Inkjet Printing for Biofabrication

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A. Ovsianikov et al. (eds.), *3D Printing and Biofabrication*, Reference Series in Biomedical Engineering, https://doi.org/10.1007/978-3-319-45444-3_26

Abstract

Inkjet printing is a noncontact printing technology with high resolution, high throughput, and considerable reproducibility. Instead of printing normal ink, inkjet technology is also applied in the field of biofabrication to print living cells and other biological factors. Cell viability and function were demonstrated to be sustained after printing. Besides two dimensional cell patterns, three-dimensional cell-laden hydrogel structures can also be inkjet printed through cross-linking. Special phenomena such as the temporary permeability change of cell membranes were also observed during printing procedures, thus making it possible to achieve gene transfection through inkjet printing. Inkjet-printed biomolecule patterns with gradient concentration were also used to direct cell fates. Since the diversity of bioink and the capability of fabricating complex structures, inkjet bioprinting behaves as an effective tool in the field of biofabrication. The applications of inkjet printing include but not limit to drug formulation, tissue repair, and cancer research.

1 Introduction

Inkjet printers, based on a noncontact printing technology, are widely used to print computer data onto paper for family users or print information onto cans and bottles for industrial users, which account for major part of printers used for color printing in offices (Le 1998; Svanholm 2007). Figure 1 shows the technology map of inkjet printing (Fig. 1).

In 1878, Lord Rayleigh first described the mechanism of a liquid stream breaking up into droplets, establishing the theoretical foundation for liquid jets (Rayleigh 1878). In 1931, Weber illustrated the formation of droplets from the breakup of viscous liquid jets (Weber 1931). In 1951, Rune Elmqvist of Siemens-Elsa patented the first inkjet device on the basis of the Rayleigh and Weber's breakup inkjet theories (Rune 1951). Henceforth, many researchers paid much attention on controlling the drop break-off mechanism to improve the image quality.

In 1965, Dr. Sweet from Stanford University achieved droplets with uniform size and spacing from the ink steam by applying a pressure wave pattern to the orifice (Sweet 1965). Then, one of the first continuous inkjet (CIJ) printers was produced by Dr. Sweet. The continuous inkjet printer systems can be divided into two categories, including binary deflection system and multiple deflection system. For the multiple deflection system, some droplets are charged to deflect to the media at different levels to form patterns as they pass through an electric field, while uncharged droplets fly straight to a gutter for recirculation (Le 1998). This idea was commercialized by IBM; IBM 4640 inkjet printer was introduced in 1976. Continuous inkjet printing is mainly used for high-speed printing such as textile printing and labeling (De Gans et al. 2004).

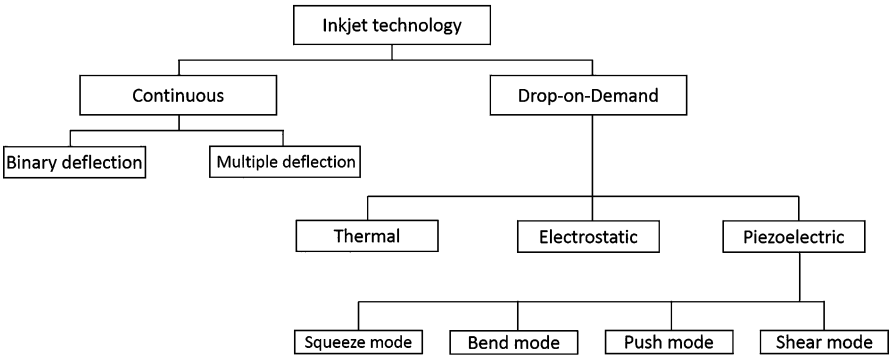

Fig. 1 Inkjet printing technology map

While continuous inkjet technology was commercialized, the development of drop-on-demand inkjet method also emerged. Unlike the continuous inkjet printers, the drop-on-demand inkjet printers eject ink droplets only when they are used for imaging on the media. This new method overcomes the disadvantages of continuous inkjet such as the complexity of drop charging, the deflection hardware, and the unreliability of the ink recirculation system (Le 1998). In addition, drop-on-demand inkjet printers selectively generate droplets, which can lower the cost and are easy to control and user friendly (Gudapati et al. 2016). Zoltan et al. composed the first group to study drop-on-demand inkjet systems (Zoltan 1972; Kyser and Sears 1980). Many drop-on-demand ideas were commercialized in the 1970s and 1980s. It turned out that drop-on-demand inkjet systems were more reliable than continuous inkjet systems.

According to different driving force, drop-on-demand inkjet methods can be divided into three categories, such as thermal inkjet printing, electrostatic inkjet printing, and piezoelectric inkjet (PIJ) printing (Cui et al. 2012a).

The thermal inkjet printer is not the first commercial product but the most successful printer on the market today. The thermal inkjet system consists of an ink chamber, a thermal actuator, and nozzles (Fig. 2a). A short current pulse is applied to the thermal actuator located in the ink chamber near the nozzle to generate ink droplets (Dababneh and Ozbolat 2014). Consequently, the temperature of the ink near the thermal actuator increases to 300 °C, which is higher than the bubble nucleation temperature, and lasts for a few microseconds during printing (Hudson et al. 2000). Then, the bubble emerges and forces the ink out of the nozzle orifice. The droplet formation could be controlled by adjusting the current pulse. The size of droplets varies due to the applied temperature gradient, usually by current pulse and ink viscosity (Hudson et al. 2000; Hock et al. 1996; Canfield et al. 1997).

