Nanomedicine and printing technologies

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1 Introduction

Nanomedicine is application of nanotechnology to medicine. Nanotechnology defined as technologies with at least one dimension under 100 nm and taking advantage of phenomena present in small length scales. Nanoparticle properties depend their material, size and shape, changes even in the latter can lead different properties. Nanotechnologies can be used for pharmaceuticals, diagnostics and regenerative medicine, as many biomolecules and cell parts are in the same size range. Nanomedicine lends itself to personalized medicine and as a highly interdisciplinary field it takes not only from biosciences but also from physics and chemistry along other engineering fields.

Currently, nanomedicine is in its infancy as available clinical applications are mainly drug delivery systems, but the research side has many things in the pipeline and some developments are for research applications only. Regenerative medicine utilizes nanostructures mainly as scaffolds to support and direct tissue growth.

Drug delivery with nanotechnologies can enhance the effectiveness of therapy and reduce side effects as the drug is not released immediately upon entry to body. The spatiotemporal release profile of the drug can adjusted with the nanomaterial choice. For diagnostics, nanoparticles can be used as contrast agents or nanotechnologies could be used with the point of care diagnostics devices. The nanoparticles can act as carriers or be treatment agents as themselves. Nanoparticle can carry multiple drugs and imaging agents simultaneously, this combination of imaging and therapy is referred to theranostics.

Both organic and inorganic materials are used. Nanomaterials and structures can be fabricated with top-down or bottom-up methods. In top-down methods bulk material is broken down into nanoparticles or patterned by removing material to acquire nanostructures. Typical patterning methods are different lithography techniques combined with etching done with toxic chemicals. Fabricating 3D structures is difficult with top-down methology. In optical lithography, mask and tool costs increase steeply as feature sizes decrease towards nanoscale, but it has high throughput. Nanoimprint lithography suffers from high master costs. With electron and ion beam lithographies are high tool costs are the main issue.

Bottom-up methods start from molecules and atoms build wanted material or structure from these precursors. They are flexible methods but many of can not achieve high throughput patterning with under 100 nm feature size. Methods based on scanning probe microscopy can achieve resolution around 1 nm but are extremely slow. Self-assembly has a lot of potential but producing arbitrary structures with it is not possible. DNA based patterning is another possibility.

Printing technologies could provide flexible and low-cost fabrication methods for nanostructures used in nanomedicine, but feature sizes are usually above 1 µm. In nanomedicine, usually 3D structures resembling biological structures that have complex shapes are wanted. The additive manufacturing approach is the solution to these issues. Usually, nanomaterials are used to print larger objects.

Small feature sizes do not guarantee that distance between neighboring structures is close to structure size as multipatterning techniques and/or using two different materials with other removed after pattering.

2 Printing Technologies

This chapter introduces printing technologies used for bioprinting. The most widely used printing technologies are extrusion based including the inkjet, but electric and laser based methods are also used. While nanostructures are difficult to fabricate with printing methods, nanoinks and bioinks would allow the use of nanotechnology regardless of feature size of final structure. Development focus has shifted to 3D and 4D printing, in the latter fabricated 3D structures are dynamic.

Table 1: Comparison of different printing methods, some methods have multiple variants with different resolutions. Data collected from various sources, the cost and viscosity values electrohydrodynamic process are guesses based on similarities to electrospinning. Abbreviations: EHD/electrohydrodynamic, ES/electrospinning, SL/stereolithography, mod/moderate, med/medium, via./viability. [5, 9, 10, 13, 19, 25, 35].

