Postprint: Application of Piezoelectric Microjet Technology in Cell Printing

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Abstract

The processes of cell acquisition and culture constitute a critical step in tissue engineering construction; 3D bioprinting technology can support the development of tissue engineering. The piezoelectric micro-jetting technology, which utilizes "sound waves" to enable pulsed flow of microfluids under the interaction of inertial forces and fluid viscous forces, represents an emerging process when applied to the field of cell printing, characterized by high precision, high efficiency, and low cost. Based on an introduction to the system and principles of piezoelectric micro-jetting technology, this paper analyzes the influences of piezoelectric driving mode, piezoelectric parameters, pulsed driving voltage waveforms, and bio-cellular inks on cell printing; presents application research cases of piezoelectric micro-jetting technology in high-viability cell acquisition and high-efficiency construction of three-dimensional cellular tissues; summarizes its current application status in the field of cell printing; and points out future research directions and significance.

Full Text

Preamble

The Application of Piezoelectric Micro-jetting Technology in the Field of Cell Bioprinting

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Abstract

The acquisition and culture of cells constitute a critical component in tissue engineering construction, and three-dimensional bioprinting technology can support the advancement of tissue engineering. Piezoelectric micro-jetting technology, which realizes pulsating flow of trace fluid through the interaction of inertial force and fluid viscous force via "sound waves," represents an emerging process in cell bioprinting with characteristics of high precision, high efficiency, and low cost. Based on an introduction to the micro-jetting system and its principles, this paper analyzes the effects of piezoelectric drive mode, piezoelectric parameters, pulse drive voltage waveform, and bio-cell ink on cell printing. Application research cases demonstrating high-viability cell acquisition and efficient construction of three-dimensional cellular tissues are presented. The current application status in cell bioprinting is summarized, and future research directions and significance are identified.

Keywords: micro-jetting technology; cell bioprinting; influence factors; application research

Introduction

The shortage of tissues and organs for transplantation and repair represents a major global challenge. Tissue engineering, which integrates biomaterials science, cell biology, and clinical medicine, can construct biomimetic human tissues and organs to address this problem. However, current challenges in tissue engineering primarily involve cell acquisition and culture, as well as structural scaffold fabrication. The emergence and development of biological 3D (Three-Dimensional) printing technology offers solutions to these issues by enabling precise cell printing and scaffold construction.

Biological 3D printing is a computer-aided additive manufacturing technology that processes bioactive materials including biomaterials, growth factors, and cells to repair and reconstruct complex functional human tissues and organs. It represents an interdisciplinary and cross-domain regenerative medical engineering technology. Based on technological timeline and material biocompatibility, bioprinting development can be divided into four stages: (1) printing materials without biocompatibility requirements, such as in vitro medical devices; (2) printing materials with biocompatibility requirements but non-degradable, such as permanent implants made of metals and ceramics; (3) printing materials with biocompatibility and degradability, such as implants promoting tissue regeneration; and (4) using living cells, proteins, and extracellular matrix as materials to print three-dimensional biological structures, including the latest 4D printing with self-assembling materials that adds a temporal dimension.

Cell printing technology represents the fourth stage of bioprinting development. It utilizes medical imaging and 3D modeling software to create digital models, enabling precise deposition of cells and biomaterials to form complex 3D cellular structures. Major cell printing technologies include laser-guided direct writing,

stereolithography, bioplotting/bioprinting, direct three-dimensional controlled assembly, inkjet printing (also called micro-droplet method), and batch cell printing. This paper focuses on piezoelectric micro-jetting technology based on inkjet printing principles, analyzing its influence on cell printing, implementation methods, and practical applications.

