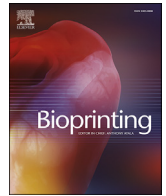




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Review article

Bioprinting for combating infectious diseases

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ABSTRACT

Infectious diseases have the ability to impact health on a global scale, as is being demonstrated by the current coronavirus disease 2019 (COVID-19) pandemic. The strenuous circumstances related to this global health crisis have been highlighting the challenges faced by the biomedical field in combating infectious diseases. Notably, printing technologies have advanced rapidly over the last decades, allowing for the incorporation of living cells in the printing process (or bioprinting) to create constructs that are able to serve as *in vitro* tissue or virus-disease models in combating infectious diseases. This paper describes applications of bioprinting in addressing the challenges faced in combating infectious diseases, with a specific focus on *in vitro* modelling and on development of therapeutic agents and vaccines. Integration of these technologies may allow for a more efficient and effective response to current and future pandemics.

1. Introduction

The World Health Organization (WHO) declared a pandemic March 11, 2020 due to novel coronavirus disease 2019 (COVID-19) [1]. COVID-19 is an infectious respiratory disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [1–3]. As of September 15th, 2020, there are over 29 million confirmed cases of COVID-19 in 188 countries [4]. Although the majority of individuals diagnosed with COVID-19 have mild/moderate symptoms including fever, dry cough, fatigue and shortness of breath, severe cases of the disease can lead to acute respiratory distress syndrome (ARDS) and damage other organs such as the heart and kidneys [1,3,5–7]. These more severe symptoms, and the inability to effectively treat them, have led to over 930 thousand deaths globally [4]. Although therapeutic screening and vaccine development has been accelerated, no specific antiviral therapeutics are currently validated for treatment of COVID-19, and no vaccine is currently available [2,3,8,9]. Remarkable progress has been made on identifying and sequencing the virus, and development of diagnostic tests; however, significant research and innovation is still required to combat the global crisis [2,3,8,10,11]. Specifically, better understanding of host-virus interactions at the molecular level, methods for screening therapeutics, and safe clinical translation methods are urgently needed to increase the efficiency of the global pandemic response [2].

Three-dimensional (3D) printing technologies provide a flexible

methodology for fabrication of precise and consistent structures through layer-by-layer deposition, binding or polymerization of material [12–14]. Various 3D printing techniques have been developed including stereolithographic, ink-jet, laser-assisted, and extrusion based printing systems; all of which follow similar procedures, i.e., the use of computer aided design (CAD) software to develop a printable design, conversion of the design into layered slices, processing of raw materials to facilitate printing, and layer-by-layer fabrication of the desired construct [13]. Notably, recent advancements in 3D printing technologies have allowed for bioprinting where the biomaterial(s) with appropriately incorporated biologically viable cells or biomolecules are used for printing to create 3D constructs [15–17]. Bioprinting technologies are currently applied in medical and pharmaceutical fields spanning from high throughput drug screening to organ transplantation and repair [2,18]. Applications of both printed and bioprinted constructs related to infectious diseases are mainly related to *in vitro* modelling, and drug and vaccine applications, including fabrication of compartmentalized tablets, nano/micro carriers and scaffold-based vaccines [2]. This paper provides a brief review of commonly used bioprinting technologies and their applications in combatting infectious diseases, using COVID-19 to highlight where further research and innovation is vital.

2. Printing and bioprinting technologies

Since the early 21st century, 3D printing techniques have been

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incorporated into medical applications, ranging from surgical guides to orthopedic implants, and tissue engineering research due to the ability to fabricate 3D structures with reproducible architecture [15,16,19]. Notably, 3D printing technologies address flaws in conventional tissue engineering fabrication methods by avoiding the use of organic solvents and production of unconnected pore systems, both of which reduce the ability to incorporate biological materials and limit vascularization upon implantation [15,16]. Biocompatible materials such as polymers, hydrogels, and composite materials can be printed to form biocompatible constructs that mimic the complex architecture and mechanical properties of natural tissues [15,16]. Bioprinting is a subset of 3D printing where the biologically viable cells and/or biomolecules are incorporated in printing constructs [15–17].

In order to fabricate a biocompatible scaffold, knowledge of the architecture of the tissue being targeted is obtained through medical imaging such as computed tomography (CT) or magnetic resonance imaging (MRI) [16,17,19,20]. Then, information obtained from imaging is used to form a CAD that mimics the external and internal geometry and architecture of the tissue, biocompatible materials are selected to mimic the mechanical and biological functions of the tissue being repaired or influenced, and 3D printing technologies are used to print constructs or scaffolds that are subsequently seeded with living cells, or in the case of bioprinting, cells are directly incorporated into the construct through the biomaterial. This direct incorporation aids in controlling the spatial distribution of cells in the material, while seeding constructs limits cell interactivity to the surface of the scaffolds [15,16]. Various 3D printing technologies have been developed with stereolithography, inkjet, laser-assisted and extrusion printing technologies commonly used in biomedical applications (Fig. 1) [15,16,19–21].

2.1. Stereolithography

Stereolithography (SLA) is a vat polymerization technique that uses UV light to cure photosensitive polymer resins with high resolution (1 μm lateral resolution) [14,15]. SLA systems consist of a reservoir filled with photosensitive resin, a laser, and a printing stage [15,19]. The UV laser operates in the x-y axis directions, and photopolymerizes the top layer of the resin in the reservoir causing solidification [15,19]. In bottom-up

printing, the printing stage is then lowered in the z-axis direction to allow for the next layer of printing [19,22]. Top down printing approaches are also possible by having the UV light directed upwards from under the vat. This approach allows for a smoother surface, removes the need for a coating blade and reduces the volume of material required [22]. The use of a support material may be required for both bottom-up and top-down printing [23]. Bioprinting can be accomplished using SLA by filling the reservoir with cells and photocurable hydrogel, while using a laser with a specific wavelength to avoid damaging the cellular materials [19]. SLA and other vat-polymerization techniques are limited in material choices, as the solution used must be photo-reactive and photocurable, and the process required to ensure biocompatibility may cause loss of drug activity [14]. Exposure to excessive external light sources may also reduce printing accuracy [23].