Electrostatic inkjet printers generate droplets by changing the volume of the fluid chamber (Fig. 2b). The driven force depends on the coulomb force between charges. The charged pressure plate could lead a brief increase in the volume of the fluid chamber so that the ink in reservoir flows into the fluid chamber. When voltage is turned off, the pressure plate restores to the original shape. Consequently, the

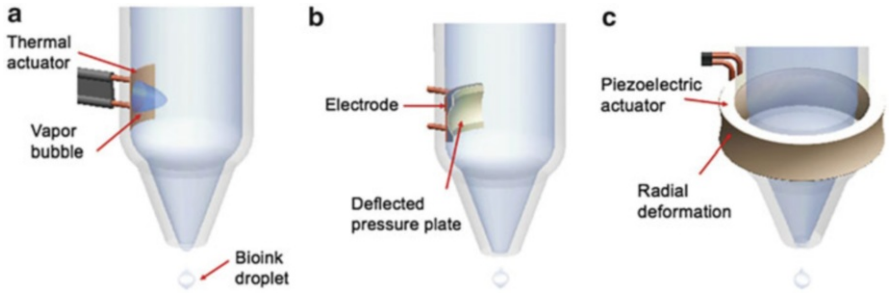

Fig. 2 Mechanisms of droplet-based printing. (**a**) Thermal inkjet printing, (**b**) electrostatic inkjet printing, (**c**) piezoelectric inkjet printing (Reprinted from A comprehensive review on droplet-based bioprinting: Past, present and future, 102, Gudapati et al., Tissue engineering and regeneration. 3d printing and biofabrication, Copyright (2016), with permission from Elsevier)

suddenly increased pressure forces droplets out (Kamisuki et al. 2000). Electrostatic inkjet printing method is also suitable for solid ink. Kamisuki developed static-electricity actuator inkjet in 1998 (Kamisuki et al. 1998). The electric power consumption is quite low due to its electrostatic driven force. Generally, electrostatic inkjet printing is still in its infancy that commercial electrostatic inkjet printers are still rather rare.

Piezoelectric inkjet printing method is similar to thermal inkjet printing method (Fig. 2c). The voltage pulse makes piezoelectric actuator change its shape and then deforms the fluid chamber (Gudapati et al. 2016). The sudden change in the fluid chamber volume leads a pressure variation. Consequently, a droplet is ejected by overcoming the surface tension of the nozzle orifice. Since Zoltan developed the first piezoelectric inkjet printer, there have been four different types of printheads used in piezoelectric inkjet printing. They are squeeze mode, bend mode, push mode, and shear mode printhead, respectively.

It can be concluded from the previous part that the two most dominant drop-on-demand inkjet printing methods are based on thermal and piezoelectric effects. In the initial stage, thermal inkjet printing was more popular due its low cost. However, the application of thermal inkjet printing is limited by the bubble mechanism which is only suitable for part of the ink. Piezoelectric inkjet printing method is more commonly used when printing functional materials, as there is no risk of thermal degradation and damage of the ink or need to use ink only with a specific nucleation temperature.

In recent years, much effort has been invested in expanding the application of inkjet printing via replacing conventional ink with conductive materials, polymers, biomaterials, and cells (Cummins and Desmulliez 2012). As mentioned above, inkjet printing has many advantageous characteristics, such as drop-on-demand printing, precise printing with very small ink droplets, noncontact printing, high-speed printing with multiple nozzles, and completely digitalized printing as an output of computer. On the other hand, scaffold-based tissue engineering was the most major approach in tissue engineering in the 1990s. After Langer and Vacanti

Table 1 Characteristics of inkjet technology and advantages for tissue engineering (Nakamura 2012)

	Characteristics of inkjet technology	Advantages for tissue engineering
1	High resolution Extremely small ink droplets	For manufacturing of microscopic structures with cellular-sized resolution Micro to macro, multiscaled fabrication
2	Drop-on-demand printing	Enabling on-demand direct cell printing
3	Direct printing of ink droplets	For direct arrangement of cells and materials for biofabrication
4	Color printing	For fabrication of composite products with different cells, materials, and growth factors
5	High-speed printing more than 10 kHz Per one nozzle Multi-nozzle system can be integrated	Handling massive amount of individual cells Rapid fabrication Lessens cell damage during fabrication
6	3D fabrication using hydrogels	Enabling 3D construction by layer-by-layer printing Enabling to printing living cells For prevention from dying Enabling 3D fabrication into the liquid
7	Linkage to digital data sources	For digital printing Easy to apply to computer-aided biofabrication For CAD-, CAM-, and CAE-based biofabrication
8	Noncontact printing	Usability of reactive materials Preventive effects for friction or contact damages
9	Printability of several inks; aqueous inks, pigment inks, suspension of several materials, and reactive solution	Printing biological materials; cell proteins, DNAs, biopolymers, humoral factors, drugs, and nanomaterials
10	Printability onto several subjects; papers, solid mass, disc, dishes, gels, and aqueous solution	Printable onto gels, aqueous solution, cell sheets; directly printing onto the tissues, organs, and wounds during surgical operation

proposed the concept of tissue engineering (Langer and Vacanti 1993), some breakthrough were desired to overcome several limitations, which included difficulties in the control of cell position and distribution, composition of multicell types, inner structures of 3D constructs, distribution of growth factors, and induction of blood vessels. Then, the challenging studies using inkjet printing were started in fabrication of 3D scaffolds and indirect printing of cells. In this way, bioprinting was started, which is sometimes called organ printing, jet-based tissue engineering. Nakamura has summarized the characteristics of inkjet technology and its advantages for tissue engineering in Table 1 (Nakamura 2012).

