability to print multiple structures and cell types it is very convenient for printing complex tissues with great accuracy.

Adaptation of current 3D printing methods for biological applications has enormous importance for future fabrication of tissue grafts and artificial organs. Besides tissue engineering, 3D printing is also used in the area of drug delivery, analysis of chemical and biological agents and organ-on-a-chip devices [19].

Despite its huge potential in regenerative strategies, the main challenges are related to necessity of improved resolution, increased speed and printing that enables cells survival [18]. Current efforts in improvement of printing resolution in lithography assume the development of methods like electron beam lithography and multi-photon absorption polymerization, because these methods are suitable for creation of scaffolds with extremely precise feature sizes, of the order of only of tens of nanometers [20].

Materials used for 3D bioprinting must meet the following criteria: should be biocompatible, support cell growth and differentiation and retain its shape long enough to preserve scaffold integrity until solidification locks in scaffold geometry. The most commonly used materials for such purposes are collagen, gelatin, hyaluronic acid, alginate, modified copolymers, and photo-polymerizable macromers [21].

For design of complex scaffolds that mimic tissue, additional research is necessary for accurate mapping of complex tissues to be able to make well-reproduced scaffolds with required structures and biological properties. One of the main challenges in future in 3D printing is...
direct „in situ” bioprinting, or printing cells and biomaterials directly onto or in a patient. Some recent research showed capabilities of bioprinting directly into wounds or burn defects [22]. Further improvements of the printing speed and resolution are needed for „in situ” printing that will enhance tissue regeneration and reduce patients recovery time.

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Različite metode 3D štampanja i bio-štampanja

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KRATAK SADRŽAJ

Danas postoji sve veća potreba za sintetičkim materijalima za zamenu tkiva dizajniranih na način koji imitira složenu strukturu tkiva i organa. Među različitim metodama proizvodnje implantata (skafolda), 3D štampanje je veoma moćna tehnika jer omogućava kreiranje skafolda sa složenim unutrašnjim strukturama i visokom rezolucijom, zasnovanim na medicinskim skupovima podataka. Ova metoda omogućava proizvodnju skafolda sa željenom makroporoznošću i mikroporoznošću i potpuno povezanom mrežom pora. Brz razvoj tehnologija 3D štampanja omogućava različite primene – od kreiranja anatomskih modela za uvežavanje složenih hirurških procedura do štampanja konstruktata za tkivno inženjerstvo. Cilj tekućih istraživanja je razvoj kompatibilnih štampača i materijala (bio-mastila) za dobijanje biomimičnih skafolda, koji omogućavaju štampanje živih celija bez značajnog gubitka njihove vijabilnosti. Napredni nivo takvoga štampanja pretpostavlja štampanje in situ, tj. štampanje celija i biomaterijala direktno na pacijentu ili u pacijenta, što će smanjiti vreme oporavka. Ključne reči: 3D štampanje; bioštampanje; skafold; biomaterijali

UVOD

Strategija tkivnog inženjerstva obično podrazumeva kombinaciju čelija i bioaktivnih faktora i 3D skafolda kako bi se dobio koristan implant [1, 2]. Idealno, skafold treba da se potpuno resorbuje tokom integracije tkiva. Skafoldi izgrađeni od biomaterijala treba da imitiraju važne aspekte tkiva, obnavlja njihove funkcije i obezbeđuju okruženje pogodno za diferencijaciju i proliferaciju čelija [3, 4]. Tradicionalne tehnike koje se koriste za proizvodnju takvih skafolda su gasna pena, širenje rastarača, vezivanje vlaknima, fazno odvajanje, čišćenje čestica i tehnike zamrzavanja, koji obezbeđuju makroskopsku strukturu skafolda, ali često nemaju osobenosti prirodnog tkiva [5]. Metode koje omogućavaju dobijanje složenih geometrija imaju značajne prednosti jer omogućuju proizvodnju skafolda nepravilnog oblika koji se savršeno uklapaju u geometriju defekta. Pored toga, mogu da imitiraju složenost tkiva kroz precizno pozicioniranje višestrukih materijala i tipova celija. Kao što je poznato, skafoldi treba da obezbede ne samo makroskopski strukturni dizajn već i mikroskopske karakteristike potrebne za sofisticiranu čelinsku kontrolu nad formiranjem novog tkiva [6]. Ova istraživanja otvaraju vrata bezbrojnim pristupima inženjerstvu tkiva kroz sukcesivnu izradu slojeva (deponovanje ekstruzijom, očvršćavanje, polimerizacija, sinterovanje ili vezivanje korističkih mnoge druge metode) [8, 9]. Prvi korak je kreiranje modela u kompjuterskom programu (CAD) i prebacivanje u format datoteke koji opisuje površinu ili mrežnu površinu u 3D prostoru kao što su *.stl (stereolitografija), *.obj (objekat), ili *.amf (adiitivno proizveden fajl). Drugi korak je pretvaranje 3D podataka u „kriške“ koje će biti precrtane od strane programa za štampač, što se obavlja pomoću programa poznatog kao „slicer". Ove tehnike omogućavaju korisniku da konfiguriše algoritam koji određuje šablon koji se koristi za popunjavanje slojeva, a zatim program izračunava potrebne parametre kako što su brzina ekstruzije, vreme sušenja ili brzina lasera kako bi se tačno popunio šablon.