2.2. Inkjet printing

Inkjet printing is a material jetting technology based on the same concept of commercial printers and was first able to support bioprinting in 2006 [14,16,19,20]. Droplets of the biomaterial solution are propelled onto a printing stage by thermal, acoustic, electromagnetic or piezoelectric mechanisms [16,19,20]. This drop-on-demand technique can form droplets with diameters as small as 20 μm , and provides fast and precise deposition [16,19,20]. Thermal deposition techniques are most commonly used due to the provision of high cell viability in bioinks. Local temperature is elevated up to 300 $^{\circ}\text{C}$ for several microseconds causing the formation of a bubble which forces the ink out of the nozzle [20]. Although elevated temperatures are used, the speed of dispensing maintains high cellular viability [20]. Inkjet printing is flexible and inexpensive; however, there are material limitations, as well as problems with clogging, and slow printing speeds [20].

2.3. Laser-assisted printing

Laser-assisted printing, also known as laser-induced forward transfer (LIFT), removes the need for needles, reducing issues such as clogging [16,19]. Laser-assisted printing systems consist of a laser source, a transparent substrate, a material to be printed, an energy absorbing layer,

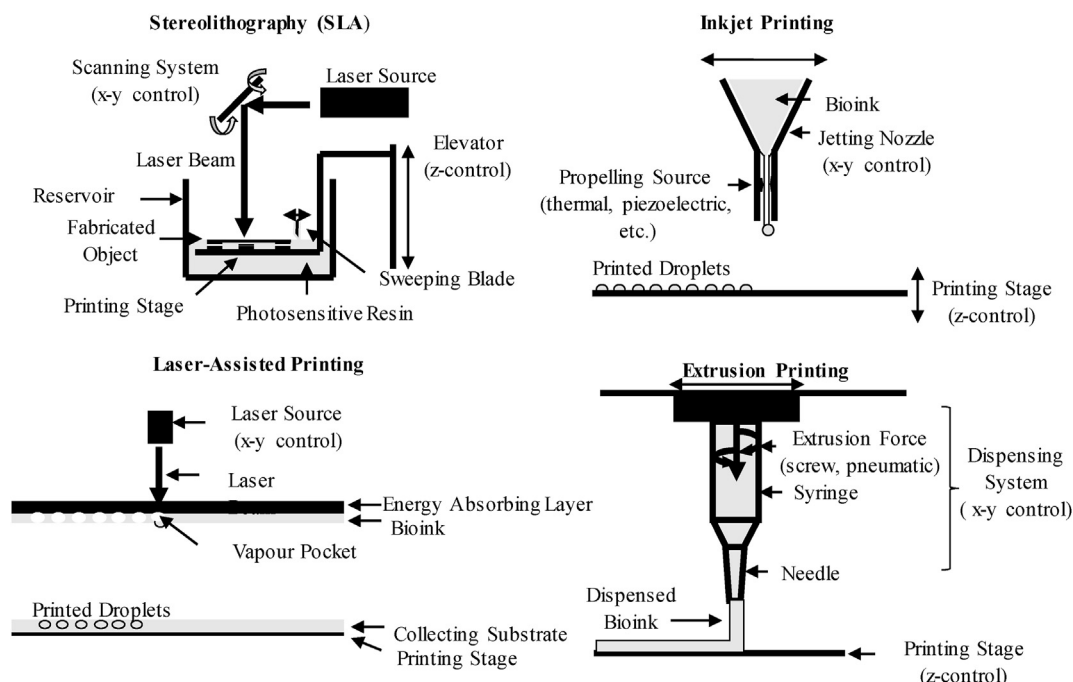


Fig. 1. Schematics of 3D printing technologies.

and a collecting substrate [16,19]. Laser pulses are focused onto the biomaterial and energy absorbing substrate generating high pressures, which propel droplets of the biomaterial onto the collecting substrate [16,19]. 2-photon polymerization is a related laser printing technology that allows for resolution in the nanometer range [14]. Laser-assisted printing is less common than other bioprinting techniques; however, it has advantages such as high resolution and high cell viability [16,19,20]. Unfortunately, the trade-off for the high resolution is reduced printing speed and limited material selection [20].

2.4. Extrusion printing

Extrusion printing, which includes fused deposition modelling (FDM), pneumatic and mechanical extrusion technologies, is the most common, and cost-effective, of the 3D printing technologies [14,19]. A typical system consists of a printing head, a printing stage and a control system responsible for controlling printing speed, temperature and location [16]. Axis control varies depending on the system, with combinations of printing head and stage movement allowing for movement in three directions. Continuous strands of biomaterials are extruded through a syringe and needle by gravitational and/or mechanical force, such as those imposed by a screw or pressurized air [16,24]. The resolution obtained through extrusion printing varies depending on the inner diameter of needle used and is limited by the mechanical force required [16]. When bioprinting, the incorporated cells undergo pressure and shear forces, which may cause cell membranes to lose their integrity if the process-induced forces exceed the cell membranes' threshold [16,24,25]. Therefore, strand resolution is normally limited to 100 μm to balance the force required for printing, needle strength and forces experienced by incorporated cellular material [16,20]. Extrusion printing can be used to deposit physiologically relevant cell densities and has a fast deposition speed, making it a viable technique for large scaffolds [16]. Unfortunately, extrusion printing has poor resolution when compared to other techniques, and experiences clogging problems [16,26]. The temperature required for FDM may also reduce the ability to print biologically viable materials; however, pneumatic and mechanical extrusion systems do not tend to require elevated temperatures, and are considered highly compatible with bioprinting applications [14]. The structural integrity of constructs fabricated through extrusion printing is also heavily dependent on the printability of the materials used [16,19,26].