Then, several innovative concepts of bioprinting were proposed, such as the concepts of tissue engineering based on blueprint (bio-CAD), bioink (cells, biomaterials, and bioactive factors), bioprinter (bio-CAM, bio-CAM

machine), biopaper (printing onto some designed scaffolds or bio-substrates), and bio-products (2D and 3D bio-constructs) as final products. This process is just compatible for the concept of computer-aided tissue engineering (Sun and Lal 2002; Mironov et al. 2003). In addition, laminated printing developed 3D bioprinting or digital biofabrication or bottom-up tissue manufacturing, too. Although recently 3D printing procedure is called additive manufacturing, this concept is just same as inkjet 3D bioprinting in tissue engineering. In this way, several concepts and strategies have been proposed and many challenging researches on bioprinting were started.

The increasing studies showed that printed cells retained their growth-promoting properties which provided new approaches in regenerative medicine (Xu et al. 2005, 2006; Cui and Boland 2009).

2 Inkjet Cell Printing

Xu et al. used a modified commercial inkjet printer to deliver viable cells for the first time (Xu et al. 2004). In their study, *Escherichia coli* DH5α cells were blended with sterilized water at a concentration of 3×10^7 cells/ml to form the bioink and then printed on soy agar substrate by a modified HP DeskJet 550C printer. The printing pattern, a colony array, was edited by Microsoft PowerPoint software, while a single colony had a circular shape with an approximate diameter of 500 μm. Some complex patterns, such as a cartoon tiger paw, were also successfully printed through this method (Fig. 3a). Xu et al. further used this printer to deposit Chinese hamster ovary cells, which showed high viability (>90%) after printing (Xu et al. 2005). This study demonstrated that mammalian cells could be printed with high viability by inkjet method for the first time, which means that inkjet printing has the potential to be utilized in tissue engineering. Based on this, cell function maintenance after inkjet printing was also proved by Xu et al. (2006). In their study, rat embryonic hippocampal and cortical neurons were printed. Immunostaining and patch-clamp analysis were further implemented to test cell function post-printing (Fig. 3b). Their results showed that printed neurons exhibited strong immunoreactivity to specific antibodies, which indicated high cell marker expression. Besides, voltage-gated potassium and sodium channels were detected on cell membrane by patch-clamp. Thus, neuronal phenotypes and electrophysiology were proved to be retained. This series of studies elucidated that living cells can be printed into predefined patterns by inkjet printing, while cell viability and cell function were maintained after passing the hot and narrow nozzle. These findings formed the foundation of inkjet cell printing.

Planar cell patterns were first created by inkjet printing in early studies (Cui and Boland 2009; Nakamura et al. 2005; Saunders et al. 2008). Briefly, cell suspensions, sometimes blended with biocompatible materials, were inkjet printed on pretreated or non-pretreated substrates. Agar, collagen (Xu et al. 2005), albumin (Yamazoe and Tanabe 2009), and fibrinogen (Cui and Boland 2009) were pre-coated on substrates