Method	resolution	speed	viscosity	cost	cell via.	3D
Inkjet	>5 µm	high	<10 mPa s	low	≈ 90 %	poor
Extrusion	>5 µm	low	30–6 × 10^7 Pa s	mod	90 %	good
Laser-assist	>5 µm	high	1–300 mPa s	high	≈ 90 %	med
EHD	100 nm, 5 µm	med	5–4 × 10^3 mPa s	mod	≈ 90 %	med
ES	<50 nm	med	5–4 × 10^3 mPa s	mod	≈ 90 %	poor
SL	2–100 µm	high	NA	med	>90 %	good
Scanning probe	≈ 5 nm	low		mod	NA	poor

2.1 Inkjet methods

The output can be a continuous flow of droplets or individual drops as needed, forced out with electrostatic, acoustic or thermal means. The printhead is based on microelectromechanical system (MEMS) technologies especially as non-continuous output requires up to 1000 printheads. The inkjet technology used for fabrication is the same than used in digital printing though some modifications are usually required. [16, 24, 28]

The continuous method uses an external pump for ink flow, which broken to droplets with piezoelectric crystal. Electrostatic field controlling droplet size, the speed and drop ratio charges the droplets allowing location where droplets hit substrate controlled with charged deflector. [16, 28]

In the non-continuous method, also known as the drop-on-demand, the thermal method (Figure 1a) heats the ink locally with a microresistor increasing pressure within ink reservoir expelling droplet. Another approach is to cause vibrations with piezoelectrics (Figure 1b) to overcome liquid surface tension leading to droplet ejection. Electrostatic inkjet printing has a small chamber connected to the ink reservoir and printhead, the droplet is ejected by chancing the volume of the chamber with electrostatic actuation. [16, 28]

The achievable resolution depends on droplet size, ink and movement accuracy of printhead and is with current systems at most micrometers. Piezolectric printing is usually favored for thermally sensitive solutions that most bioinks are. [9, 16, 28]

The inkjet printing of 3D objects is possible but sets additional demands for ink and printhead movements. Inks used in 3D printing have to have high viscosity so that ink does not flow away, also usually supportive medium is used. Inkjet printing is a quite versatile high speed method with low costs but requirement for low ink viscosity limits its applicability (Table 1). [16, 28]

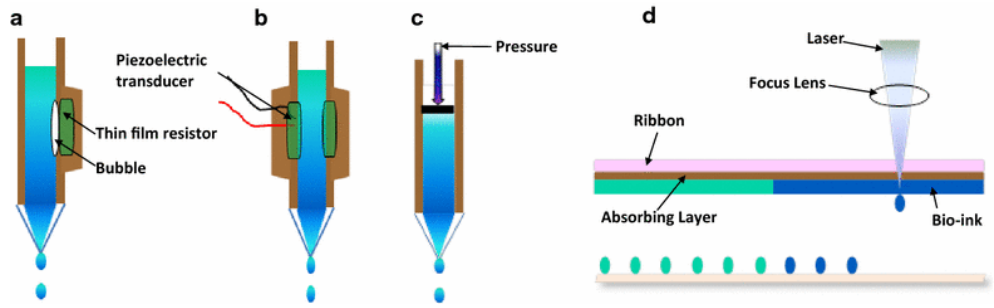

Figure 1: Diagrams thermal inkjet (a), piezoinkjet (b), extrusion (c) and laser assisted (d) printing methods. Source: Li *et al.* [19], license: CC-BY-4.0.

2.2 Extrusion methods

In extrusion (Figure 1c), the ink is forced out from printhead with external force as continuous flow with external force. The external force can be air pressure, a piston or screw. One method is known as Fused Deposition Modeling (FDM), where the filament is melted before going though nozzle to substrate. It is one of the main methods in 3D printing. Extrusion allows ink viscosity be several orders of magnitude higher than in the inkjet but printing is slow and resolution much lower than with the inkjet, also the lowest possible ink viscosity is high (Table 1). Wanted shape is retained due increased material right right after printing. [9, 10, 16]

As with all other printing methods nozzle clogging and other process issues can be issue with high-viscosity or nanocomposite inks. Some extrusion methods can reach about 1 µm resolution, but FDM is limited to about 40 µm. In some variants, curved substrates are used for complex patterns. Printing resolution depends on movement accuracy of printhead or table, nozzle size and extrusion parameters. [9, 10, 16]

