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Review

Conceptual Framework of a Multi-Nozzle Extruder Bioprinter for Advanced Tissue Engineering

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Abstract: This paper focuses on a particular programmable bioprinter which has been developed by using a dual-nozzle extruder and is intended to expand the capabilities of traditional single-nozzle systems in constructing complex biological structures. In contrast to single-nozzle designs, which limit the number of biomaterials that can be utilized, the dual-nozzle approach makes it possible to quickly deposit various biomaterials, thereby improving the construction of multi-material and multi-cellular structures significantly. This functionality can be said to be of importance to areas such as tissue engineering and regenerative medicine as well as in pharmaceutical applications which require multi-material construction to create structures that closely resemble natural tissue environments. The introduction of an engineered dual-nozzle configuration on the bioprinter significantly advanced the efficiency of fabricating structures with design features that are like tissues while allowing for more freedom with regards to the deposition process. This paper comprehensively assesses the bioprinter design, its parameters, and its operation, giving a range of medical applications for the technology. It also mentions the current limitations in the bioprinting processes for example, creating constant flow of materials and ensuring the absence of cross-contact of materials used.

Keywords: Bioinks, Multi-material printing, Regenerative medicine, Tissue engineering, Dual-nozzle extrusion, and Bioprinters.

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Introduction

Printing has become a talking point now-adays due to use in biological sciences and more recently by aerospace and automotive industry as well. Bioprinting, which is a complex subsector of 3D printing that aims to develop biological tissues using biomaterials such as hydrogels, cells (living) and various supporting matrices. This technology allows for the creation of tissue models, personalized organ replacements and drug screening platforms. In the Biomedical field it is now considered one of the revolutionary technologies to create biological tissues using 3D bioprinting. The

heterogeneity in tissue architecture, which often consists of multiple cell types and extracellular matrix components have long been replicating as one of the biggest obstacles for tissue engineering. Single-nozzle 3D printers restrict the multiple biomaterials in one structure, thereby hampering complex integration of tissue that functionally mimics local biology. In contrast, with a dual nozzle bioprinter is allows for two materials to be deposited at the same time making it simpler to build complex, multi-material tissue constructs and supports better mimicry of native tissue architecture.

Over the past few years, research has geared towards generating bioprinting processes with a lower chance of errors and higher precision, resolution and more material compatibility. The double-nozzle extruder bioprinter is an option, allowing different biomaterials to be mixed and thus increasing the range of applications in bioprinting in tissue engineering, regenerative medicine and pharmaceutical research.

Bioprinting and Technological Progress

Introduction to Bioprinting: Derived from additive manufacturing principles, bioprinting is the process of creating biological tissues layer by layer using cells, bioactive molecules and biomaterials. The end goal is creating tissue or organ models for in-vitro-based research, or ultimately transplantation into a living organism. Having two nozzles means that the printer can in practice print multiple materials in a single build, enabling more complex designs.

Although the early bioprinting devices were based on single nozzle, it holds back material printing time. For example, you must use layers of various biomaterials with specific mechanical properties if you want to create skin or cartilage tissue. This process is very slow with only one nozzle, and it needs to be done in several prints, which could cause problems as structural failures or getting misalignment. A dual-nozzle printer, on the other hand, can extrude two completely different materials simultaneously to ensure smooth material integration and significantly increase print speed and accuracy.

Material and bioink development: This is the first step all bioprinting researchers must deal with and thus it´s almost everywhere in bioprinting papers. Bioinks, the materials that are extruded from the bioprinter to generate a biological structure, typically contain living cells encased within an alginate hydrogel matrix for support. For bioinks to support tissue growth, they must be biocompatible, possess suitable mechanical properties and degrade at a rate that enables cell expansion. Natural polymers, like collagen, gelatin, alginate, and fibrin, hold potential as bioactive materials due to their inherent physiochemical properties and their similarity to the original extracellular matrix (ECM). This resemblance to the ECM provides a natural guide for cellular self-assembly and behavior. On the other hand, synthetic polymers, such as poly (ethylene glycol) (PEG) and poly (lactic-co-glycolic acid) (PLGA), offer precise control over mechanical properties and can be customized for specific applications. The ability of a twin-nozzle bioprinter to print synthetic and natural polymers at the same time opens new avenues for synthesizing a combination structure with high benefits from both material types.

