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Review Article

Journal of Veterinary and Animal Sciences
ISSN (Print): 0971-0701, (Online): 2582-0605
https://doi.org/10.51966/jvas.2025.56.1.1-7

Advances in Infectious Disease Modeling: A perspective on 3D-Bioprinted Tissue Models to Study Host-Pathogen Interactions

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Citation: Soman, S.S., Kuchipudi, S.V. and Kumar, S. 2025. Advances in Infectious Disease Modeling: A perspective on 3D-Bioprinted Tissue Models to Study Host-Pathogen Interactions.
J. Vet. Anim. Sci. **56** (1):1-7

Received: 27.02.2025 Accepted: 14.03.2025 Published: 31.03.2025

Three-dimensional (*3D) bioprinting* is a revolutionary biomedical technology that allows researchers to create custom 3D tissue models to study human organ physiology and disease pathobiology. Bioprinting utilizes bioinks containing living cells, biomaterials, and essential growth factors to construct complex, 3D tissue-like structures with remarkable precision. Their application in infectious disease research is particularly significant, as they replicate organs such as lungs, liver, skin, and intestines, allowing scientists to analyze pathogen-host interactions at cellular and tissue levels closely. By employing 3D bioprinting, researchers have successfully developed tissue models to study viral and bacterial infections, offering insights into pathogen evolution, immune responses, and therapeutic interventions. These models play a critical role in drug discovery by providing a physiologically relevant platform for testing the efficacy and safety of antimicrobial and antiviral drugs. Additionally, bioprinted tissues can minimize reliance on animal testing and improve species-specific drug response predictions. 3D bioprinted models are poised to transform infectious disease research and therapeutic development. Here, we give a perspective on 3D bioprinting and its applications in infectious disease modeling.

Keywords: 3D bioprinting, Infectious Diseases, Disease modeling, Organoids, Bioengineering

Introduction

Disease modeling enables researchers to replicate human and animal diseases in laboratories, facilitating a deeper understanding of pathogenesis and treatment responses. Traditionally, disease models such as animal models, cell cultures, and computational simulations are used to study disease biology and treatment responses (Fig.1). Animal models provide physiological relevance data. Still, they often fail to mimic human-specific conditions. 2D cell cultures offer controlled environments but lack the 3D tissue complexity. Computational models aid predictions but require experimental validation, highlighting the need for advanced alternatives.

Three-dimensional (3D) bioprinted tissue models are innovative tools that accurately replicate human tissues, providing researchers with cutting-edge techniques to study organ physiology and disease mechanisms. Scientists can fabricate intricate, tissue-like structures with remarkable precision by utilizing bioinks composed of living cells, biomaterials, and growth factors. These models are particularly valuable for investigating infectious diseases, as they

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closely mimic specific organs such as the lungs, skin, or intestines, enabling researchers to observe pathogen-host interactions at a cellular level. For instance, bioprinted tissue models have effectively studied infections such as COVID-19 and influenza. Furthermore, bioprinted models play a pivotal role in drug discovery, offering an efficient platform for evaluating the efficacy and safety of antimicrobial and antiviral compounds. By reducing dependence on animal testing and improving the accuracy of human response predictions, 3D bioprinted models have the potential to reform infectious disease research and therapeutic development, paving the way for more effective treatments and a deeper understanding of disease pathobiology(Soman and Vijayavenkataraman, 2020). This paper provides a perspective on 3D bioprinting techniques, their applications in disease modeling, and their potential in advancing infectious disease research.