2.3 Laser-assisted

Derakhshanfar *et al.* report that in laser-assisted printing (Figure 1d), 1 ns-1 fs laser pulses are used to separate droplets from the ink layer below one or two protective and absorptive layers that support the ink layer and protect it from the energy of the pulse. When the laser pulse hits the absorptive layer, high-pressure bubbles form in the ink layer. This is a high resolution method but pulse lasers required are expensive. As no nozzle is used, viscosity limitations or clogging are not issues. In

one variant, instead of absorptive the laser pulse evaporates a coating below of the matrix containing cells to be printed causing a droplet to deposit on substrate due kinetic force and gravitation. [9]

Laser energy causes damage to ink layer due thermal stress, which can be somewhat reduced by increasing film thickness and ink viscosity. Use of multiple cell types can be difficult due work needed to fabricate the ink layer for printing. [22]

2.4 Electrohydrodynamic methods

According to Ru *et al.* in electrohydrodynamic printing, ink flows though microcapillary nozzle due electric field between substrate and nozzle with pump moving ink to the nozzle. This requires conductive substrate. Printing modes and droplet properties can be varied though pump pressure, voltages and nozzle-substrate distance. Printing resolution is dependent on printing parameters, composition of the ink and nozzle size. Electrohydrodynamic methods can not print as viscous inks than extrusion methods, but based on Table 1 could fill a gap between the inkjet and extrusion with 3D printing capability and resolution higher than the inkjet. [28]

Wang *et al.* have developed a coaxial focused electrohydrodynamic jet printing method to avoid issues caused by needle diameters below one micron, such as a needle blockage. The coaxial technique uses two needles, so functional ink comes from inner needle and high viscosity ink from the outer needle. This stabilizes the jet allowing the inner jet be at nanoscale while the inner needle diameter is 100 µm. Insulating outer solution means that voltage applied to needle cause surface charges at the exterior and inner interfaces of outer solution generates shearing forces on both solutions as surface charges move. The electric shearing forces focus the outer solution and induce internal pressure and high viscous shearing force to the inner ink decreasing size of inner jet below 100 nm. [34]

2.5 Electrospinning

In electrospinning (Figure 2), fibers are created by ejecting charged solution from nozzle to substrate, the output spins on the way to substrate due electric field between the nozzle and substrate. The setup is similar to the electrohydrodynamic method. This technique can produce nanofibers but due the whipping motion on output the end result is normally a nonwoven mat of randomly oriented fibers. [20]

To increase the usability of electrospinning various techniques have been developed for fiber positioning and alignment. Pre-patterned substrates have been used along with modified electrode setup as improvements. With some materials changing the polarity of the DC voltage generating the field can affect fiber concentration and size along with deposited pattern. Higher voltages lead increase bending instability and jet stretching, which could result finer fibers but cases with an increased fiber diameter has been reported. [20]

AC voltages and a rotating mandrel for fiber collection could result aligned nanofibers. Surface charge accumulation is one factor affecting fiber alignment. Adding DC bias to AC voltage can eliminate jet instability. [20]

Many groups have fabricated uniaxially aligned fiber arrays with different collector designs. Varied electrode patterns can lead varying fiber patterns. Various tip modifications and multitip systems have been demonstrated for the fabrication of structured fibrous membranes. As electrospinning and electrohydrodynamic jetting equipment have similar structure similar modifications can be done to both, such coaxial, tips. [35]