1. Piezoelectric Micro-jetting Technology

Micro-jetting represents an important application of microfluidic control. The principle of piezoelectric micro-jetting utilizes the expansion and contraction deformation of piezoelectric ceramic materials to eject "ink" from the nozzle, forming droplets. The piezoelectric micro-jetting printing system [Figure 1: see original paper] consists primarily of an electrical controller, pneumatic controller, piezoelectric nozzle, and visual observation component. The piezoelectric nozzle [Figure 2: see original paper] comprises a glass capillary with piezoelectric ceramic material PZT (piezoelectric transducer) attached to its outer middle section.

The jetting mechanism works as follows: negative pressure from the pneumatic controller balances the jetting ink at the nozzle position. The electrical drive controller applies a pulse waveform. During the rising and holding phases of the pulse voltage, the piezoelectric ceramic material inside the nozzle generates slight deformation, which contacts the nearby glass capillary wall and creates a "sound wave" that squeezes and ejects solution from the nozzle. When the voltage drops, the piezoelectric ceramic relaxes, the glass capillary expands, and the ink at the nozzle recesses to "cut off" the extruded solution. Under surface tension, the extruded solution gradually aggregates into a single droplet, achieving regular jetting printing.

Initially applied in micro/nano-electronic printing and biosensors, micro-jetting technology has rapidly advanced in biological applications such as cell acquisition and organ printing in recent years. Cai Renye successfully printed yeast cells using a self-developed cell printing device based on piezoelectric jetting, analyzing the effects of pulse voltage, bio-ink concentration, viscosity, and buffer medium on cell viability. Xu C et al. studied droplet formation during drop-on-demand inkjetting of fibroblast cells and sodium alginate mixed bio-ink. Kim Y K et al. successfully obtained mouse fibroblasts and human embryonic kidney cells using piezoelectric nozzles, verifying through subsequent culture and proliferation that piezoelectric micro-jetting does not damage mammalian cells. Kim J D et al. printed human adipose-derived stem cells using piezoelectric micro-jetting technology. Ng W L et al. validated the printing feasibility of human foreskin fibroblasts (HFF).

In organ printing, Lee J H et al. reported a microfluidic 3D bone model where micro-jetting prepared microstructures containing antibiotics and biphasic calcium phosphate nanoparticles, combined with microfluidic chips and cell culture to accelerate bone-implant integration while preventing bacterial infection.

Zhang J et al. precisely prepared sodium alginate microstructures on glass slides using self-developed micro-jetting equipment, forming microfluidic chips combined with corresponding cell array structures to simulate in vivo environments. These developments demonstrate that piezoelectric micro-jetting technology has achieved significant progress in cell bioprinting, providing foundations for cell acquisition and tissue regeneration.

2. Influence of Piezoelectric Micro-jetting Technology on Cell Printing

Factors influencing piezoelectric micro-jetting cell printing include technical conditions and bio-ink properties.

2.1 Influence of Piezoelectric Drive Mode on Cell Printing

Piezoelectric micro-jetting cell printing employs two drive modes, which The R et al. compared [Figure 3: see original paper]. Mode A (expand-then-squeeze) begins with non-zero drive voltage where solution is pre-compressed before entering the nozzle. When voltage decreases, the piezoelectric ceramic relaxes and the nozzle expands; subsequent voltage increase compresses the nozzle to eject droplets, which are cut off during the next waveform cycle. This mode generates higher droplet ejection speeds and more elongated droplets. Mode B (squeeze-then-expand) begins with zero drive voltage; as voltage gradually increases, the piezoelectric ceramic compresses the nozzle to extrude droplets, which are then cut off when voltage decreases and the nozzle expands. This mode produces larger droplet volumes with gentler ejection rates.

Yamaguchi S et al. conducted printing experiments on insect sf9 cells (spodoptera frugiperda cell), demonstrating that the "squeeze-then-expand" mode more easily generates single-cell droplets (with 100% of 100 droplets containing only one cell). Comparing with non-jetted cells revealed survival rates around 90%, indicating the jetting process does not significantly affect cell viability. Therefore, the "squeeze-then-expand" mode is more suitable for cell printing, particularly single-cell printing.