Traditional Disease Models Versus 3D Bioprinted Disease Models

Current *in vitro* and animal models often fail to replicate the complexity of host-pathogen interactions at the tissue and species levels. Most pathogens change their tissue tropism and species preferences as they evolve. The time lapse in understanding infectious disease biology at the cellular and species level hampers the development of effective therapies and vaccines in infectious disease emergencies. 3D bioprinting overcomes these limitations by enabling the creation of biomimetic tissue models that closely mimic host organs and tissues(Devalla and Passier, 2018). These models replicate tissue microenvironments, facilitate swifter host-pathogen interaction studies, and enable high-throughput drug screening *in vitro*. While 3D bioprinted disease models are still in the early stages of development, ongoing advancements in material science, bioink formulations, new printing technologies, and organ-on-a-chip integration are accelerating the application of 3D bioprinting for studying infectious diseases(Long *et al*., 2022)

3D Bioprinting: The Technique and its Components

3D bioprinting is a biofabrication technology that integrates—*(1) bioinks, (2) bioprinting techniques, and (3) bioprinters*—to fabricate functional tissue-like structures with high precision (Fig.2). Bioinks, the core material in bioprinting, consist of hydrogels, living cells, and supportive biomolecules that mimic the extracellular matrix (ECM) and provide scaffolds for cell growth and interaction. Bioinks can be derived from natural polymers or synthetic materials. Successful bioinks must meet critical criteria, including biocompatibility, printability, and structural stability. Bioprinters are machines that deposit bioinks in a defined fashion to construct 3D structures using extrusion-based, inkjet, and laser-assisted printing techniques. The choice of bioprinting technique influences the final construct's quality and resolution, allowing for a range of applications in regenerative medicine and disease modeling.

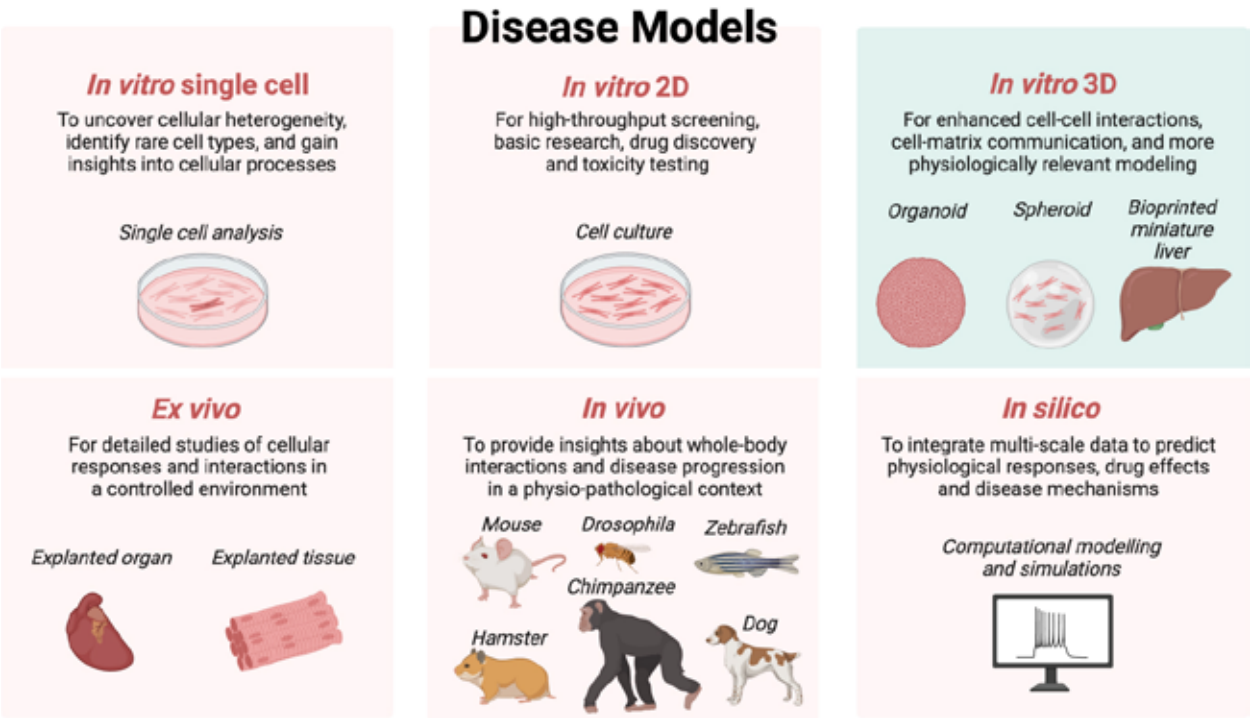

Fig.1. Various types of diesese models used in biomedical research. [Created in https://BioRender.com]