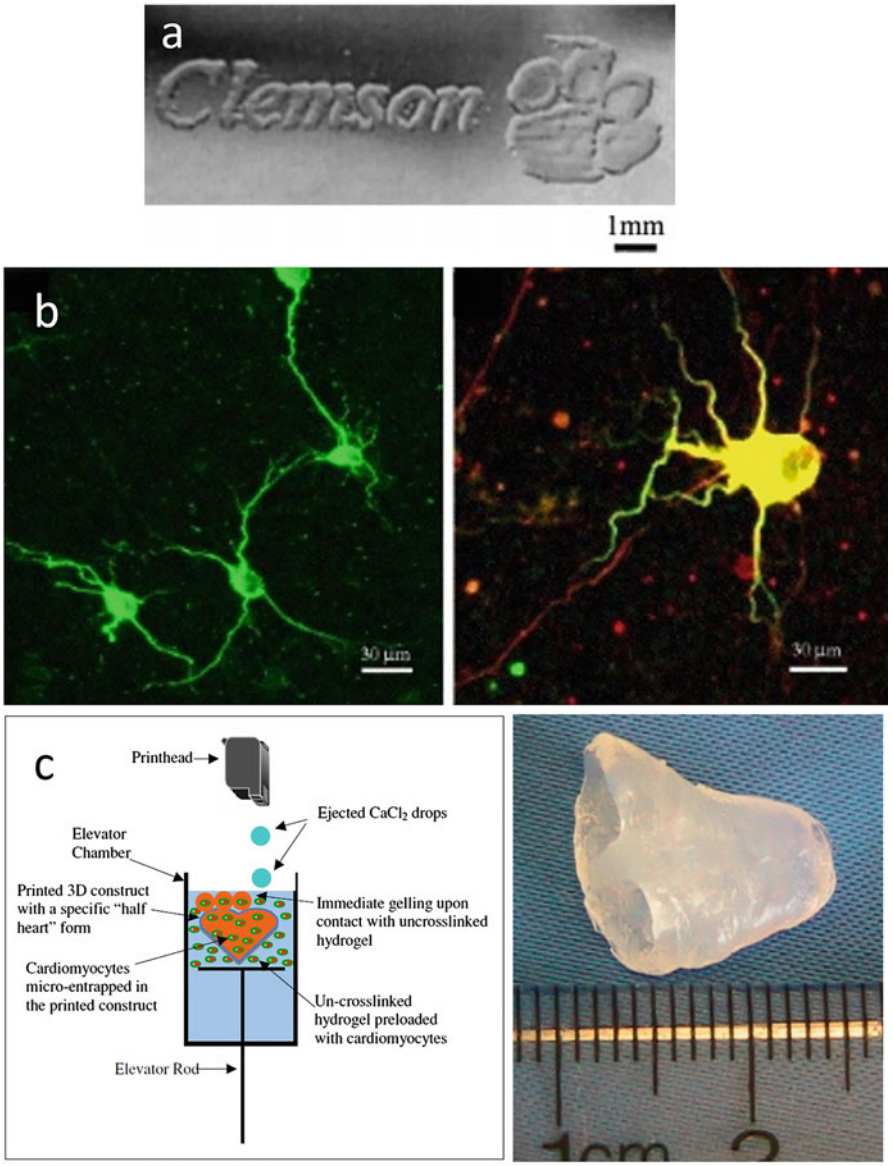

Fig. 3 (**a**) A 2D cartoon tiger paw printed with *Escherichia coli* DH5α cells (Xu et al. 2004). (**b**) Printed neurons stained with neuron markers anti-MAP 2 monoclonal antibodies (green) and anti-neurofilament monoclonal antibodies (red) after 15 days of culture (Xu et al. 2006). (**c**) 3D "half-heart" scaffold printed with cardiac cells (Xu et al. 2009a). Left: a schematic diagram of the printing process. Right: the real printed object

for optimal cell immobilization and modified microenvironment. Cell patterns were deposited on these substrates with droplet ejection from the multi-nozzle printheads. The diameter of a single printed dot was in micrometer magnitude, which is similar to the diameter of single cells (Nakamura et al. 2005). Meanwhile, by controlling the concentration of cell suspension, few cells could be contained in a single droplet. In some extreme conditions, only single cell was ejected within one droplet, which might have the potential to achieve single cell printing (Nakamura et al. 2005). Shuichi et al. realized this one-cell-per-droplet inkjet printing with a push–pull piezoelectric ejection method, and 100% accuracy was achieved (Yamaguchi et al. 2012). Cell viability and cell function post-printing were also evaluated in these researches. Cui et al. deposited bioink composed by human microvascular endothelial cells, thrombin, and calcium ions on a coverslip pre-coated with fibrin channel patterns (Cui and Boland 2009). After 21 days of culture, the printed endothelial cells proliferated and formed tubular structures inside the fibrin channels, which showed high functionality of the printed cells. In this work, to some extent, the printed fibrin channels could be regarded as 3D fibers with micro-diameter. It can be concluded that if these layers with defined patterns accumulated to a certain thickness, a so-called 3D structure is achieved.

3D cell-laden scaffold plays an important role in tissue or organ regeneration. A successfully constructed 3D scaffold must have structural integrity, proper mechanical strength, and cytocompatibility. The cytocompatibility of inkjet printing has been proved as mentioned above. Thus, the main concern turns to fabricate a 3D cell-laden structure with structural integrity while keeping mechanical strength by inkjet printing. However, in inkjet printing, to avoid nozzle clogging and to enable droplet formation, bioink must have low viscosity and low cell density (Murphy and Atala 2014). This restriction leads to proper cross-linking strategies which must be designed. The fibrinogen–thrombin system is one of the feasible cross-linking strategies. In this procedure, high-viscosity fibrinogen is coated on substrates as biopaper while low-viscosity thrombin is mixed with cells as bioink. Gelation occurs immediately after thrombin is ejected onto fibrinogen substrates and cells are trapped in the fibrin gel. There are some related works reported (Xu et al. 2006; Cui and Boland 2009). Another system is alginate–calcium system. Xu et al. printed calcium chloride into an alginate–gelatin–cell mixture to fabricate a "half-heart" with two connected ventricles (Fig. 3c) (Xu et al. 2009a). The alginate–gelatin–cell mixture was filled in an elevator chamber which could move along the z-axis. The chamber was lowered after one layer was printed and cross-link occurred between alginate and calcium ions. Meanwhile, new cell-laden gel was added for the fabrication of a new layer. The printed scaffolds showed adequate moduli and tensile strength. The cardiac cells embedded in the scaffold exhibited contractile properties under mild electrical stimuli in vitro. With a same system, collagen can also be used as extracellular matrix. Xu et al. printed an alginate–collagen tissue construct containing multiple cell types (Xu et al. 2013). This study also demonstrated the feasibility to fabricate heterogeneous tissue constructs by inkjet technology. In contrast, low-concentration cell-laden alginate solution could also be printed into calcium ion solution (Pataky et al. 2012; Arai et al. 2011). In this method,