Prethodno su ove tehnike bile prilagođene za livenje kalupa, ali je nedavni brzi razvoj povećao njihovu svestransnost i preciznost. Uz pomoć današnjih tehnika mogu da se dobiti skafoldi koji u potpunosti imitiraju makroskopsku geometriju organske kanalne strukture. Cilj ovih istraživanja je razvoj kompatibilnih štampača i materijala (bio-mastila) za dobijanje biomimičnih skafolda, koji omogućavaju štampanje živih celija bez značajnog gubitka njihove vijabilnosti. Naprednik nivo takvoga štampanja pretpostavlja štampanje in situ, tj. štampanje celija i biomaterijala direktno na pacijentu ili u pacijenta, što će smanjiti vreme oporavka.

Ključne reči: 3D štampanje; bioštampanje; skafold; biomaterijali

ACELARLINE METODE ZA DOBIJANJE SKAFOLDA

Tehnike brzog prototipovanja koriste višeosnove sisteme za pozicioniranje i jednu od različitih metoda za generisanje 3D konstrukcija kroz sukcesivnu izradu slojeva (deponovanje ekstruzijom, očvršćavanje, polimerizacija, sinterovanje ili vezivanje korističkih mnoge druge metode) [8, 9]. Prvi korak je kretanje modela u kompjuterskom programu (CAD) i prebacivanje u format datoteke koji opisuje površinu ili mrežnu površinu u 3D prostoru kao što su *.stl (stereolitografija), *.obj (objekat), ili *.amf (adiitivno proizveden fajl). Drugi korak je pretvaranje 3D podataka u „kriške“ koje će biti precrtane od strane programa za štampač, što se obavlja pomoću programa poznatog kao „slicer". Ove tehnike omogućavaju korisniku da konfiguriše algoritam koji određuje šablon koji se koristi za popunjavanje slojeva, a zatim program izračunava potrebne parametre kako što su brzina ekstruzije, vreme sušenja ili brzina lasera kako bi se tačno popunio šablon.

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Ključne reči: 3D štampanje; bioštampanje; skafold; biomaterijali

Stereolitografija

SLA tehnike koriste skrenuti laserski zrak ili projektovani izvor svjetlosti za očvršćavanje datih područja fotopolimera na površini nekog materijala [Slika 1] [8, 10]. Razni fotopolimeri su odgovarajućim viskozitetom i sposobnošću očvršćavanja mogu
se koristiti za pravljenje konstrukata sa SLA. Kuk je koristio SLA za izradu 3D skafolda za inženjerstvo koštanog tkiva koristeći biorazgradive polimere, kao što su dietil-fumarat i polipropilen fumarat [10]. Takođe, fotostabilna keramička akrahilna suspenzija je korišćena da se formira konstrukcija šupljje kosti i koštanih skafolda korišćenjem hidroksiapitate [7].

Nedostatak SLA metoda je taj što je rezolucija ograničena prečnikom laserskog zraka (oko 250 μm), iako su laserski sistemi, koja se menjaju, digitalna projekcija obrade svetlosti proizveli detalje veličine oko 70 μm. Ove tehnike se takođe mogu koristiti za dizajniranje skafolda od hidrogelova prirodnih i sintetičkih polimera koji se kreiraju u vodi i znatno su manje kruti od tradicionalnih SLA konstrukata. Hidrogelovi su postali veoma popularni kao bijomaterijali iziuše i oboje, a dodatno sinterovanje je neophodno za očvršćivanje delova koji sadrže pukotine i druga oštećenja. Fokus tekućih istraživanja je na razvoju novih materijala za FPF i usavršavanju parametara štampanja radi poboljšanja površinskog izgleda skafolda.