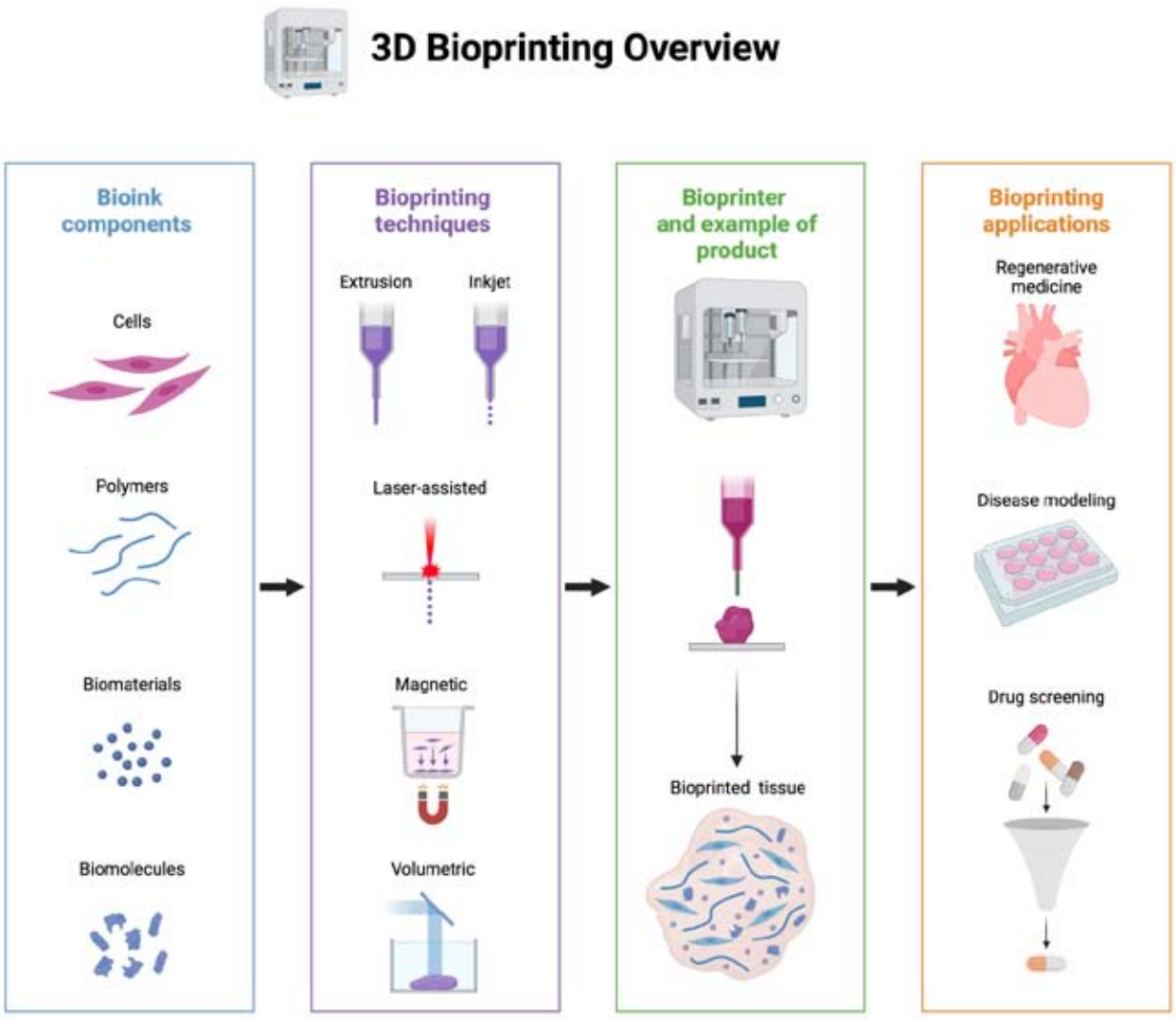

Fig.2. The essential components of bioprinting.[Created in https://BioRender.com]