Recent technological advancements have incorporated multi-head and core-shell nozzle systems, allowing for the printing of multiple biomaterials at once [15]. Multi-head systems allow for the loading of multiple syringes with different materials. This provides the advantage of being able to combine a structural material with a more bioactive material in alternating layers; however, this technique is still limited as only one material can be printed at once, making the fabrication of multi-material scaffolds relatively slow [15]. Core-shell systems utilize coaxial nozzles to incorporate one material within another or to fabricate hollow strands [15]. This allows for further tailoring of mechanical and biological properties of printed or bioprinted constructs [15].

3. Bioprinting in combating infectious diseases

Bioprinted constructs have been used widely in the repair of tissues and recently in combating infectious diseases. With the recent viral outbreaks of SARS-CoV-2, severe acute respiratory syndrome coronavirus (SARS), Ebola, middle eastern respiratory syndrome coronavirus (MERS) and Zika, there has been significant interest in applying 3D printing technologies to combat infectious diseases [2,16,27–30]. Infectious diseases are caused by pathogenic microorganisms such as bacteria, viruses, parasites or fungi [31]. Although the most recent epidemics have been viral with mainly respiratory implications (SARS-CoV-2, SARS, MERS), bacteria have also been responsible for disease outbreaks such as the Haitian cholera outbreak and *Escherichia coli* outbreak in Germany, which impact other physiological systems such as the gastrointestinal

tract [32]. Antibiotic resistant bacterial strains pose a serious threat due to the lack of effective therapeutic modalities [32]. Infectious diseases have the ability to impact all physiological systems and as such, the model and treatment required for a specific disease is often unique to the specific disease. Pathogens can be transmitted by various means including contact with animals or insects carrying the pathogen, ingestion of contaminated food and/or water, contact with contaminated surfaces or bodily fluids, or inhalation of airborne pathogens or respiratory droplets exhaled by an infected individual [32].

Proactive approaches related to coping with infectious diseases have highlighted the requirement for research and understanding of pathogens and pathogen-host interactions, more efficient and targeted development of therapeutics, and the production of effective vaccines [32]. Due to this, research and innovations in bioprinting applications such as *in vitro* modelling of various systems, and therapeutic and vaccine development and delivery have the potential to significantly impact the management of current and future pandemics [2]. The fabrication of bioprinted constructs for such applications requires the consideration of many factors (Fig. 2), mainly including the selection of appropriate cell type(s) and biomaterial(s), structure design, selection of a fabrication method that allows for the creation of the structure as designed, and evaluation of the structure to ensure it provides clinically relevant results. More factors must also be considered for the fabrication of therapeutics, such as dosage, release kinetics, and bioavailability, which can also be affected by the selected biomaterial, active medicinal ingredients, and structure design. The importance of these factors in applying 3D bioprinting to combating infectious diseases is discussed further below.

3.1. *In vitro* models

In vitro modelling is commonly implemented before studies proceed to *in vivo* testing in order to investigate cellular interactions in applications such as safety pharmacology, drug screening, and modelling of disease pathology and disease progression [20,33–37]. Conventional 2D cell culture techniques involve the adherence of cell monolayers to a flat surface such as a Petri dish [38]. Techniques such as sandwich cultures (cells are placed between two layers of extracellular material (ECM)), micro-patterning (2D surface topography is patterned in a controlled manner), and varying substrate stiffness, have been developed to improve 2D cell culture results [38]. However, 2D cell cultures lack relevance to certain conditions and diseases due to their inability to recapitulate the microenvironments of natural tissues [33,37]. 2D cellular environments are incapable of demonstrating physiological cell-matrix and cell-cell interactions, and may cause the display of different phenotypes and genomic profiles than what naturally occurs in physiological environments [34,37].

Animal models including mouse models, ferret models, and non-human primate models are commonly used in preclinical trials to assess viral and host factors that contribute to disease pathology and transmission *in vivo* [39]. Factors considered in selecting which animal model to use include susceptibility to viral infection, ability to support viral replication, and clinical manifestations [40]. Unfortunately, animal models are limited in clinical translatability due to differing cellular microenvironments, with approximately 80% of therapeutics assessed as effective in animals failing in human clinical studies [37,41,42]. In viral studies, the species used for animal testing is often not a natural host for the virus being studied, requiring modification of the virus or genetic modification of the animal in order to allow for susceptibility to infection [37,41]. Animal models also suffer from uncontrolled variables and ethical concerns, motivating the development of more effective and humane alternatives [33].

3D *in vitro* models allow for the consistent production of an architecture and cellular spatial arrangement that more closely mimics physiological conditions in a way that can be standardized and scaled as required [33]. Use of multiple cell types, physiologically relevant architecture, and advanced biomaterials has allowed for significant