All hydrogels are heterogeneous network structures of polymer chains, formed by physical or chemical crosslinking in three-dimensional space. In one of polymeric biomaterials spheres, cells can preserve their size and shape for a long time. And most important is that the pores in hydrogels contribute to the nutrient exchange between cell-flocks and the surroundings due to their high-water content ultimately leading to physiological properties inherent in animal tissues. These advantageous characteristics of living organoids mean that they require special carriers; such biocompatible polymers serve that function. One of the key advantages of dual-nozzle systems is their ability to print cell-rich bioinks together with other supporting materials (e.g., sacrificial scaffolds that can be removed afterwards to make hollow structures such as blood arteries). As an example, to create the blood vessels that are necessary for nutrition delivery in larger tissue constructs, alginate gels are commonly combined with Pluronic F127 acting as a fugitive material which can be washed out easily after printing.

The Dual-Nozzle Bioprinter's Design

This dual nozzle, bioprinter is equipped with a 3D motion platform that enables precision control over the X, Y, and Z axes. Each nozzle has actuators controlling both the flow rate and extrusion temperature independently, ensuring the

most accurate printing of each bioink. And the nozzles can be placed on different carriages to move independently from each other across the print area. As a result of its autonomous control, the system can print more complex designs because it can adjust the extrusion parameters and position per nozzle in real time.

The printer's extrusion system is actuated by stepper motors coupled to high-precision belts and pulleys. A system of high-level feedback-controlled mechanisms lets an intricate dance take place, ensuring that the nozzle travels precisely enough to lay down each layer with extreme accuracy. One of the key improvements of the design was to integrate a real-time feedback mechanism in the system, that continuously monitors the performance of nozzles and adapts flow rates accordingly to prevent issues like clogging or under-extrusion.

If material flow is controlled by a peristaltic pump, the bioinks are extruded gently and without intermediate pressure changes. The layer thickness is made uniform, and not only does this reduce the waste of bioink but in addition, accuracy and reproducibility are also increased by moving around using peristaltic pump systems that control the flow rate. The system also includes an internal cooling mechanism to regulate the temperature of high-cell-dense bioinks, which are sensitive to temperature variations. This can really help the customers who are struggling with dual-nozzle nozzles where both the nozzles move together, making it difficult to print complex structures accurately. In contrast, the independent nozzle movement of the dual nozzle bioprinter explored in this article provides tuning opportunities for more complex geometries. Independent nozzle movement allows the printer to print different materials in adjacent areas of a 3D model at the same time (e. g. scaffold material in one area and cell-laden bioinks in an adjacent region).

This autonomous movement also reduces the dreaded inefficiency of printing. One of the nozzles can be set aside at any time (either to rest or prepare for the next layer) to reduce idle time; this ensures optimal throughput per bioink. Together these two aspects dramatically enhance print performance, particularly for complex multi-material prints at a large scale, such as tiered organ models or vascularized tissues.

Utilization in Regenerative Medicine and Tissue Engineering

Complex tissue fabrication: The dual-nozzle technology is most advantageous because it will be able to print multicellular and multi-material constructions, which is an absolute necessity for tissue engineering applications. Constructing skin, the separate materials for the dermis and epidermis are again an example. A durable, scaffold like material would be necessary for the dermis layer, yet a more compliant biocompatible layer is needed for cell growth to occur from within the epidermal surface. Such elements can be spun together on a dual nozzle bioprinter and deposited simultaneously to offer benefits in manufacturing time, as well as providing the structure with improved coherence.