Bioinks

Bioinks are the foundational materials in 3D bioprinting, made up of living cells, cell growth, and differentiation-promotingadditives and biomaterials to create tissue-like constructs. These inks typically consist of hydrogels that mimic the extracellular matrix, with natural materials such as collagen, gelatin, cellulose, and alginate offering excellent biocompatibility(Ouyang *et al*., 2020). Synthetic polymers like polyethylene glycol (PEG) and polycaprolactone (PCL) provide tunable mechanical properties. Bioinks often incorporate ECM proteins like matrigel, laminin, or fibronectin to enhance cell adhesion, proliferation, and differentiation(Soman *et al*., 2022). Crosslinking agents stabilize bioprinted structures through physical or chemical methods, such as ionic crosslinking for alginate or UV-activated reactions for synthetic polymer ECMs. Bioinks must exhibit optimal biocompatibility, mechanical fidelity, and degradability while allowing for efficient nutrient diffusion to support cell survival, tissue maturation, and tissue differentiation(Ho *et al*., 2022). Recent innovations have led to hybrid bioinks that combine natural and synthetic materials for better functionality, scalability, and stability. In addition, emerging "smart" bioinks respond dynamically to external stimuli such as temperature, pH, or light, enabling the creation of adaptive tissue models for advanced applications in organ transplantationmedicine(Kim and Cho, 2024).

Bioprinting Techniques

Bioprinting techniques, including extrusion-based, inkjet-based, laser-assisted, and volumetric bioprinting, are integral in creating 3D tissue models. Extrusion-based bioprinting is the most common, involving the layer-by-layer deposition of high-viscosity bioinks to create robust tissue structures like cartilage, tissue fillers, and vascular networks(Govindharaj *et al*., 2024). Inkjet bioprinting deposits fine droplets of bioink, offering high resolution and rapid deposition, making it ideal for delicate tissues like skin and neural networks, though it is limited to low-viscosity bioinks(Choudhury *et al*., 2018). Laser-

assisted bioprinting uses laser energy to precisely transfer bioinks, creating intricate, high-resolution tissue models beneficial for small-scale, delicate designs. Volumetric bioprinting, a newer approach, permits the creation of geometrically complex, centimeter-scale constructs at an unprecedented printing speed(Jing *et al*., 2023). A new method, sacrificial writing into functional tissue (SWIFT) using embedded three-dimensional bioprinting was reported to create high cellular density 3D tissue constructs(Skylar-Scott *et al*., 2019). Other methods, such as magnetic and acoustic bioprinting also prevail in the field.Each technique has distinct advantages, allowing for a wide range of applications in creating complex tissues for biomedical research(Fritschen *et al*., 2024).