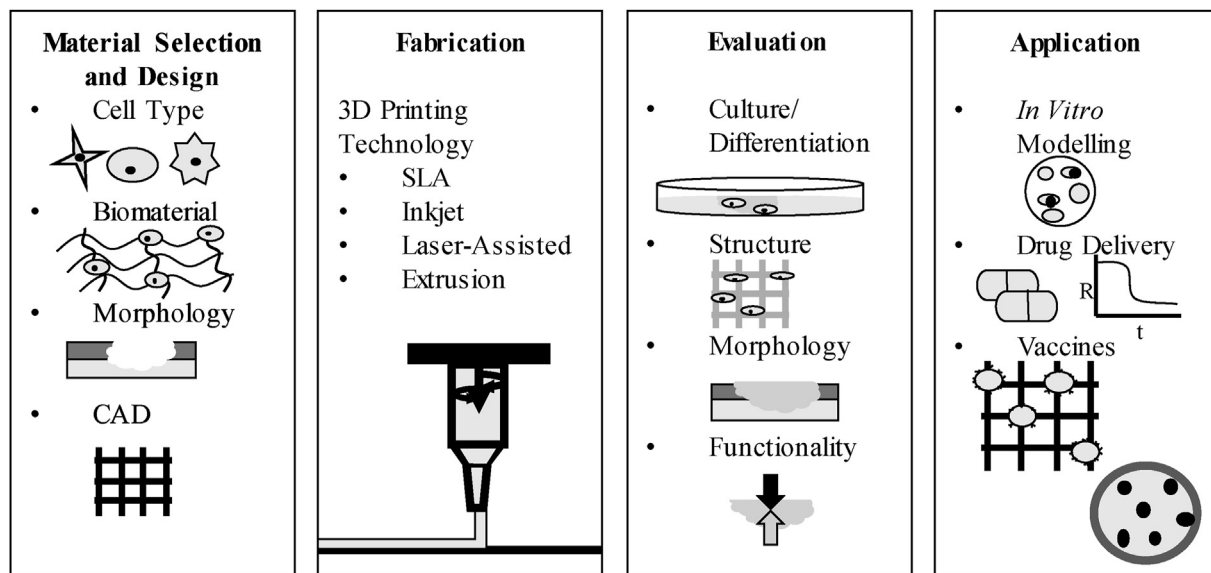


Fig. 2. Bioprinting pathway for combating infectious diseases.

improvement in biofabrication of constructs for *in vitro* testing [34]. As cell behavior is affected by substrate topography, substrate stiffness, mechanical forces and density, the control of these features provided by 3D bioprinting allows for improved accuracy of *in vitro* studies [36]. 3D bioprinting has been effectively applied to the creation of a wide range of tissues, including hepatic, bone, vascular, myocardial and respiratory tissues (Table 1) [37,43–51].

As infectious diseases often require specific cellular architectural features and polarized orientations with receptors in order to attach and enter the host cells that are often not present in typical 2D cultures, 3D cultures have demonstrated improved ability to study host-pathogen interactions [52–55]. 3D bioprinting has been successfully applied to modelling various conditions and infectious diseases such as influenza A virus (IAV), adeno-associated virus (AAV) and human noroviruses [41, 56–60]. The use of 3D extrusion bioprinting has been demonstrated to be a promising approach for developing lung tissue models through use of chitosan-collagen scaffolds cultured with primary human small airway epithelial cells (HSAEpCs) [59]. The developed 3D model demonstrated increased cell viability and morphologically resembled the lower airway

epithelium. Infection of this lung model with H1N1 and H3N2 influenza strains caused changes in marker protein expression and the release of proinflammatory cytokines, as would be expected in physiological conditions. Extrusion bioprinting has also been used to form a liver tissue model for infection studies with AAV [60]. The model demonstrated viral replication and is being considered for use in development of antiviral compounds. Recent works have focused on designing a 3D tissue model for SARS-CoV-2 [55].

While many disease models require different cellular components and morphology, many of the considerations required for design of 3D bioprinted models can be generalized. Fig. 3 demonstrates the considerations and iterative pathway required for *in vitro* disease modelling.

In the design stage of disease modelling, methods for recapitulating *in vivo* conditions of the specific tissue are determined [56]. CAD software can be employed to design relevant microarchitecture while biomaterials and cell types are selected to form a bioink suitable for the 3D printing technology selected [56]. Fabrication via bioprinting can then be used to create the designed structure for culture [56]. Once culture is complete, the bioprinted construct can be challenged with a virus and analyzed

Table 1
3D printed constructs of various tissues.

Tissue	Biomaterials	Cells	Printing Technology	References
Respiratory	extracellular matrix (ECM) Matrigel	EA.hy926 endothelial cells A549 epithelial cells	extrusion printing	[43]
Hepatic	gelatin methacrylate (GelMA) glycidol methacrylate-hyaluronic acid (GMHA)	hiPSC-HPCs	digital-light processing (DLP)	[44]
Bone	acrylated poly (ethylene glycol) (PEG)	human mesenchymal stem cells (hMSCs)	ink-jet	[45]
Bone	polylactide (PLA) GelMA	hMSCs	FDM SLA	[46]
Bone	nanohydroxyapatite (nHA)	collagen	multipotent mouse bone marrow stromal cells (D1 cells)	laser-assisted printing [47]
Ocular	recombinant human laminin human sourced collagen 1	human embryonic stem cell derived limbal epithelial stem cells (hESC-LESC) human adipose tissue derived stem cells (hASCs)	laser-assisted printing	[48]
Myocardial	poly-ethylene glycol-diacrylate (PAG-DA) Alginate	porcine aortic valve interstitial cells (PAVIC)	extrusion printing	[49]
Myocardial	methacrylated collagen (MeCol) carbon nanotubes (CNTs) Alginate	human coronary artery endothelial cells (HCAECs)	ink-jet	[50]
Vascular	Gelatin Fibrinogen	human umbilical vein endothelial cells (HUVECs) human neonatal dermal fibroblasts (HNDFs) hMSCs	extrusion printing	[51]