gelation time and droplet deposit position were precisely controlled to obtain fine pattern quality. 3D tissue such as lumen structure was successfully built through this way (Xu et al. 2012; Xu 2014; Chiristensen et al. 2015). Poly(ethylene glycol) dimethacrylate (PEGDMA) is also inkjet-printable for its water-soluble nature, and it can be further photocross-linked to get more reasonable mechanical strength than that of cross-linked alginate or fibrinogen. Therefore, PEGDMA or similar photocross-linkable synthetic biomaterials may play an important role in bone or cartilage tissue reconstruction (Cui et al. 2012b, 2014). Besides, novel materials such as gellan gum and Pluronic F127 have been successfully used as bioink for inkjet printing (Ferris et al. 2013; Biase et al. 2011). Gellan gum has similar cross-linking mechanism as alginate but can form gels at lower concentrations. This property may allow the content of bioink to be kept at low levels while cell content will be more.

Although cell viability and phenotype have been proved to be retained after inkjet printing process, damage occurred to the cells by thermal heat or stress when passing the nozzles still exists and could not be neglected (Xu et al. 2009b; Cui et al. 2010). Thus, cell viability loss could not be eliminated and varies according to the printing parameters, droplet size, printing velocity, cell concentration, etc. Several studies focused on adjusting the printing parameters to optimize cell viability. Hendriks et al. established a model describing the cell viability as a function of droplet impact parameters (Hendriks et al. 2015). The model will certainly help to modulate the parameters to maximize cell viability. On the other hand, some changes to cells may also be caused by inkjet printing process. Tse et al. presented a study to print Schwann cells using a piezoelectric inkjet printer (Tse et al. 2016). Schwann cell is a kind of neuronal-related cell which exists in peripheral nervous systems. They have neurites elongated which will form sheaths surrounding the neuron axons. In Tse's study, inkjet-printed Schwann cells were found to generate neurites earlier than normal Schwann cells, and the neurites were longer. This result may either due to a piezoelectric effect or a transient high shear stress during ejection. At all events, this finding indicates that inkjet printing may be benefitted to produce fine neuronal networks in neural tissue engineering. Moreover, the precisely aligned cell patterns generated by printing may be more similar to their natural states, which may provide higher stage of biomimicry. Cui et al. presented a study to deposit evenly aligned mouse myoblasts onto micro-sized cantilevers by thermal inkjet printing (Cui et al. 2013). The printed myoblasts formed myotubes – a function unit in muscular system – within a few days, while randomly deposited cells took more than 14 days.

Though inkjet printing has been successfully used in 2D and 3D cell pattern fabrication and some beneficial impacts on cells have been revealed, inkjet printing technology is still not the best choice to fabricate complex 3D cell-laden constructs for several drawbacks. First, the small inkjet orifice is easy clogged, which limits a wide majority of bioinks to be used. Second, the ejected droplet may gel in air before assembling to the substrates, though printing parameters may be well controlled before printing procedures. Third, structures with high mechanical strength and structural integration could probably not be achieved by inkjet printing because of the low bioink viscosity and droplet ejecting mechanism. It is noteworthy that porous structures can hardly be fabricated by inkjet printing, which is vital to the

supply of nutrition and oxygen needed by the cells loaded in a hydrogel scaffold. Take these into account: other printing technologies, such as extrusion printing and laser-assisted printing, may be more suitable to build 3D cell-laden constructs (Gudapati et al. 2016). In our opinion, the future directions of inkjet cell printing should be built on its superiorities, for instance, multichannel, high-throughput, rapid printing speed and noncontact printing style. Multicellular or cell–molecule dot arrays can be easily printed by inkjet printing in a high-throughput and reproducible manner. Researches on cell–cell interactions or cell–drug resistance may be facilitated by this model (Choi et al. 2011; Matsusaki et al. 2013; Rodríguez-Dévora et al. 2012). On the other hand, based on its noncontact style, no strict distance limitations exist between the printing nozzle and substrates. When the surface morphology of the substrate becomes irregular, cells could also be deposited on the preset location by inkjet printing with high accuracy. This may indicate that inkjet technology could achieve in situ printing to directly deposit cells onto superficial wounds, thus accelerating wound healing and reducing processing steps. Finally, stem cell functionality after inkjet printing has been proved to be preserved (Xu et al. 2013). Since stem cell bioprinting has attracted significant attention due to its pluripotency and in vitro expansion ability, inkjet may also play a part in stem cell array printing and in situ stem cell printing.

3 Inkjet-Mediated Gene Transfection and Inkjet Printing Biology Molecule

In addition to print cells, inkjet printing also have capacity to print proteins, cell guidance, and combination biologics. Several reports have demonstrated the interesting side effect of thermal inkjet printing technology and apply it to gene transfection and intracellular delivery (Xu et al. 2009b; Cui et al. 2010; Shattil et al. 1992; Owczarczak et al. 2012). Xu et al. firstly introduced a novel inkjet-mediated technology that gene transfection and cell delivery can be simultaneously achieved (Xu et al. 2009b). In this study, porcine aortic endothelial (PAE) cells and green fluorescent protein-coding (GFP) plasmids were co-printed into fibrin gel substrate. The co-printed plasmids could be transfected into cells and then expressed. They found that the viability of printed cells was over 90% and transfection efficiency was over 10%. The transfected cells could then be precise printed into predefined positions, and GFP could be expressed in in vitro and in vivo experiments. The author also postulated the mechanism of inkjet-mediated gene transfection. When cells and plasmids pass through the channel of printing head during the co-printing process, the high shear stress and heat may produce transient membrane pores. Plasmids can then get into the pores and expressed in cells (Fig. 4). Combining gene modification and cell delivery can benefit the field of tissue engineering and regenerative medicine, because it is important to facilitate the cell with certain function to form functional tissue and organ. Cui et al. further studied the thermal inkjet printing induced gene transfection (Cui et al. 2010). This study had a more comprehensive understanding on the influence of printing procedure on printed