**Fuziona depozicija**

FDM tehnike omogućavaju korisnu platformu za kreiranje skafolda pomoću preciznog XYZ sistema pozicioniranja za usmernavanje položaja mlaznice prilikom nanošenja materijala [8, 15]. Materal se deponuje u slojevima i očvršćuje u prethodno definisanih obliku. Tradicionalni SFF štampači često se koriste za brze prototipove korišćenjem akrilatril-butadien-stirena malog prečnika, koji se ubacuje kroz mlaznicu koja se zatvara do temperature veće od 200°C.

Biorazgradivi polimeri koji se koriste u inženjerstvu tkiva uglavnom se rastvaraju na nižim temperaturama i mogu se oblikovati na sličan način kao i SLA konstrukcije. One su posebno pogodne za izradu složenih oblika. Osim toga, neki materijali koji se prirodno nalaze u kostima, kao što je trikalcijum-fosfat, takođe mogu biti odštampani korišćenjem PFP tehnika, omogućavajući stvaranje složenih oblika skafolda, uključujući unapred konstruisanu međusobnu povezivanje poroznosti. Rezolucija i minimalna veličina pora ograničenu su karakteristikama praha, a dodatno sinterovanje je neophodno za očvršćivanje delova koji sadrže pukotine i druga oštećenja. Fokus tekućih istraživanja je na razvoju novih materijala za PFP i usavršavanju parametara štampanja radi poboljšanja površinskog izgleda skafolda.
omogućava uspješnu izradu klinički relevantnih skafolda za tkivno inženjerstvo, jer je idealno prilagođena biološkim materijalima zahvaljujući svojoj sposobnosti da deponuje više materijala sa širokim opsegom svojstava. Skafoldi dobijeni ekstruzionim bio-štampanjem su obično mekani, zbog visokog sadržaja vode, što ih čini ograničenim za primenu samo kod mekih tkiva.

Laserski potpomognuto bio-štampanje

LAB, ili biološko lasersko štampanje, grupa je laserskih tehnika koje koriste lasersku energiju kako bi olakšale densifikaciju materijala skafolda (Slika 6) [8, 17]. Jedan tip LAB-a podrazumева upotrebu laserskog pulsa (LDV) za lokalno zagrevanje slajda sa slojem koji apsorbuje energiju i rastvorom čeliјa. Laserski pulsu indukuje sublimaciju ili isparavanje materijala, protejeruјući rastvor čeliјa na suprotnoj strani i precizno ih nanoсеći na podlogu. Ova metoda uključuje laserski indukovani transfer i lasersko isparavanje pomoћu matrice, koji su mogući koristiti za depoziciju fibroblasta, keratinocita, humanih mezenhimalnih matičnih čeliјa, različitih kancerskih čeliјa i biopolimera.

Kako laserske tehnike omogućavaju visoku preciznost, ova metoda je pogodna za bio-štampanje najmanjih detalja nativnih tkiva i organa. Ova tehnika pruža mogućnost direktnog štampaњa čeliјa, ali uz nekoliko ograničenja, kao što su štetan efekat na čeliјsko preživljavanje i njihovo dugoročno ponašanje.

Inkjet bio-štampanje

Inkjet bio-štampanje omogućava preciznu depoziciju čeliјa i biomaterijala, koristeći neke pogodnosti 2D inkjet štampanja kako bi nastali 3D skafoldi [8, 18]. U ovom postupku ogrenišćena napregena tečnosti pada u precizan kalup kreiran pomoću odgovarajuћeg softvera. Jedna od najvažnijih prednosti ove tehnike je brzina kojom se mogu proizvoditi skafoldi sa vrlo kompleksnom 3D arhitekturom. Ova velika brzina ograničava broj polimernih materijala koji se mogu koristiti za bio-štampanje, jer njihovo vreme geliranja mora biti veће ili jednako vremenu depozicije kapi.

Inkjet bio-štampači se mogu prilagoditi za štampanje materijala sa poveћanom rezolucijom i brzinom. Oni koriste termičku ili piezoelektricnu energiju da deponuju kapljice mastila sa više mlaznica i sa odgovarajuћeg softvera. Jedna od najvažnijih prednosti ove tehnike je brzina kojom se mogu proizvoditi skafoldi sa vrlo kompleksnom 3D arhitekturom. Ova velika brzina ograničava broj polimernih materijala koji se mogu koristiti za bio-štampanje, jer njihovo vreme geliranja mora biti veће ili jednako vremenu depozicije kapi.