2.2 Influence of Piezoelectric Parameters on Cell Printing

The matching between image information transmission during printing and the excitation pulse in the nozzle' s piezoelectric ceramic affects micro-jetting stability and accuracy. As mentioned, the driving force of piezoelectric nozzles originates from piezoelectric ceramic deformation, which is primarily determined by the pulse voltage from the drive power supply. Consequently, nozzle performance directly depends on the pulse voltage and frequency provided by the electrical controller, ultimately affecting droplet size, ejection speed, droplet uniformity, and post-ejection trajectory linearity, which collectively influence cell printing outcomes.

Zhang M et al. investigated pulse voltage effects on cell distribution using 3T3 mouse fibroblasts and sodium alginate mixed solution, finding that at 40V pulse voltage, the difference between actual and theoretical cell distribution values was 3%, while at 50V, the difference reached 18%. Additionally, Saunders R E et al. studied pulse voltage and duration effects on cell viability using fibroblast cell solution, concluding that increased pulse voltage and duration raise droplet velocity, increasing impact force between cell-laden droplets and the substrate, thereby reducing cell survival rates. These results demonstrate that piezoelectric parameters directly affect cell printing.

2.3 Influence of Pulse Drive Voltage Waveform on Cell Printing

The electrical controller can provide various drive voltage waveforms to piezoelectric nozzles. Gan H Y et al. studied the effects of different waveforms—including unipolar, bipolar, M-shaped, and W-shaped waves—on droplet size. Results showed bipolar waves are more suitable for Newtonian or near-Newtonian fluids and have greater effect on reducing droplet volume, while W-shaped and M-shaped waves have smaller adjustable ranges but significantly affect non-Newtonian fluids, reducing droplet volume and line width.

The most commonly used waveform in piezoelectric micro-jetting is the bipolar trapezoidal wave [Figure 4: see original paper], where the X-axis represents time (s) and Y-axis represents pulse voltage (V). Parameters include pulse voltage amplitudes (Dwell Voltage and Echo Voltage in V), positive/negative voltage holding times (Dwell Time and Echo Time in s), pulse voltage rise time (Rise Time in s), and fall time (Fall Time in s).

As micro-jetting technology advances in cell printing, higher precision requirements and smaller cell droplets can be achieved using smaller nozzle diameters. However, excessively small nozzles are prone to clogging, significantly reducing printing reliability and repeatability. To address this, Kwon K S et al. proposed a high-speed waveform design method based on wave propagation theory, finding that for high-speed micro-jetting printing, improved pulse waveforms can effectively suppress residual pressure waves after ejection, enhancing printing stability. Lee Y I et al. formed fine line patterns on polyimide surfaces through piezoelectric micro-jetting printing, improving post-printing resolution by modifying pulse waveform design to shorten the piezoelectric ceramic deformation cycle, thereby assisting in ejecting faster and more precise droplets. For cell printing, improved pulse drive voltage waveforms can modify piezoelectric ceramic deformation frequency, alleviating clogging issues caused by high-viscosity cell inks while simultaneously increasing printing speed.

2.4 Influence of Bio-cell Ink on Cell Printing

Research on bio-cell ink effects on droplet stability has yielded significant results. Fromm J E introduced a critical dimensionless parameter Z (related to solution density, surface tension, viscosity, and nozzle diameter) through testing various

solutions, predicting that droplet ejection requires Z > 2. Reis N et al. and Derby B confirmed this, finding stable droplet formation and inkjet printing occurs when ink Z values are between 1 and 10. Jang D et al. further refined this definition to 4 Z 14 by examining kinetic changes during stable droplet formation and analyzing printing characteristics (single degree of freedom, minimum distance, positional accuracy, maximum jetting frequency). Solutions with Z < 4 exhibit tailing that prevents stable droplet aggregation, while Z > 14 cannot form stable droplets for printing.