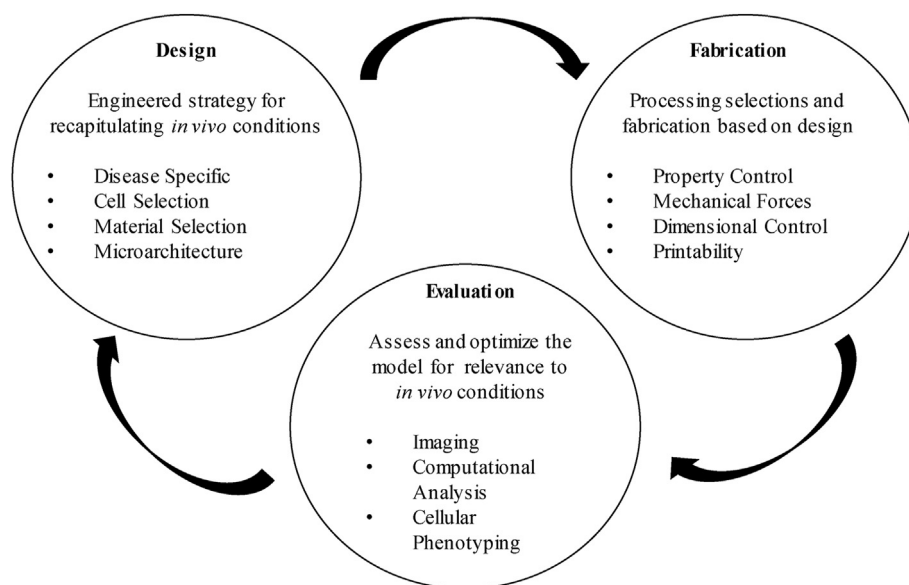


Fig. 3. Relationship between model designs, fabrication techniques and assessment for disease models.

using assessment technologies.

3D models of infectious disease have demonstrated increased physiological relevance when compared to 2D *in vitro* models, by producing infection patterns similar to what is observed in biological conditions and mimicking basic immune responses as shown by the IAV infected respiratory model produced by Berg et al. [41]. This allows for better understanding of disease pathology and may lead to new findings related to treating and protecting against infectious diseases. However, current use of 3D *in vitro* models is limited by imaging and assessment technologies which are designed for imaging and screening 2D cultures and integration into pharmaceutical systems is limited [20,21,34,56]. The lack of standardization of these models also currently limits their broad integration and implementation.

Due to the ability of 3D models to more closely represent physiological conditions, it has been proposed that these models could bridge the gap between cell culture and *in vivo* studies, leading to more successful clinical translation of therapeutics [33]. High-throughput screening assays are commonly used in the pharmaceutical industry to assess the toxicology of drug candidates [34]. Recent advances in additive manufacturing technologies allow for the automated bioprinting of 3D constructs in assay format, providing constructs for testing that exhibit responses more similar to natural physiological responses compared to 2D assays (Fig. 4) [34,61].

In order to apply 3D bioprinting to high-throughput screening, cells of interest are selected and loaded into a bioink [34,61]. The bioink can then be printed into a multi-well plate containing a matrix material, or printed as a structurally stable construct in order to form a 3D cellular model [34,61]. Drug compounds can then be added and their effect can be assessed in a more physiologically relevant format [34,61]. However,

technologies used to screen 3D constructs used in assay format are not currently available or integrated into pharmaceutical screening systems [34].

It is seen from the above discussion that for combatting infectious diseases, bioprinting has the ability to efficiently and economically produce *in vitro* models for disease modelling, drug optimization, and drug screening, thus allowing for more efficient testing of drug candidates particularly in the circumstance that a new disease arises [33]. Many companies such as Organovo, Aspect Biosystems and Nano3D Biosciences have already started working towards providing commercially 3D printed tissue for drug screening with the goals of reducing costs, shortening timelines and reducing the need for animal research in drug development [62].

3.2. Therapeutic agents

The development of therapeutic agents for COVID-19 is currently ongoing with repurposing and development of antivirals and immunosuppressants considered some of the main requirements for successfully resolving the pandemic [63]. These therapeutic agents are used for the treatment of individuals already infected by the disease in order to manage symptoms and aid the body's immune system in killing the virus [63]. While high-throughput screening of therapeutics through use of 3D bioprinted *in vitro* models is one application of 3D bioprinting for combating infectious diseases, these technologies can also be applied in the formation of drugs themselves. In 2015, the US Food and Drug Administration (FDA) approved the first 3D printed pharmaceutical tablet, sparking significant interest in the application of 3D printing technologies for drug delivery [12–14,64]. The ability of bioprinting

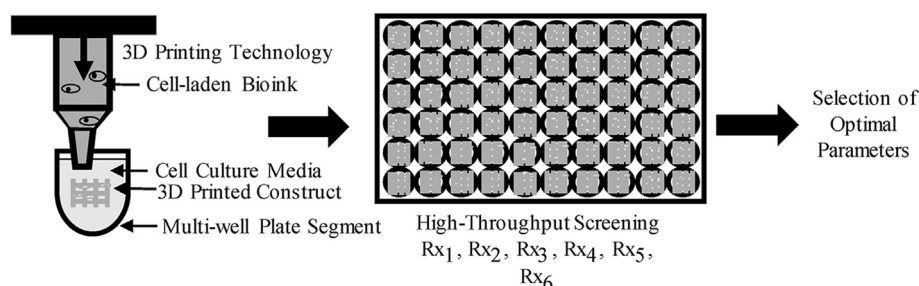


Fig. 4. Schematic of application of 3D bioprinting in high-throughput screening assays.

technologies to provide control over spatial arrangement, geometry, and density of 3D printed constructs provides the ability to tailor features such as drug release kinetics, targeting, and delivery pathway [12,13,64–66]. Utilizing 3D printing technology for pharmaceutical drug delivery systems has the ability to replace large scale manufacturing with on-site, on-demand production of personalized medications, allowing for reduction of cost and material waste [13,14,64,66]. In time and resource constrained settings such as those of a pandemic, these effects may have a significant benefit [13].

By tailoring a drug's release kinetics, the required frequency of administration can be reduced [2,13]. In the case of infectious diseases, reducing the required frequency of administration minimizes the risk to healthcare workers responsible for administering the therapeutic [2]. Bioprinting technologies have been used to develop therapeutics with extended, immediate, or multi-rate release kinetics [14]. Factors such as biomaterial composition, geometry, and infill density all affect release kinetics, and the ability of bioprinting to efficiently control these factors allows for flexible and rapid tailoring [14].