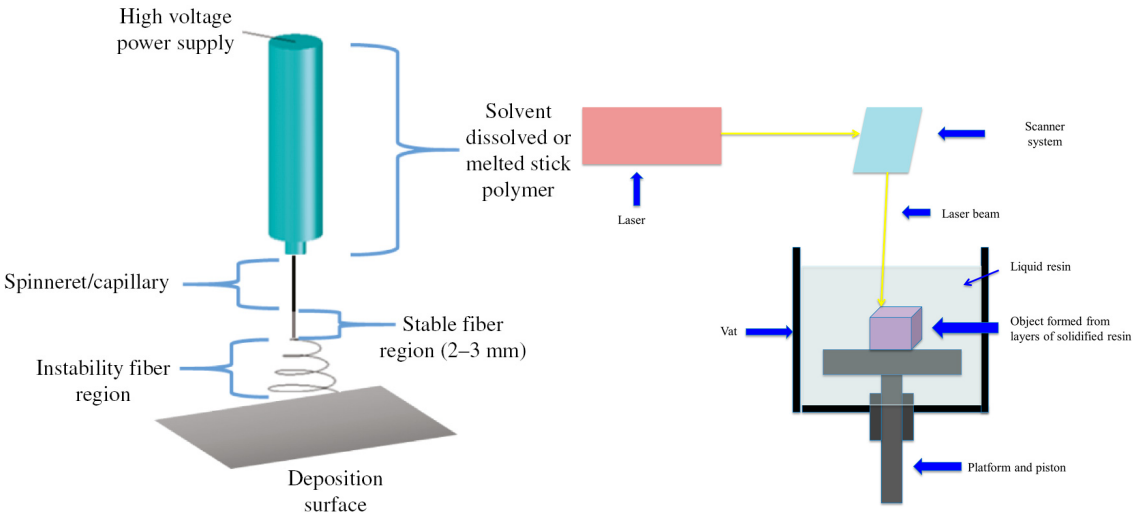

Figure 2: On left, a diagram of electrospinning setup. Source: Skinner *et al.* [29], license: CC-BY-NC-ND-3.0. On right, a diagram of stereolithography process. Source: Konta *et al.* [16], license: CC-BY-4.0

2.6 Stereolithography methods

Farahani *et al.* state that stereolithography (Figure 2) is a 3D printing method where an object is formed by curing resin with UV light layer by layer. In the basic variant only one light source is used which scanned to expose specific pattern before the platform is lowered to expose the next layer. After all layers are exposed the non cured resin has to be removed. By using laser and micromirror array with photo-polymerizable resin, higher resolution could be achieved and resin consumption decreased. The micromirror array is computer controlled so the tilt of each mirror is adjusted individually for each layer. Due the resolution limit on the basic variant, the method was further developed to challenge other methods listed in the Table 1 and take advantage of the excellent 3D printing ability. [10]

According to Lee, stereolithography variant using two photon absorption the resin polymerizes when it absorbs simultaneously two identical photons whose combined energy equals energy required to initiate the polymerization. This technique requires two lasers, located on the top and bottom of resin. Higher resolution and thinner layers are possible than with the standard variant with a single laser. Resolutions about 100 nm has been reported for the two-photon technique and few tens of nanometers might be reachable though novel photopolymers and better laser sources. [14]

2.7 Scanning probe microscope based

Technologies used in scanning probe microscopy can be used write patterns on substrate. Ink can flow on the surfaces of the tip or a capillary could act as the tip. Devices with the capillary inside the cantilever and tip also exists. Resolution of a few nanometers is reachable in 2D-printing, but z-dimension is more problematic. Low printing speed is significant issues with main method of increasing speed is to use multiple probes for parallel printing. De Souza *et al.* have tested 3D printing with AFM based setup and were able to achieve layer heights 5.13 nm [33].

Dip-pen nanolithography is a nanofabrication method, where ink on AFM tip reacts with substrate and attaches to it. Ink flow happens due ink condensed between the tip and substrate creating capillary forces. Ink composition has to be chosen so that required reactions are possible. [28]

In the capillary as tip variant, a glass or quartz capillary with tapered tip and an aperture of a few hundred nanometers is used. Only when tip contacts the substrate can ink flow out of the capillary due surface tension of the droplet at the tip. [28]

2.8 Bioink

Bioink is solution containing biomolecules and or cells. The biomatter sets strict limitations to the printing process as biomolecules and especially cells are sensitive to process conditions, such as the temperature and pressure. Some bioinks have to also contain scaffolding material. Inks also contain many supporting additives, such as growth factors and signaling molecules. [9]

Depending on biomatter to be printed, printhead geometries might have to meet certain criteria and/or hydrophobic/philic coatings might be needed to prevent ink attaching to printhead surfaces. Composition of bioink has to be chosen to fit the selected printing method, as their requirements for suitable ink have major differences.