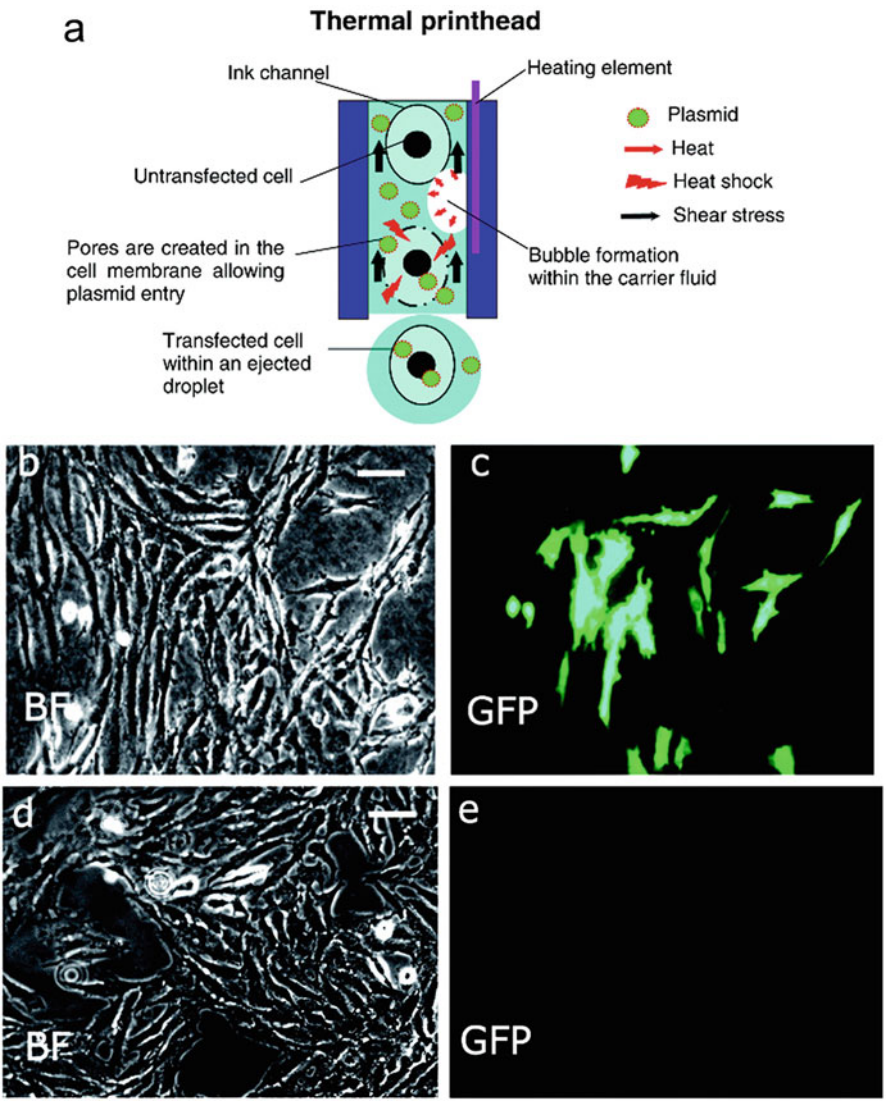

Fig. 4 (**a**) Schematic drawing of the postulated mechanism for inkjet-induced gene transfection. (**b**) The co-printed PAE cells after 2 days. (**c**) The green fluorescence expressed in co-printed cells. (**d**) The controlled nonprinted cells. (**e**) No fluorescence showed in the nonprinted cells (Xu et al. 2009b)

cells, such as cell viability and the size of cell membrane pores. Cell concentration was also optimized. Chinese hamster ovary (CHO) cells and fibrillarin- or GFP-fused plasmids were co-printed to achieve transfected cells, and it was observed that the transfection efficiency was above 30%, while cell viability was 89%. Furthermore, the study evaluated the membrane pore size and membrane repair time by incubating

and staining the printed cells with dextran molecules (Shattil et al. 1992). Dextran can only penetrate the membrane pores, so the average Stokes diameters of the dextran molecules were used to indicate the size of membrane pores. Finally, it was observed that transient membrane pores can be repaired within 2 h. The study introduced that inkjet cell printing technology creates transient membrane pores during the printing process which holds possibility to be applied in intracellular delivery, such as genes, proteins, and factors' transfection. Owczarczak et al. reported how to use a standard inkjet printer to process cells with fluorescent G-actin transfected (Owczarczak et al. 2012). Other researchers can follow their video instruction to convert a standard HP DeskJet 500 printer to an inkjet bioprinter. The printer has the capability to print cells and defined cellular microenvironments, leading to defined functional tissue structures.