Chahal D et al. specifically studied concentration effects on droplet stability using fibroblasts mixed with sodium alginate and polystyrene solutions, finding that increased bio-cell ink concentration raises droplet volume and ejection speed, suppresses satellite droplet generation, but increases tailing elimination time. Compared with particle suspension solutions, bio-cell inks produce relatively smaller droplet volumes and ejection speeds. Moon S et al., Liberski A R et al., and Cheng E et al. investigated cell ink properties using breast cancer MCF-7 cells mixed with Ficoll PM400 and PBS (phosphate buffer saline), revealing that while micro-jetting precisely controls droplet size, cell distribution within droplets is non-uniform and does not follow Poisson distribution. Modifying cell ink viscosity can improve cell deposition, though cell deposition at the piezoelectric nozzle affects final droplet distribution and causes clogging. Adding suspensions to cell inks can modify rheological properties, enabling uniform cell dispersion, approximating Poisson distribution, and reducing cell aggregation and nozzle clogging. Table 1 summarizes the effects of several bio-inks on cell printing.

3. Advantages of Piezoelectric Micro-jetting Technology for Cell Printing

As a non-contact printing method, piezoelectric micro-jetting technology has found applications in tissue engineering, biomedicine, flexible wearable devices, and optical components. Its advantages in cell printing primarily manifest in two aspects:

3.1 High-Viability Cell Acquisition

Cell acquisition and culture are crucial for final tissue formation, making cell printing a major focus in biomedical tissue engineering. Early concerns suggested that piezoelectric micro-jetting might rupture cell membranes and cause cell death. However, extensive experimental data demonstrates that the slight deformation, "sound waves," and acceptable ejection speeds during piezoelectric micro-jetting cause minimal cell damage and do not lead to cell death. Instead, its wide parameter adjustability, low cost, and high cell viability have made it a mainstream trend in cell printing.

Xu T et al. applied micro-jetting technology in 2005 to print mammalian hamster ovary cells, with over 90% maintaining viability and proliferating during 25

days of culture. Subsequent studies increased human fibroblast viability to 94-98%. A Cambridge University research group in 2013 used a piezoelectric inkjet printhead to "print" adult rat glial cells and retinal ganglion cells through a sub-1mm nozzle. Although many cells were lost due to sedimentation at the inkjet bottom, the printed cells remained healthy, with fragile cell membranes surviving nozzle ejection and maintaining normal biological characteristics in culture.

Ferris C et al. printed mouse myoblasts using endotoxin-free low-acyl gellan gum suspension as bio-ink, achieving >95% viability. Since 2016, Detsch R et al. analyzed piezoelectric inkjet parameters for colorectal epithelial cell printing, achieving 98% viability in cultured bone marrow stromal cells. Kim Y K et al. printed mouse fibroblasts and human embryonic kidney cells using different nozzle diameters, achieving >94% viability. Ng W L et al. printed neonatal foreskin fibroblasts using a 50 m nozzle, achieving 95% viability. These studies demonstrate that piezoelectric micro-jetting enables cell printing with high viability. Table 2 summarizes several cell printing cases using piezoelectric technology.

Cryopreservation is a common cell culture method where ice crystal formation during rapid vitrification and toxicity from high-concentration cryoprotectants can damage cells, though high transformation rates can reduce ice crystal formation. Therefore, micro-droplets generated by jetting printing are crucial. Dou R et al. applied piezoelectric micro-jetting to print mouse fibroblasts and human neural stem cells for cryopreservation [Figure 5: see original paper]. Compared with conventional liquid cryopreservation, picoliter-scale cell droplets ejected by piezoelectric micro-jetting reduce thermal diffusion length, increase freezing rate, and significantly decrease cryoprotectant concentration, thereby minimizing cryopreservation-induced cell damage.