In bioink design, taking into account controlling cell functions, such as adhesion, proliferation and differentiation is very significant issue. Not only biochemical issues, but also biophysical interactions within the ink influence cell fate. As printability and cytocompability set major and often conflicting factors for bioinks, they are usually composed of multiple materials. Hydrogels, highly hydrated 3D polymeric networks, are usually a major component of bioinks, as they can act as support for printed cells. As the use of multiple inks simultaneously increases, intercompability between them becomes a significant factor. [6]

By including nanoparticles and nanoengineered hydrogels in bioinks printability, degradation resistance and support structure properties can be significantly altered. For printability, viscosity changes under shear due nanotechnological features are important. Nanoparticles have functions for cell differentiation, conductive structure, drug release and studying inside of the printed structure. Supramolecular bioinks could be used as replacements for traditional hydrogels, whose bonds can break under repeated stress. [6]

In supramolecular polymers, monomers have functional groups interacting non-covalently with each other. These interactions lead to large polymer-like entan-

glements, under high stress energy can dissipate though reversible breaking the non-covalent bonds. When bonds are open bioink is thinner and easier to print, but after it has been deposited on substrate bonds form again and viscosity increases. Therefore, printed objects retain their shape better. [6]

2.9 Nanoink

Nanoinks are solutions containing nanomatter, typically nanoparticles or nanofibers. Viscosity of the ink increases significantly when nanocomponent is added so the solution contains additives along with different nanomaterials. The mixture is naturally chosen so that end result meets specified criteria. Nanoink solutions are usually stabilized for the prevention of aggregation and precipitation with polymeric materials and surfatants, or with electrostatic interactions. [15]

Inks with carbon nanomaterials are interesting group as they can be conductive, semiconducrive or insulating. Nanoinks can also contain metallic nanoparticles for conductive structures, quantum dots for optoelectronics, or plasmonic nanomaterials. Metals used in the nanoinks can not be susceptible to oxidation, so for example aluminum is out of question with gold and silver being favored due their high conductivity. Nanocellulose and nanogels are also frequently used. [15, 17]

Print quality can be increased by using either directed or self-assembly methods. The assembly can be driven by external forces or without for example by using co-solvent to generate surface tension gradient to force particles away from the edges of the droplet. Other potential approaches are changing particle shape to increase inter-particle interactions or adjusting solvent evaporation kinetics. [15]

For extrusion methods, the ink can be very stiff and FDM uses a continuous filament, which can contain nanomaterials mixed with a thermoplastic polymer.

3 Applications

Printing technologies are currently under extensive development, so clinical applications are rare but potential is significant. The main applications are in regenerative medicine, medical devices and different research systems. Printed structures might have nanostructures, but usually nanocomponent is in the printing material. Most of the potential applications are not mature enough to start clinical trials. 3D printing has also been used encapsulate drug molecules to pills.

3.1 Scaffolds

Scaffolds can mimic an extracellular matrix that supports cell structures in the body, they can also contain drugs, growth factors, etc.. Regenerative medicine also uses scaffolds. In the scaffold design and fabrication, ensuring cell adhesion, profilation and differentation are critical tasks. The scaffold has to withstand stresses present in the tissue of question, such as the repeated contraction cycles of heart tissue. After the tissue has renegerated the scaffold has to degrade or be dissolved. [5]

Chronakis states that currently nanofiber scaffolds are used in the wound care and other skin replacement treatments, as they can increase the skin growth rate and have antimicrobial properties. These electrospun membranes can control water evaporation, have high oxygen permeability and promote fluid drainage, while preventing the invasion of exogenous microorganism that can not pass though ultrafine pores. Fibrinogen protein is good material for these membranes as it can be left to the wound to prevent blood loss and encouraging the natural healing process. See Figure 3 for the SEM images of electrospun scaffolds. [8]