One of the main limitations of tissue engineering is Vascularization. Tissues thicker than a few millimeters require a network of blood arteries to transport the nutrients and oxygen. Dual-nozzle allows for the simultaneous printing of sacrificial materials as well as cell-laden bioinks, to form hollow channels that can be seeded with endothelial cells for future blood arteries. This result is demonstrated in the studies where functional vascular networks inside of tissue constructs have been engineered by adding the bioink such as alginate and sacrificial materials like Pluronic F127. Dual **Nozzle Bioprinting:** An area of Application Double-nozzle bioprinting is suitable for personalized medicine, one of the most frequently hyped mills in aid. It permits the production of tissue transplants or organ models derived from cells originating in an individual patient using bioinks. This project represents a new opportunity to develop patient-specific drug test models or even custom implants that have less chance of rejection. Recent breakthroughs in stem cell science have led to potential solutions for this by harnessing the power of induced pluripotent stem cells (iPSCs) into generating many desired cell types critical for bioprinting. The dual-nozzle technology enables one to deposit multiple and different cell types simultaneously in a single construct. For example, cardiomyocytes combined with fibroblasts can be printed nearby other to fabricate a functional heart tissue model. One of its applications in regenerative medicine research is to produce tissues for transplantation and repair of damaged or degenerated tissues or organs.

Pharmaceutical Research and Drug Testing: Dual nozzle bioprinters enable a novel approach to generating tissue models that better replicate human physiology in the drug development process. Failure of conventional 2D cell

cultures to mimic human tissue complexity results in poor preclinical study translation to clinical trials. With the aid of several cell types and matrix components, dual nozzle bioprinting method enables more physiologically relevant 3D tissue models for drug testing.

Bioprinter liver and kidney models are already being used by researchers to investigate drug metabolism and toxicity. By printing tissues of different layers and compositions, scientists can study how drugs move in and interacts with different cell types. This, in turn, can help to reduce errors that occur during preclinical studies and further curb the use of animals for toxicity screening of new drug candidates.

Error mitigation and process optimization

Print Parameter Optimization: The printing precision required for successfully building complex tissue structures. There are many factors that affect the quality of 3D prints, such as material flow rate, layer thickness and extrusion speed. These are carefully controlled by powerful software algorithms in the dual-nozzle bioprinter, adjusting printing parameters on-the-fly. This includes ability to vary the extrusion speed for each nozzle in its own system, based a well on mechanical and viscosity characteristics of bioink. Some bioinks that are less viscous, such as Pluronic F127, can be extruded faster to reduce printing time with a reverse case for more viscous ones like alginate which may need to be printed at even lower extrusion rate to maintain single droplet deposition. The system also incorporates temperature control to maintain the extrusion conditions, from printing of thermoplastic filament. This is of particular importance to temperature sensitive materials, such as cell-laden hydrogels.

Detection of Error with Correction: Bioprinting never eradicates print failure, as the material might be misaligned, or the nozzle is blocked. The double-nozzle bioprinter has several error-detection systems to avoid these problems. Individual nozzles are monitored with real-time sensors for flow rate and extrusion pressure so that any variation from the theoretical parameters can be quickly recalibrated.

If it detects a clog, the device may immediately stop printing and either retract the nozzle or start a cleaning cycle to clear any obstacles. In addition, the printer has a feedback control system that monitors the location of each nozzle and corrects any misalignment to ensure that every single layer is deposited with the required precision. These kind of error corrections are necessary for large, multi-layered objects that print with tolerable quality and function reliably.

Prospects and Difficulties

Bioinks: Although bioinks for extrusion bioprinting have greatly advanced, more research is needed to develop bioinks that accurately replicate the properties of real tissues. Often, the stabilities of printed tissue structures with current bioinks are limited by their mechanical characteristics. This poses a challenge for the design of future bioinks, where one must balance mechanical strength required for tissue architecture hold and biological properties necessary to support cell viability and proper integration into tissue host. The cutting edge of this research is making bioinks which can signal in response to the surrounding environment, for instance with changes in temperature or pH differences, ultimately speeding up tissue maturation following printing. Additionally, bioinks that include bioactive molecules such as peptides or growth factors may further enhance tissue performance and cell differentiation.

Clinical Scaling: Transferring dual nozzle bioprinting from research to clinic presents several challenges. There are limits to the size of tissues that can be produced with current bioprinting techniques; For large-scale tissues or whole organs, vascularization & integration with the patient's body are still among the greatest challenges. In general, improving the vascularization methods such as in bioprinting bed using endothelial cells or the introduction pre-vascularized networks will be necessary to overcome this limitation. Getting past regulation is an extra hurdle. Before being used in the clinical context, bioprinter tissues must comply with rigorous safety and efficacy standards. All this needs to be well tested beforehand to ensure that the printed tissues function like real ones and the bioinks used for this purpose do not produce allergic reactions or any undesirable effects.