Immediate release profiles are required for quick onset medications such as analgesics [14]. To maximize dissolution rate, 3D printing has been used to develop high surface area to volume geometries [67]. The development of 3D printed tablets with channels for accelerated release has been explored using hydrochlorothiazide (commonly used to treat high-blood pressure) as the active ingredient [68]. Hydrochlorothiazide was chosen as the model drug as its enhanced dissolution is required for the drug to be effective when taken orally. The incorporation of multiple short channels into the 3D extrusion printed tablets were found to be optimal in enhancing immediate release of the active ingredient.

In other cases, zero-order kinetic profiles are preferred as they allow for sustained release, improving tolerability, and reducing the incidence of adverse effects [14]. In order to achieve a sustained release profile, complex geometries, and scaffolds with controlled pores for drug release have been developed [69–72]. Microspheres or nanoparticles with tailored release kinetics have also been developed, which can be loaded into 3D bioprinted constructs for controlled drug release [71,72]. Other approaches for achieving zero-order release kinetics through 3D printing include using a drug-release barrier on all but one side, and biomaterial selection based on degradation rate [73,74]. The high resolution spatial arrangement provided by 3D printing technologies has also allowed for the development of therapeutics with multiple release profiles within a single dose by use of compartmentalization and radial gradients (Fig. 5) [14,65,69,75,76].

In designing compartmentalized tablets or capsules with controlled release kinetics, the active pharmaceutical ingredients (APIs) are selected and separated with personalized dosages [76]. The thickness of the

capsule at various locations, and the biomaterial used in the casing is selected based on the degradation rate required to ensure release occurs at the proper time interval [76]. Once the CAD has been completed, 3D printing technologies are utilized to fabricate the capsule [76]. This process been investigated for use of treating infectious diseases such as tuberculosis (TB), which is caused by the bacterium *Mycobacterium tuberculosis* [77]. Short-term, combination drug therapy is the current recommended procedure for treatment of TB; however, the interactions of the drugs used in combination therapy must be closely monitored. The use of 3D printing of compartmentalized tablets for controlled release has been demonstrated to reduce systemic drug exposure, dosing frequency and anti-TB drug interactions through specific design of orally administered tablets [77].

The bioavailability of an administered drug is very important in maintaining therapeutic efficacy [14]. Oral dosages are the most commonly administered due to convenience; however, oral drug delivery leads to challenges related to bioavailability as absorption of drugs varies depending on interaction with the gastrointestinal tract [14]. Polyethylene glycol (PEG) coatings, solid dispersions, and the use of FDM printing technology to manipulate the infill of an extrusion printed tablet, have been shown to optimizing floatability and bioavailability of orally administered therapeutics, but 3D printing technologies have also been used to address these challenges through providing alternate delivery routes such as vaginal, transdermal and implantable pathways [14,65,69,73,78,79].

Transdermal patches release therapeutic agents through the skin, bypassing the enterogastric system [14]. 3D printing has been applied to the manufacturing of transdermal patches, as it is capable of producing individually contoured patches for greater patient comfort, greater dose control, and allows for the production of microneedles for local permeation [14]. Inkjet printing has been used to develop biodegradable microneedles with varied geometries and therapeutic agents while extrusion printing has been used to develop a flexible drug delivery patch for combatting pancreatic cancer [80,81]. The use of inkjet printing in forming an antiviral and anticancer film, for administration through the cervix for treatment of human papilloma virus (HPV)- related cervical cancer, has been proven to be a successful approach [82]. It was found that release times could be prolonged and dose could be accurately controlled, indicating that 3D bioprinting could be used as an approach for combating HPV-related condition.

Bioprinting technology has been used to form and treat various personalized biodegradable drug-eluting implants such as stents, bone scaffolds and catheters [14,64,83]. The use of drug-eluting implants has been shown to be capable of sustaining drug release over extended periods, reducing biofilm formation and increasing the biological compatibility [14,64,83]. Bioresorbable implants have demonstrated success in alleviating long-term risk of permanent devices and allowing for the regeneration of natural tissues [14,64,83]. The use of 3D bioprinting to fabricate an antibiotic eluting liner for use in treating periprosthetic joint infections has been proposed [84]. These infections, commonly caused by bacteria could be controlled through the incorporation of antibiotics into the joint liner, which could be 3D bioprinted according to the autonomy of the patient and incorporate built-in release channels. As 3D printing provides a wide range of possible administration pathways and methods for increasing bioavailability, it should be considered as a method for optimizing therapeutic efficacy.

As diverse patient populations have different therapeutic needs due to dissimilar ages, masses, and metabolisms, the ability of 3D printing to produce personalized doses could remove challenges caused by the current mass manufacture system used for pharmaceuticals [12–14,64,66]. Additive manufacturing technologies allow for greater individual customization of therapeutics such as the creation of polypills which combine all of the drugs required by an individual into one pill [13,85]. Overall, the use of 3D printing technologies in drug delivery has the ability to reduce treatment times by reducing the required frequency of administration by optimizing release rate, bioavailability, and providing

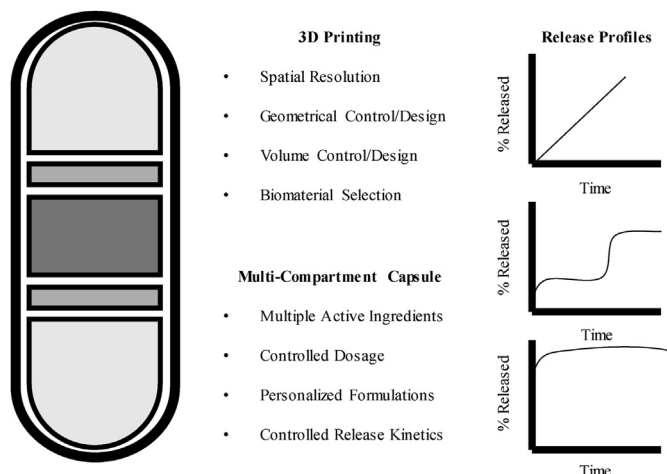


Fig. 5. Schematic of application of 3D printing in designing compartmentalized capsules for tailored release kinetics.

personalized dosage options [2,66]. These benefits could free up hospital space, and help ensure the safety of healthcare workers when combatting future pandemics and infectious diseases [2].