Scaffolds are often fabricated from hydrogels or their nanoengineered variant nanogels, as their biocompability is high and nutrient and gas exchange works well but they poor cell seeding. [7]

Electrospinning is a good method for scaffold fabrication as it produces 3D structures mimicing the extra cellular matrix (ECM) with appropriate porosity and nanofibers. Scalability to mass production is also major advantage, along with ease of use. Composite material consisting of CaP nanoparticles within biopolymer is favored for bone scaffold fabrication with electrospinning. [1]

Lee *et al.* have printed multiwalled carbon nanotube scaffolds for nerve regeneration. The nanotubes were functionalized with amine group and blended with hydrogel bioink. A stereolithography based printing method was used, nanotubes embedded in the scaffold make it conductive. The printed scaffold had retangular holes and lines are around 200 µm wide. Increased tensile strength was noted with added nanotubes and enhanced neurite outgrowth from neural stem cells placed in the scaffold. [18]

According to Rezende *et al.*, electrospinning has been used to fabricate vascular scaffolds as resulting structures are good for cell adhesion. However, cells are unable to invade and seed the nanofiber meshes, but increasing the mesh porosity could solve that issue. Custom fabricator with two nozzles using two different polymers has been shown as possible approach to getting material properties closer to natural. [27]

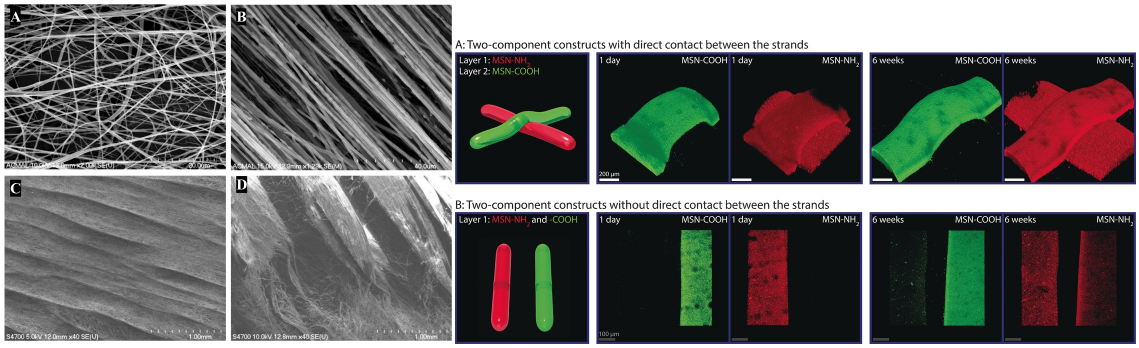

Figure 3: On left, SEM images of electrospun scaffolds with different morphologies and orientations. Source: Nagam Hanumantharao and Rao [23], license: CC-BY-4.0. On right, extruded hydrogel layers containing nanoparticles with different functionalizations and particle migration after printing. Source: Baumann *et al.* [3], license: CC-BY-NC-ND-4.0

3.2 Drug delivery

Nanopharmaceuticals can be printed by dissolving them. Printing allows creating systems with individual release profiles and geometries. Multiple ingredients could be packaged to the same dosage form, while nanoparticles could carry multiple drugs, the possibility of using multiple nanoparticle types simultaneously might have advantages. [2]

Yu *et al.* have reviewed the use of electrospinning for creating dosages from poorly water-soluble drug molecules, which are incorporated to the polymer for the electrospinning process. Drug distribution in the nanofibers can be varied and other compound can also be present. Nanofibers can consists of at least two different materials, one for the core and another for the sheath. The fibers have to meet certain demands for post-processing, as they are packaged to capsules or broken into pulver for tablet fabrication. Advantages are increased stability, which eases strorage requirements, enhanced dissolution and complex release profiles. [36]