Inkjet-mediated gene transfection (IMGT) may avoid the drawback of normal stem cell induction process, such as low efficiency, low throughput, and teratoma formation during transgene reactivation. Paquian et al. co-printed plasmid with stem cells to test whether IMGT would work in stem cells (Paquian et al. 2016). This study found that stem cells remained viable after printing, and one of them can be transfected by GFP-coding plasmid. However, it was unable to deliver 4-factor encoding plasmids into stem cells, likely due to the large size of the plasmid. Meanwhile, considering that different cells may produce different levels of membrane disruption, the match of vector size and membrane pore size may be well considered before transfection.

In current years, there are some reports focusing on the combination of bioprinting technology and stem cell research. Spatial patterns of biology factors have been printed to guide the fate of stem cells. Campbell et al. initiated the study of using inkjet to print spatially controlled growth factor to affect cell behavior (Campbell et al. 2005). In this study, the number of preosteoblastic cells increased as the concentration of fibroblast growth factor-2 (FGF-2) increased. In another report, the proliferation of MG-63 cells responded to the concentration of FGF-2 (Miller et al. 2006). Ilkhanizadeh et al. modified a thermal inkjet (TIJ) printer to print spatially defined gradients of biology molecules, including FGF-2, ciliary neurotrophic factor (CNTF), and fetal bovine serum (FBS) on a polyacrylamide gel (Ilkhanizadeh et al. 2007). The response of neural stem cells (NSCs) cultured on biological molecule patterns was observed, and it was found that NSCs differentiated into astrocytes when CNTF presented and undifferentiated when FGF-2 presented (Ilkhanizadeh et al. 2007; Hermanson et al. 2002; Johe et al. 1996). When cultured on the adjacent regions of FGF2 and CNTF, stem cell fate was controlled. The number of glial fibrillary acidic protein (GFAP) expressed cells increased correlated with CNTF concentration (Hermanson et al. 2002; Johe et al. 1996; Kazuhiko et al. 2002). Similar phenomenon was seen when NSCs were cultured on the FBS substrate, which led NSCs to differentiate into smooth muscle cells (Kazuhiko et al. 2002). Phillippi et al. observed the differentiation of muscle-derived stem cells (MDSCs) responded to the spatial patterned gradients of bone morphogenic protein-2 (BMP-2) (Phillippi et al. 2008). They used a piezoelectric printing system to print BMP-2 onto fibrin substrates. The same as NSC studies

mentioned above, MDCS fate can be controlled by different spatial gradients' pattern of proteins. However, this report demonstrated that MDSC can differentiate into two different fates as osteogenic and myogenic lineage.

Currently, several biology molecule printings which applied in cell migration (Miller et al. 2011), combination of multiple growth factor gradients (Miller et al. 2009), multilineage stem cell differentiation (Ker et al. 2011a), and control of cell alignment (Ker et al. 2011b) have been reported. All these researches provide us a new way to better understand stem cells. Fabricating spatial gradient of protein pattern would help us better study the role of protein in tissue and organ on influencing tissue repair, regeneration, and even cell fate.

4 Application of Inkjet Bioprinting

Inkjet printing combined with biotechnology has been exploited in various application areas such as high-throughput screening and cancer research, drug formulation, and tissue repair and organ regeneration (Gudapati et al. 2016; Scoutaris et al. 2016).

High-throughput screening and cancer research. Because of the highly controlled accuracy, repeatability, and uniformity of 3D manufactured microarrays, inkjet printing was first applied to high-throughput screening (HTS). HTS usually requires testing and collecting hundreds or more samples and performing subsequent analysis. Due to the need of repeatability and accuracy, HTS requires highly automated and convenient sample preparation, which can be totally featured by inkjet printing (Scoutaris et al. 2016). One of the first works related to inkjet printing in HTS was Silzel's spotted monoclonal antibodies that retain specificity and affinity on specific recognition of four human immunoglobulins and human myeloma proteins (Silzel et al. 1998). In another study, Rodríguez-Dévora et al. presented inkjet printing to assemble a high-throughput miniature drug screening platform (Rodríguez-Dévora et al. 2012). Using a modified Hewlett Packard model 5360 compact disc printer, *Escherichia coli* cells' expression green fluorescent protein, along with alginate gel solution, has been arrayed on a coverslip chip. Different antibiotic droplets were patterned on the cell spots to evaluate the inhibition of bacteria for antibiotic screening. The results revealed that thermal inkjet bioprinting, comparing with micro-pipetted samples, is a powerful method to generate high-throughput arrays of samples for drug screening applications. Matsusaki et al. presented piezoelectric inkjet (PIJ) bioprinting of multilayer liver tissue models for drug screening and high-throughput applications (Matsusaki et al. 2013). Rapid and automatic development of three-dimensional human micro-tissue chips is carried out by droplet printing technology. Xu et al. introduced a high-throughput automated cell printing system to bioprint a 3D co-culture model using cancer cells and normal fibroblasts micropatterned on Matrigel™ (Xu et al. 2011). This approach can support the research on the unknown regulatory feedback mechanisms between tumors and stromal cells and provide a tool for high-throughput drug screening in cancer research.