Currently, the use of 3D printing for pharmaceutical manufacturing is focused on proof-of-concept research, with technologies suitable to replacing the current mass manufacture system lacking [14]. This is in part due to regulatory requirements related to materials and serializability of the printing systems used; however, with the FDA having released its initial considerations on the use of 3D printing technologies in late 2017, researchers are now focusing on implementing these technologies as the scalability, cost effectiveness, and personalized nature of 3D printed drug delivery makes it likely that these technologies will be implemented into the pharmaceutical industry in the coming decade, and the benefits of controlled release kinetics, increased bioavailability, and possibilities of utilizing alternate delivery routes makes 3D printing technologies a powerful tool in combatting various diseases [14,86].

3.3. Vaccination strategies

One of humanity’s main methods of combatting infectious diseases is the development of vaccines [2,87,88]. Vaccines function by introducing antigenic particles to the immune system, triggering a primary immune response which allows the body to be better prepared to initiate a secondary immune response if it encounters the same antigen again [89]. In order for a vaccine to be effective, it is important that the immune response triggered by a vaccine is strong enough to induct long-term immune memory [89]. SARS-CoV-2 can be classified as an antigenically variable pathogen (AVP) making it difficult to vaccinate against due to its genomic and antigenic instability [90]. However, a vaccine remains the most likely method for successfully controlling the COVID-19 pandemic; therefore, the production of a vaccine for COVID-19 has become a major area of research globally, with a variety of vaccines and vaccine strategies being investigated [9]. At this time initial results from Phase 1 clinical trials are beginning to be released demonstrating tentatively positive results; however, there is currently no approved vaccine [9,91,92]. Due to the different objectives between therapeutic drugs for treatment, and vaccines for pre-emptive protection, different factors must be considered in vaccine design. The application of 3D printing technologies in the creation of micro/nano particles, micro-needles and scaffolds for immunomodulation is a rapidly growing field driven by the need for improved vaccination technologies (Fig. 6) [93].

Targeted delivery of antigens via polymer micro/nano particles is commonly used in vaccination strategies due to high biosafety, loading capacity and controlled release profiles [17,94]. Recent advancements in

bioprinting technologies have allowed for printing resolutions in the nanometer scale allowing for the use of 3D printing in fabrication of consistent micro and nanoparticles [95]. Using multiphoton lithography, an advanced additive manufacturing technology, antigen nanoparticles have been developed for vaccine delivery, while soft lithography has been used as a fabrication method for microdisk vaccines [17,96]. The use of these advanced manufacturing techniques provides greater consistency in the dimensions and geometries of the particles formed, which could allow for controlled and repeatable effects leading to more efficient clinical translation in the future [96].

As previously discussed, 3D bioprinting technologies have been applied in the fabrication of microneedles for drug delivery; however, microneedles are also being investigated as a vaccination strategy due to their ease of use, transdermal application and benefits such as extended release kinetics [97]. Antigenic cargo can be loaded into the microneedles to be painlessly pressed into the skin, removing the need for a medical professional for application [94]. Influenza vaccines using microneedles have recently been demonstrated to produce a similar level of immunity as compared to intramuscular immunizations [97]. An implantable chitosan-loaded microneedle system which was implanted by use of a dissolvable support, degraded over time allowing for sustained intradermal delivery of the influenza vaccine [97]. The microneedles caused sustained antigen exposure and immune stimulation evoking long-lasting protective immunity.

The use of implantable biomaterial scaffolds as part of a vaccination strategy has been investigated due to the ability for localized delivery and controlled release kinetics as well as the ability to recruit immune cells into the scaffold and activate them through provision of favorable microenvironments [88,98–101]. In order to fabricate a scaffold-based vaccine, a biomaterial scaffold is designed and printed with the immunological cargo encompassed in the selected biomaterial [94]. The biomaterial is selected for its ability to create a microenvironment that enhances immunomodulatory activity by acting as a vaccine adjuvant, strengthening the likelihood of the vaccine stimulating production of long-term immune memory, and allowing for the implant to act as a local immune system depot [94]. The strength of the immune response triggered by a foreign body, or an implanted scaffold-based vaccine, is related to the strength of the patients’ immune system, and the immunogenicity of the antigen expressed [89]. Scaffold-based vaccine immunogenicity is further related to the implants size, shape, and surface chemistry [102–107] (Fig. 7).

Larger diameter implants, with smooth surfaces, and controlled pore sizes have been shown to reduce immune cell response [102,105,106]. Surface modifications have also been shown to regulate immune cell infiltration of scaffold-based and biomaterial selection and release kinetics has been shown to influence vaccine efficacy [103,104]. As 3D printing technologies can be used with a wide range of biomaterials and provide the ability to control all of these variables, it is a likely candidate for the production of optimized scaffold-based vaccines. The application of biomaterial scaffold-based vaccines is especially pronounced in the field of cancer immunotherapy, as implantable scaffolds allow for

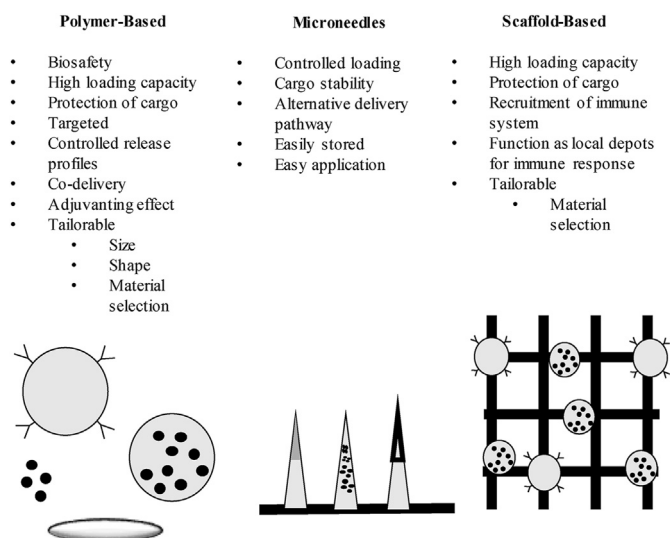


Fig. 6. Biomaterial and 3D printing-based strategies for vaccine development.