Baumann *et al.* have used extrusion based 3D printing to fabricate a hydrogel matrix containing mesoporous silica nanoparticles (MSNs) (Figure 3). The nanoparticles were functionalized with either amino or carboxyl groups and then mixed the separate batches of hyaloronic acid-based hydrogel for printing. MSN release was faster and cell uptake higher with carboxyl than with amino functionalization, which has been attributed to their positive charge. [3]

Tao *et al.* 3D printed a mold for preparing a hydrogel containing paclitaxel nanoparticles to cavity caused brain tumor removal, the shape was determined with MRI during surgical planning. The hydrogel allows the slow release of the drug to tumor cavity for inhibiting tumor cell proliferation with the goal of eradication of any residual tumor cells. [31]

3.3 Devices

3D printing could be used to fabricated individualized implants from nanomatter, leading to increased biocompability with possibility using bioinks to improve attachment and use of biomimetic designs. Microneedle arrays could be printed with the drug encapsulated. Printing diagnostics devices for point-of-care applications shows potential to ease their production as reagents and test area could be printed with readout systems including the electronics and battery, allowing 3D shapes used without the need to use bonding and other top-down methods. Implantable devices could be coated with electrospun thin films with suitable surface design to allow them to better integrate into body. [4, 15]

3D printed microfluidic systems could be used in production of nanopharmaceutical agents. Fabrication of nanorobots for repairing body from inside with printing is more futuristic prospect currently, but if eventually realized they would be a major breakthrough.

Gou *et al.* have 3D printed poly(ethyleneglycol) diacrylate hydrogel matrix containing polydiacetylene nanoparticles with acrylamide groups to chemically link particles to hydrogel. Chosen printing method was dynamic optical projection stereolithography, the hydrogel matrix was then fabricated layer-by-layer photopolymerization. The hydrogel matrix degrades extremely slowly so nanoparticles stay within the matrix. The nanoparticles trap toxins within the matrix by interacting with them, enabling the use as a filter device for detoxification. The 3D microstructure is biomimetic inspired by liver structure and the targeted use case is liver failure patients. [11]

Kong *et al.* have reviewed the use of 3D printing with nanoinks in bionics, which combines biological systems with engineered mechanical/electronic ones. Advanced prostheses need electronic systems including sensors and flexibility is a major requirement, due biological materials used and the individualized nature of these devices printing technologies are the best fabrication method. Metallic nanoparticles allow the printing of interconnects and other devices, such as sensors or antennas. Integrated circuits could potentially be printed directly into bionic devices. Bionics could also give humans capabilities they do not have, such as “hearing” radio frequencies. [15]

3.4 Regenerative

For regenerative applications usually a scaffold with stem cells is printed. Tissue engineering offers solutions for issues with the current organ and tissue replacement methods as it would allow the replacements grow and change with time. Combining multiple bioprinting technologies could compensate the disadvantages of individual methods. The combination of electrospinning for the scaffold and inkjet for bioactive layers has been reported. Vascular network creation is one of the largest challenges present as printing small diameter vascular does not show good results and requires much higher resolution, which is slow, so researchers are going to use angiogenic growth factors to get vascular grow with tissue. [5]

Published studies show the viability of printing technologies for tissue engineering, but cells within do not always work well compared to other bioinks. By printing the scaffold separately seems to have in many cases led to better results. Stereolithography techniques have been used for scaffold fabrication for bone and cartilage repair. Optimizing resin composition and high porosity so cells fit into pores might require additional particles. [9]

Hasan *et al.* have reviewed fabrication efforts of tissue-engineered heart valves, they note that scaffolds are usually fabricated with the electrospinning of nanofibers. Cells encapsulated in microporous hydrogels are potential inks for printing the scaffold and cells simultaneously. However, the seeding of cells to the structure is often done to the prefabricated scaffold. The printing process for tissues engineered constructs can incorporate nanoparticles for gene delivery, diagnostics and drug release. Modifying surface morphology at nanoscale increases area to volume ratio and allows nanoscale coatings for drug release. [12]