Ekstruziono bio-štampanje

Ekstruziono bio-štampanje je jedna od najekonomičnijih tehnika brzog prototipovanja (Sliка 5) [8, 17]. Ekstruziono bio-štampanje tipično uključuje pritisak ili vijčano/klipno aktiviranje kertridža komercijalno dostupnog inkjet štampača za kreiranje i glia čeliјa izolovanih iz centralnog nervnog sistema odraslih mиševa. Pokušaj indukuje transfer i leseći kristali indukuju povećanje pritiska, što dovodi do izbacivanja kapljice mastila na podlogu.
Glavni nedostatak inkžet štampanja je zahtev da biološki agensi budu u tečnom stanju, kako bi se omogućila depozicija. Odložene kapljice zatim očvršćuju u potrebnu geometriju, preko unakrsnog povezivanja na osnovu fizičkih, hemijskih, pH ili ultraljubičastih metoda. Zbog hemijskog unakrsnog povezivanja, mnogi prirodni materijali često menjaju svoje hemijske osobine. Osim toga, neki mehanizmi unakrsnog povezivanja indukuju smanjenje ĉelijske vijabilnosti i funkcionalnosti (Slika 7).

Iako inkžet bioprinting omogućava enkapsulaciju živih ĉelija, njihova koncentracija mora da bude relativno niska da bi se omogućilo formiranje kapljice i sprečilo začepljenje dizne. Uprkos brojnim manama, ovaj metod ima veliki potencijal zbog svoje niske cene, visoke rezolucije i visoke kompatibilnosti sa mnogim biomaterijalima. Ovakvi štampači omogućavaju taĉnu depoziciju finih kapljica precizne zapremine da bi stvorili skalofle visoke kvalitete sa netaknutim ĉelijama. Veličina kapljice može biti podešavana izmeĊu 1 i 300 pL sa brzinama depozicije od 1 do 10.000 kapljica u sekundi. Prema tome, ovaj metod omogućava stvaranje skalofa sa preciznošću od 100 μm, što je veoma obećavajuće za stvaranje složenih skalofa. Iako zbog tipičnih svojstava gel-mastila ne može proizvesti vrlo visoke strukture, vrlo je pogodan za štampu kompleksnih struktura sa velikom preciznošću zbog svoje sposobnosti da štampa višu strukturu i tipova ĉelija.

**OČEKIVANA SUDBINA OVIH METODA U BUDUĈNOSTI**

Adaptacija postojećih metoda 3D štampanja za biološke prime-nje je od ogromnog znaĉaja za buduću proizvodnju tkivnih graf-tova i veštakštih organa. Pored inženjerstva tkiva, 3D štampanje se takoĊe koristi u oblasti isporuke lekova, analizi hemijskih i bioloških agenasa i organa na ĉipu ureĊaja [19].

Uprkos svom velikom potencijalu u regenerativnim strategijama, glavni izazovi se odnose na poboljšanje rezolucije, povećanje brzine i štampanja koje omogućava preživljavanje ĉelija [18]. Tekuci napori u unapređenju rezolucije štampanja u litografiji podrazumevaju razvoj metoda kako ćelija, litografija elektronskim snopom i multifotonska apsorpciona polimerizacija, jer su ove metode pogodne za izradu skalofa sa veoma preciznom veličinom detalja, reda samo nekoliko desetina nanometara [20].

Materijali koji se koriste za 3D bio-štampanje moraju zadovoljiti sledeće kriterijume: treba da budu biokompatibilni, da podrţe rast i diferencijaciju ĉelija i da zadrţe oblik dovoljno dugo da bi se oĉuva integritet skalofa dok se ne završi proces oĉvršćavanja unutar geometrije skalofa. Najviše korišćeni materijali za ove svrhe su kolagen, želatin, hijaluronska kiselina, alginate, modifikovani kopolimeri i fotopolimerizujući makromeri [21].

Za dizajniranje kompleksnih skalofa koji imitiraju tkivo neophodna su dodatna istraţivanja za taĉno mapiranje kompleksnih tkiva da bi mogli da se naprave reprodupcinli skalofi sa zahtevanim strukturnim i biološkim osobinama. Jedan od glavnih izazova u budućem 3D štampanju je direktno bio-štampanje ili štampanje ĉelija i biomaterijala direktno na pacijentu ili u pacijenta. Neke nedavne studije su pokazale mogućnost bio-štampanja direktno na rane ili opekotine [22]. Dalja unapređenja brzine štampanja i rezolucije su neophodna za in situ štampanje, koje će unaprediti regeneraciju tkiva i redukovati vreme oporavka pacijenta.