Organ Printing: The ability to print entire organs for transplantation is the goal of bioprinting. Even though it will be a while before we can produce our organ models for drug discovery and disease modeling, there has been significant

advancement in that direction. Scientists, for example, have developed bioprinter models of the liver that better represent a variety of important aspects of hepatic function than traditional 2D tissue culture systems for more-accurate investigation of drug metabolism. Progress in multi-material printing and improving bioink composition are needed to print entire organs to more accurately replicate organ tissues that present complex mechanical characteristics. Due to the capability of embedding various materials and cell types into one single construct, the dual-nozzle architecture will provide a strong foundation for that advancement.

Summary

This research is focused on developing a dual-nozzle extruder as a stand-alone device for biological 3D printers. While single nozzle extruders have made some progress, they still cannot be used for many needs, like printing various bioinks or support materials simultaneously. Most of the existing dual-nozzle extruders have fixed nozzles, which decreases the flexibility. These constraints can be solved by using dual-nozzle extruders. The new design derived from in-depth analyses of the existing fixed dual-nozzle systems. On the same lines, printer platform is driven along the Z-axis and then moved to where precise extrusion takes place when two nozzles are moving independently on X-axis and in sync on Y-direction for coordinated extrusion. Initially it was imagined that X, Y plane mobility would be entirely free, but interference problems arose. A compromise was made to synchronize this motion in one plain to reduce the interferences and preserve useful motion. Crucial components such as the filament handling components, the printer frame, motion systems, and nozzle materials were carefully designed to ensure proper bioink extrusion and to be biocompatible. Key advantages of this stand-alone dual-nozzle architecture are the simultaneous multi-bioink processing capabilities as well as the ongoing removal and inclusion of sacrificial support materials to seed more complex, multi-material tissue designs. Further investigation is needed to enhance synchronization, redesign for productivity, and enable dependable profile extrusion. The new bioinks and materials could lead to broader uses of biological 3D printing, with a payoff on tissue engineering and regenerative medicine.

To sum up, creation of fully autonomous dual-nozzle extruder that provides the advantages over fix-nozzle systems is a remarkable achievement in bioprinting. This leads to more sophisticated capabilities in building biological structures for medical research and healthcare applications. The aim of this study is to build a standalone, dual-nozzle extruder using biological 3D printer. Single-nozzle extruders have improved over the years but are still unable to meet certain requirements, as they tend to be less capable of multi-material or multi-bioink work. Many dual nozzle extruders are fixed at the nozzles, which limits its application. To circumvent these physical limitations that are imposed on extruder nozzles, dual-nozzle extruders are necessary to be employed. The research into the current fixed dual-nozzle system which resulted in many suggestions to improve is led to the proposed new design. The X-axis moves each of the nozzles separately and when they are synchronized in the Y-direction, those independent movements grant for more advanced playing with new levels along the Z-axis. Although full X and Y plane mobility was initially considered, this proved problematic because of interference issues. To reduce noise and still maintain fundamental operations, the solution was to make all processing-one dimensional. Critical components, such as filament manipulation mechanisms, the printer frame itself, motion control systems and nozzle materials were carefully designed to enable bioinks to be extruded accurately and with compatibility for biological material. The independent dual-nozzle design also confers powerful advantages, enabling printing with multiple bioinks simultaneously and supporting the addition of removable support materials to build complex, multi-material tissue structures. More research is necessary to make it possible to improve synchronization, design optimization, and operational reliability of the extrusion process. The future continues to flourish with the research on more kinds of bioinks and materials used for biological 3D printing, which only means more opportunities ahead both from tissue engineering and regenerative medicine.

In the end, the innovation of an independent dual-nozzle extruder that overcomes limitations fix-nozzle systems in bioprinting is a notable application. It will make it easy for medical researchers to develop better biological structures.

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