Thermal inkjet fabrication PEGDMA (poly(ethyleneglycol) dimethacrylate) scaffolds for cartilage has been reported with the subsequent bioprinting of ink containing stem cells, bioactive glass and hydroxyapatite nanoparticles. Printed stem cells were noted to be more uniformly distributed than pipeted stem cells. [26]

3.5 Research

Using nanomatter and 3D printing, researchers could fabricate different assays for screening purposes or organ models for disease modeling and drug development. As with regenerative medicine, proper microenvironment is required for models to work. The printing of large biomolecules, such as proteins and DNA in microarrays has been reported in the micrometer scale with electrohydrodynamic method, nanoparticles could be printed simultaneously for labeling and sensory purposes. [25, 32]

Trampe *et al.* have bioprinted hydrogel containing sensor nanoparticles and living cells with an extrusion based method for investigating the spatiotemporal oxygen dynamics with 3D scaffolds. Fluorecence measurements were used to monitor O_2 concentration as the emitted wavelength is the function of the O_2 concentration. The goal is to monitor the metabolic activity of the cells though the O_2 distribution for the scaffold design optimization. The authors state that sensory nanoparticle properties have to be chosen so that their excitation does not damage cells, the response matches the wanted concentration range and a clear signal can be acquired. Other physiochemical parameters could also be measured with different nanoparticles and potentially simultaneous measurements of multiple parameters is possible. [32]

4 Summary

Nanomedicine will probably become part of mainstream medical science within ten years. The top-down fabrication method used in the IC and MEMS industries are not compatible with biological systems and are focused on mass production. Bottom-up methods allow fabrication of complex 3D structures that can partially consist of biomaterials and each one can be different with no significant extra cost.

Printing technologies are additive methods and divide into several subcategories with each having advantages and disadvantages. The output of printing can be continuous flow or droplet based. The simplest methods are extrusion based, where ink is forced out from the nozzle with external force. Inkjet printing can attain resolution of a few micrometers and is a droplet based method, but is limited to low viscosity inks. Electrohydrodynamic methods use an electric field between the nozzle and substrate to control how fast ink hits substrate, electrospinning produces continuous fibers. Methods based on SPM have a resolution up to a few nanometers, but are slow. In the stereolithography methods the object is fabricated by hardening the resin layer-by-layer. Laser assisted methods use laser pulses to release droplets from the ink matrix above substrate.

As printing technologies capable reaching the sub 100 nm resolution are very rare, the nanotechnological component comes from inks used. The inks often contain nanoparticles, biomolecules and even live cells. Nanofiber mats can be fabricated with electrospinning. Often the major component in the inks is a hydrogel.

Literature has countless examples of printed structures for regenerative medicine, medical devices, pharmacy and medical research, but many works do not have the nanotechnological component. Often nanoparticles make only a few percent of the used ink, high concentrations would change ink properties significantly. Scaffolds are often fabricated from nanofibers, but polymers have also been used. Electrospinning and stereolithography are favored for scaffold fabrication, porosity of the structures has to be large enough for cells to fit inside.

Drug delivery though different implants and scaffolds can be achieved by mixing the drug containing nanoparticles to the ink used. Drugs have also been encapsulated with printing for oral administration.

While nanorobots are currently futuristic dream, printing diagnostic devices with test systems and readout electronics could simplify their design and potentially increase their performance as structural design constraints from fabrication methods are much lower. Valves for major blood vessels have been fabricated with the printing of nanofibers.

On tissue engineering side the scaffold is often fabricated with different method and then the bioink containing stem cells is printed. Methods optimal for scaffold printing are often incompatible with cell printing. Researchers have printed multiple tissues with varying success.

For research and development printing assays with reagents and cells could speed up work. Optimizing scaffold structures could be done by mixing sensory nanoparticles in scaffold material and then using fluorescence to monitor parameters of interest in the structure containing printed cells.

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