Bioprinters

Bioprinters are essential components in 3D bioprinting, playing a key role in the precise deposition of bioink layers containing living cells and biomaterials. These printers employ various techniques, such as extrusion-based, inkjet, and laser-assisted methods. Extrusion-based bioprinters are widely used for handling highly viscous printing inks, making them suitable for creating robust tissue structures(Hashimi *et al*., 2022; Zhang *et al*., 2021). Inkjet bioprinters excel at providing high resolution for finer details, making them ideal for high-throughput applications where precision is critical. Laser-assisted bioprinters utilize laser energy to pattern bioinks with exceptional accuracy, allowing the creation of complex, intricate tissue structures. Volumetric bioprinting uses an optical-tomography-inspired approach that employs visible light projection, enabling the rapid, high-resolution fabrication of precise tissue architectures(Bernal *et al*., 2019). This method accelerates tissue creation and printing within a few minutes compared to the traditional layer-by-layer printing techniques. Commercially available bioprinters include the versatile RegenHu known for its extrusion-based bioprinting technology and co-axial bioprinting, the Organovo NovoGen platform, the CELLINK Bio X and INKREDIBLE printers, which specialize in handling various bioinks, and the Allevi 3D Bioprinter designed for precision cell printing. The versatility of these bioprinters makes them invaluable tools in 3D tissue engineering.

Infectious Disease Modeling in 3D Bioprinted Organoids

In infectious disease research, bioprinted models are useful for studying host-pathogen interactions, as demonstrated in COVID-19 lung models(Hwang *et al*., 2023; Lee *et al*., 2024),gut-anaerobic and aerobic pathogen interaction models(Cheng *et al*., 2023), and 3D bioprinted of *E. coli* MG1655 biofilms(Aliyazdi *et al*., 2023). These disease models give futuristic 3D microphysiological systems to enhance our understanding of disease mechanisms(Fig.3).

Researchers biofabricate lung tissue and infected it with influenza A virus. A bioink consisting of alginate, gelatin, matrigel and human alveolar A549 cells were used to bioprint lung tissues. The infection resulted in widespread distribution of the virus in the 3D model and a clustered infection pattern that is also observed in the natural lung but not in two-dimensional (2D) cell culture(Berg *et al*., 2018). The infection model also demonstrated viral replication and proinflammatory interferon release from the infected cells. In another study, human lung tissues were bioprinted using multiple lung cell types: primary human lung fibroblasts, monocytic THP-1 cells, and alveolar epithelial A549 cells. The cells were embedded in a hydrogel consisting of alginate, gelatin and collagen, and cultured for 35 days, and challenged with bacterial toxins LPS and ATP. The 3D lung cultures showed the release of the proinflammatory cytokines IL-1β and IL-8, confirming that the model can generate an immune response. The virus infection and inhibition assays using a seasonal influenza A virus strain in the same model, virus replication was reduced by the treatment with an antiviral agent in a dose-dependent manner. The bioprinted lung construct

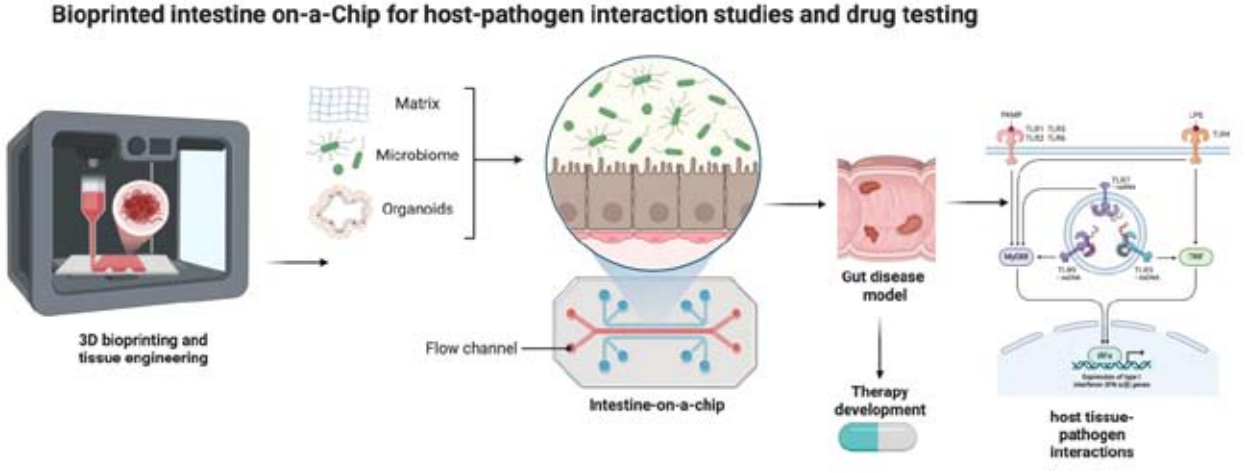

Fig.3. Illustration of a bioprinted intestine-on-a-chip model for gut microbiome interaction studies. Created in https://BioRender.com