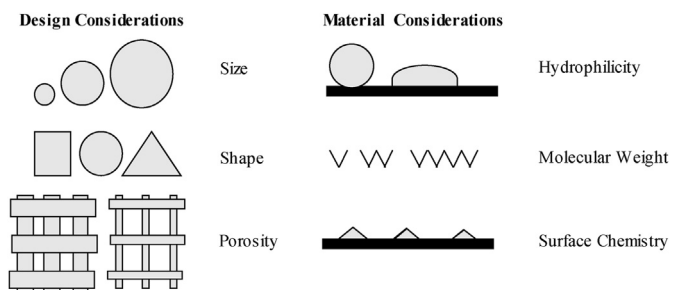


Fig. 7. Design and materials factors that influence immune response upon implantation.

patient-specific therapeutic localization and reduce toxic side effects as the therapeutic dose can be reduced without decreasing bioavailability [101]. The use of a 3D alginate scaffolds has been demonstrated to provide a high loading capacity and slow antigen release causing long-term activation of antigen-presenting cells [108]. Although this study was based on tumor suppression, the same concepts can be applied to *in vivo* modulation of immune cells for infectious diseases.

The ability of 3D printing to fabricate structures with a combination of different release kinetics has been investigated as part of vaccination strategies in order to remove the need for booster shots [100,109]. Copolymer rods with enclosed vaccine depots for use in immune priming, boosting and long-term maintenance, as well micromolded or 3D printed biocompatible and biodegradable polymeric formulations with multiple release intervals have been developed [100,109].

As 3D printing and biomaterial selection allows for precise control of many of the factors that influence immune response including size, shape, and surface chemistry, while also providing controlled and tailorable release kinetics and dosages, it is not surprising that research into 3D printing in various vaccination strategies is rapidly increasing [107]. 3D printing-based vaccination strategies provide a greater number of options for vaccine delivery and provide favorable microenvironments for recruitment of the immune system [94]. However, the use of biomaterial and 3D printed-based vaccine strategies creates new considerations and may complicate vaccine design, as the extent of the inflammatory immune response to implanted materials must be closely investigated and controlled [94]. As research in this field progresses, greater understanding of the immune response to biomaterials may lead to increased vaccine efficacy and personalization, while also removing the need for medical professionals in receiving the vaccine. The tailorability of constructs fabricated through 3D printing can aid in increasing the rate of development and efficacy of vaccines produced to combat infectious diseases, and the ability to develop modular vaccines may help reduce the time required to produce safe and effective vaccines in the future, reducing the overall impact of infectious diseases [2,99].

4. Conclusions and future research

Infectious diseases are capable of causing health care crises of global proportions as currently being demonstrated by the COVID-19 pandemic. Medical professionals and various other researchers across the globe have been working non-stop to find ways to diagnose, treat and protect against infection; however, difficulties in gaining understanding of disease pathology, developing and screening therapeutics, and developing effective drug delivery and vaccination strategies have all slowed the medical field's ability to combat this disease leading to ever-increasing mortality.

Rapid advancement in 3D bioprinting technologies has uniquely situated bioprinting technologies to be a main component in combatting current and future pandemics. The main benefits of 3D bioprinting include its ability to fabricate structures in a highly controlled and repeatable manner, allowing for rapid scaling of production. The application of 3D bioprinting technologies in fabrication 3D *in vitro* models provides physiological relevant models providing an opportunity to increase understanding of host-pathogen interactions, while also allowing for enhanced pre-clinical testing that increases the probability of therapeutics being successful in clinical studies. This may allow for more efficient response to emerging infectious diseases as host-pathogen interactions can be effectively studied, and therapeutics can be effectively assessed. The application of 3D bioprinting to drug development provides the ability to control release kinetics and optimize bioavailability of therapeutic agents through controlled design and material selection. Bioprinting technologies also allow for the personalization of doses and safer delivery of drug combinations. Improved drug delivery capabilities protect both healthcare workers and patients and dose/interaction frequency can be reduced and the drugs provided will be more effective. Vaccine development, including polymer-based, microneedles and

scaffold-based strategies may also be improved through the greater control of biomaterial adjuvants, encapsulation of antigens and increased immunogenicity which helps to induce long-term immune memory. Using these techniques, vaccine efficacy can be increased leading to greater societal protection against infectious diseases. Further research and advancement in additive manufacturing technologies will lead to a greater range of applicability to the medical field, while advancement of accessory technologies such as 3D-assay screening technologies will allow for smooth technological integration. As 3D printing and bioprinting is further integrated into healthcare and pharmaceutical applications, society's ability to respond effectively to novel diseases will increase helping to protect both healthcare workers and the general populace.

Some references have yet to be peer-reviewed and conclusions should be drawn from them with caution.

CRedit author statement

Amanda Zimmerling: Conceptualization, Investigation, Writing-Original Draft, Writing- Review and Editing, Visualization, Funding acquisition. **Xiongbiao Chen:** Conceptualization, Writing- Review and Editing, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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