During drug development, preclinical efficacy, safety, and dosage testing primarily rely on animal models. However, differences between animal and human responses often cause drug failure, prolonging development cycles. Park T M et al. printed cervical cancer cells using piezoelectric micro-jetting, quantitatively and positionally ejecting them onto nanofibrous membranes to form tissue arrays [Figure 6: see original paper]. Enzyme-linked immunosorbent assay measurements and anticancer drug testing showed nearly 100% cancer cell viability with high affinity for matrix metalloproteinases 2 and 9 and high resistance to docetaxel. These experiments demonstrate that cells cultured using piezoelectric micro-jetting maintain viability comparable to conventional culture methods.

3.2 High-Precision and High-Efficiency Construction of 3D Cellular Tissues

Layer-by-layer printing of biological 3D tissues has emerged as a novel approach to address organ shortage and culture difficulties. Current organ printing technology remains immature with various printing devices, including micro-

extrusion, solenoid valve, laser, and piezoelectric systems. Piezoelectric micro-jetting offers distinct advantages in precision and efficiency: (1) it can eject micron-scale diameter, picoliter-volume droplets with system motion accuracy up to 1 m, fully satisfying the 10 m intercellular spacing requirements of living tissue architecture; (2) it achieves frequencies up to 30kHz, enabling 30,000 cells printed per second, with multi-channel integration capabilities for enhanced efficiency.

Christensen K et al. tested complex cellular structures using sodium alginate and mouse fibroblast alginate as bio-inks and calcium chloride solution as cross-linking agent and support material, successfully printing horizontal and vertical branched vascular-like structures [Figure 7: see original paper] while maintaining >90% cell viability after 24 hours. Suntivich R et al. created cell storage "silk nest" arrays [Figure 8: see original paper] using piezoelectric micro-jetting. The nest material consisted of silk polyelectrolytes modified with polylysine and polyglutamic acid side chains, rapidly forming 70-100 m diameter, several-hundred-nanometer thick structures through multilayer inkjet printing for cell array storage, packaging, and culture.

Lorber B et al. and Owens C M et al. demonstrated the feasibility of printing mouse bone marrow stem cells and rat retinal ganglion cells to construct neural tubes and nervous systems using piezoelectric inkjet bioprinting. Lee V et al. created simulated skin tissue with dermal and epidermal structures by printing collagen, keratinocytes, and fibroblasts in separate layers using 3D inkjet printing. After 2 weeks of air-liquid interface culture, keratinocytes differentiated normally to form multi-layered epidermal stratum corneum. Xu T et al. prepared 1mm-thick tissue-engineered cartilage scaffolds using rabbit chondrocyte-laden elastic fibrin and collagen hydrogel as bio-ink through hybrid inkjet printing. After 1 week of in vitro culture, >80% of chondrocytes retained proliferative activity, and biomechanical testing revealed significantly enhanced mechanical properties with good biological performance.

Due to its droplet precision, high ejection rate, and multi-channel printing capabilities, piezoelectric micro-jetting enables not only single-cell printing but also simultaneous printing of multiple cell types, extracellular matrix, and biomaterials, offering potential advantages for high-throughput cell arrays and complex tissue structures, providing strong support for organ manufacturing and transplantation.

Conclusion and Future Directions

The high precision, efficiency, non-contact nature, and low cost of piezoelectric micro-jetting technology have attracted increasing attention in cell bioprinting with broad development prospects. Current applications in cell culture and tissue engineering have expanded beyond simple 3D frameworks to demand higher performance in cell culture, multi-cell type organ printing, biosensor fabrication, and DNA synthesis. Key challenges include further improving cell viability, en-

suring optimal culture environments before and after printing, and precisely controlling printing parameters and bio-ink effects on cell printing. Therefore, research on micro-jetting technology in cell printing, along with innovative approaches to complex structure design and post-processing, requires continued development. With advances in artificial intelligence and materials science, bioprinted tissues and organs will likely be realized through increasingly mature micro-jetting technology in the field of cell bioprinting.

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