provides an alveolar model to investigate pulmonary pathogenic biology and to support the development of new therapeutics for the influenza virus and other viruses(Berg *et al*., 2021; Shpichka *et al*., 2020).3D tissue scaffolds and 3D bioprinted tissue systems were used to study infectivity, replication kinetics, and host–viral interactions of viruses, such as influenza(Zhou *et al*., 2018), Zika(Cugola *et al*., 2016), and SARS-CoV-2(Zhou *et al*., 2020), adenovirus(Hiller *et al*.,2018), norovirus(Ettayebi *et al*., 2024), showing increased physiological relevance of 3D systems as compared to 2D models. Furthermore, 3D bioprinted tumor models provide insights into cancer progression and enable testing therapies within a context that better mimics the tumor microenvironment(Neufeld *et al*., 2022)(Neufeld *et al*. 2022)

In the investigation of host–pathogen interactions, model systems must effectively replicate not only the host organism but also the intricate dynamics between the host and the infecting microorganism. Pathogens often provoke a host response, such as inflammation, and intracellular pathogens such as Plasmodium, Trypanosoma, and Mycobacterium are good at manipulating the host immune response to favor their survival through the suppression and evasion of host inflammatory mechanisms(Behar *et al*., 2010; Dieng *et al*., 2020). Evolution has led to the development of highly specialized pathogens that engage with the host in specific ways, creating particular microenvironments or niches that facilitate their survival and replication for a prolonged period in host tissues(MacGregor *et al*., 2012).Host cell receptors are significant determinants of host susceptibility to zoonotic viruses. Animal species sharing host cell receptors that support the binding of multiple viruses can play a key role in virus spillover and the emergence of novel viruses and their variants(Kuchipudi *et al*., 2021). Making host-specific bioprinted organon-on-a-chip systems can accelerate the comparative viral infection studies and thus aid in understanding the evolution of zoonotic viruses in different species(de Melo *et al*., 2021). To accurately model these interactions *in vitro*, disease modeling systems must capture both the host's complex responses and the pathogen's strategies for modulating those responses(Aguilar *et al*., 2021). Disease modeling in 3D organoids developed using 3D bioprinting will be a game-changing strategy to understand the molecular, cellular, and tissue-level aspects of host-pathogen interactions(Chia *et al*., 2022).

Conclusions

3D bioprinting represents a promising tool for advancing the understanding of infectious diseases, mainly by replicating pathogen behavior in native cellular environments. This technology can uncover new insights into host-pathogen interactions, identify new therapeutic targets, and revolutionize disease modeling(Kolesky *et al*., 2018). *Wyss Institute for Biologically Inspired Engineering* at Harvard University, USA, *NYU Abu Dhabi*, UAE, and the *National University of Singapore*, Singapore, are the leading academic institutions with specialized laboratories for bioprinting innovations. Many commercial companies are also focused on bioprinting and clinical translation of 3D tissues and organs. 3D Systems, Frontier Bio Corporation, and Lung Bioengineering are fabricating transplant-grade lung tissues. Loreal and Poietis Biosystems are developing bioprinted skin. FluidFormmanufactures functional heart valves, regenerative ECM scaffolds, and contractile cardiac muscle patches. Organovo, San Diego, USA, is developing functional liver tissues for disease modeling and drug screening. Moreover, being a highly interdisciplinary field, 3D bioprinting fosters collaboration between engineers, biologists, and clinicians to translate biomedical innovations into impactful real-world applications.

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