Drug formulation. Inkjet printing is a new drug formulation method with several reports. Meléndez et al. first reported using thermal inkjet technology to work for the development of solid dosage forms of low water-soluble active pharmaceutical ingredients (Meléndez et al. 2008). After that, many research groups tried to improve the dissolution rates of poorly soluble drugs through inkjet printing, by dispensing nanoparticle complexes of ciprofloxacin–polysaccharide with polyethylene–glycol (Cheow et al. 2015) and naproxen/PEG 3350 (Hsu et al. 2015). With the help of inkjet printing technology, Hauschild et al. obtained drug-loaded polymer microspheres of narrow size distribution and controlled diameter of 50–200 nm which can be used as drug carriers (Hauschild et al. 2005). Totally, as a simple and convenient approach of drug-loaded polymer particles with controlled size and shapes, inkjet printing has its irreplaceable position in drug formulation.

Tissue repair and organ regeneration. Regenerative medicine aims to search for effective therapeutic strategies to promote self-repair and regeneration of tissues and organs, hopefully to restore their function. Regenerative repair includes structural and functional repair (Huang 2011). Great achievements were made in structural repair (bone, cartilage, vessel, etc.) for relatively low technical requirements, and some of the technologies and products have been applied to clinics. On the other hand, the functional repair, mainly the repair and regeneration of solid organs, usually has fewer breakthroughs. Inkjet printing is anticipated to accelerate the development of personalized regenerative medicine.

Structural repair. Cui's group evaluated bioactive ceramic nanoparticles in stimulating osteogenesis of printed bone marrow-derived human mesenchymal stem cells in poly(ethylene glycol) dimethacrylate (PEGDMA) scaffold (Gao et al. 2014). hMSCs suspended in PEGDMA were co-printed by a TIJ bioprinter with nanoparticles of bioglass (BG) and hydroxyapatite (HA) under simultaneous polymerization so the printed substrates were delivered with high accuracy in three-dimensional (3D) locations. This technology demonstrated the capacity for both soft and hard tissue engineering with anatomic structures. Campbell's group engineered stem cell microenvironments, using inkjet bioprinting technology, to create spatially defined patterns of immobilized growth factors (Phillippi et al. 2008). Using this approach, they engineered cell fate toward the osteogenic lineage by printing patterns of BMP-2 within a population of primary muscle-derived stem cells (MDSCs) isolated from adult mice. This patterning approach was conducive to pattern the MDSCs into subpopulations of osteogenic or myogenic cells simultaneously on a same chip. When cells were cultured under myogenic conditions on BMP-2 patterns, those cells on pattern differentiated toward the osteogenic lineage, whereas cells off pattern differentiated toward the myogenic lineage. Boland's group improved wound healing through bioprinted skin grafts (Yanez et al. 2015). A layer of human microvascular endothelial cell-laden thrombin was bioprinted using a TIJ bioprinter onto fibrinogen. A full-thickness wound was created at the top back of athymic nude mice and the area was covered by the graft. As a result, wound contraction improved up to 10% when comparing with the control groups. The grafts supported the formation of new skin with comparable morphological

characteristics of native skin but lacked sebaceous glands, hair follicles, and hair bulbs.

Functional repair. In the study of substantive organs, inkjet printing has a lot of related researches. With the help of modified inkjet printers, Xu et al. fabricated cardiac (heart) tissue with beating response (Xu et al. 2009a). In this study, model cardiac cells remained viable in constructs as 1 cm thickness due to the programmed porosity, which suggested that the inkjet bio-prototyping method can be used for hierarchical design of functional cardiac pseudo-tissues, balanced with porosity for mass transportation and structural support. Akashi's group bioprinted liver tissue through layer-by-layer deposition of hepatocytes, endothelial cells, fibronectin, and gelatin using a PIJ bioprinter (Matsusaki et al. 2013), and the tissue model was used for evaluating drug metabolism of antidiabetic drug troglitazone. Rothen-Rutishauser's group reported about the biofabrication of human air–blood tissue barrier analogue composed of endothelial cell, basement membrane, and epithelial cell layer with a bioprinting technology (Horváth et al. 2015). In contrary to the manual method, this technique enables automatic and reproducible creation of thinner and more homogeneous cell layers, which is required for an optimal air–blood tissue barrier. This bioprinting platform offered a tool to engineer advanced 3D lung model for high-throughput screening for safety assessment and drug efficacy tests.

Although there are more and more researches about inkjet bioprinting, most of these technologies are still kept in lab and away from clinical and commercial use. Although the technology of bioprinting with growth factors and other biologics other than cells, or used in drug testing and HTS and drug formulation, began to be used in clinics, translation of inkjet bioprinting on tissue repair and organ regeneration still remains difficult.

5 Conclusion

Due to the rapidity, high resolution, and reproducibility, inkjet printing plays a unique part in biofabrication to print cells and biomolecules. Two-dimensional and three-dimensional patterns have all been successfully built by inkjet printing. With the sustaining of cell viability and cell expression, functional biomimetic tissue scaffolds have also been achieved by this method. Furthermore, the transient heat and stress may possibly give positive affection to cells, thus facilitating cell function expression. On the other hand, the precise deposition of different biomolecules with different concentrations will direct stem cell fate by zones. Complex heterogeneous structure with different cell types may probably be achieved by this way. Though the current application of inkjet bioprinting has not reached to the clinic, commercial inkjet bioprinters have been developed to serve in the researches of drug screening, tissue and organ repairing, and cancer modeling. It is believed that with the maturation of this technique and the whole field, inkjet bioprinting may serve for clinic and benefit the human beings in the future.

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