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REVIEW ARTICLE

3D BIOPRINTING - RECENT ADVANCES IN TISSUE REGENERATION

Dhaivat Parikh, Mohit Shah*
Institute of Pharmacy, Nirma University, Ahmedabad, India.
mohit.shah@nirmauni.ac.in

ABSTRACT

The scarcity of organs for transplantation has been a significant problem for the healthcare sector for years. Despite technical advances, too many patients are still waiting for organ transplants compared to the number of qualified donors. Numerous lives are lost; as a result, every year worldwide. Successful organ transplantation is further restricted because of the expense of the procedure and the possibility of immunological rejection. Fortunately, 3D bioprinting technology has become a viable answer to this urgent need. 3D bioprinting technology can completely transform healthcare systems by making it possible to produce tissues and organs unique to each patient. These technologies make it feasible to construct complex, useful biological structures, opening the door to the production of tissues and organs that closely resemble the anatomy and physiology of the human body. With an emphasis on tissue engineering and synthetic organ printing, we examine the most recent advancements in 3D bioprinting technology and their applications in healthcare systems in this review paper. This review summarises the work of many scientists who have made significant contributions to the field of 3D Bioprinting, examining their methods for designing and printing intricate biological structures, the biomaterials they use, and the strategies they use to improve cell viability and functionality.

INTRODUCTION

The applications of 3D Bioprinting are varied and have the potential to significantly impact various fields. These includes creation of artificial organs, which could potentially eliminate the long waiting list for organ donation, wound healing by creating artificial skin cells, as well as developing neurons, and hepatocytes for use in therapeutic procedures. Additionally, the bioprinted tissues can be used in place of animal testing to provide a more ethical and

***Corresponding author E-mail:** mohit.shah@nirmauni.ac.in*

cost-effective solution for drug discovery process. Finally, 3D Bioprinting can be used to develop "Organs-on-Chips," which combine microfluidic technology with 3D Bioprinting to create 3D extracellular environments that mimic native extracellular matrix. These models can be used for disease modeling, drug discovery, and high-throughput assays. 3D Bioprinting also has the potential to develop a body-on-a-chip.

Extrusion-Based Bioprinting, Inkjet-Based Bioprinting, Pressure-Assisted Bioprinting, Laser-Assisted Bioprinting, and Stereolithography are some of the 3D bioprinting technologies that have received a lot of attention. Extrusion-based Bioprinting is a popular method for producing three-dimensional models and structures. Materials that can be converted into filaments are used in extrusion-based Bioprinting and are temperature controlled before being dispensed onto a stage via nozzles that move in all three dimensions (X, Y, and Z axes). Multiple head nozzles may aid in the deposition of different types of biomass, such as cells, over a single scaffold in order to facilitate the development of complex tissues or organs. To achieve desirable results in 3D Bioprinting, two primary mechanisms are used: semi-solid extrusion (SSE) and fused deposition modelling (FDM). SSE employs pressurised air or rotating screw gears to create a streamlined input of semi-solid mass from one or more nozzles, depositing biomass layer by layer. In contrast, high temperatures are used in FDM to melt thermoplastic filaments, which are then extruded from the nozzle and deposited as multiple layers on the platform. As a result, 3D printers are made up of two major components: the extrusion system and the positioning system, which collaborate to produce visually and geometrically structured models and structures.

The Inkjet-Based Bioprinting technique, also known as Drop-on-Demand Bioprinting, has gained significant popularity in the field of 3D printing for both biological and non-biological applications. Originally developed for 2D ink-based printing on paper, this technique has been modified to include electronically-controlled rising stages and the use of biologically relevant materials in the printer cartridge. However, it is important to note that the biomaterials must be in liquid state in order to form droplets. Inkjet-based bioprinter operates by ejecting precise liquid droplets onto a substrate using thermal or acoustic forces. With the help of an electrically heated print head, thermal inkjet bioprinting creates enough pressure to push droplets out of the nozzle. Contrarily, Acoustic Inkjet Bioprinting uses a piezoelectric crystal to create an acoustic wave inside the printer head, which separates the liquid into the tiniest droplets possible. The appropriate choice of inkjet bioprinting technology depends on the specific requirements for tissue or organ development, as well as the availability and expertise of the operator.

Using pressure to force biomaterials out of a nozzle, pressure-assisted bioprinting creates 3D bio-compositions. This method employs a variety of hydrogels, viable cells, nutrients such as proteins, ceramics, collagen, and the biodegradable polymer chitosan. The primary benefit of this method is that it allows for room temperature processing, which is advantageous for thermolabile materials, as well as direct integration of homogeneous cells onto the substrate. The printer speed, on the other hand, is limited, resulting in approximately 40% to 80% cell viability after the process. Using pneumatic pressure from a plunger or a screw-based pressure generator, the appropriate amount of pressure can be generated, resulting in a continuous filament. The biomaterials are then deposited in multiple layers on a stationary platform.

Laser Assisted Bioprinting is a method of spraying biomass over a platform that makes use of a laser gun as an energy source. This technology, which was previously used for metal bioprinting, has been updated for biomass such as cells, DNA, peptides, and others. A pulse of a unique laser-targeted beam, a device to focus, and donor transport support in the form of a ribbon, a thin layer of biomaterial suspended in liquid, and a receiving substrate for the upside-down projection are all part of the process. The biomass is made up of hydrogel, culture growth media, incubating cells, proteins, and ceramic materials. The laser propels the biomass forward over a solid platform, irradiating laser light as a ribbon over the linear structure, causing the liquid biomass to evaporate as droplets over the receiving substrate, which is a cell culture medium with the ability to adhere.

Stereo-Lithography (SLA) is a nozzle-free 3D bioprinting technology that allows for the precise printing of biological and non-biological models. This technology makes use of light-sensitive hydrogels that are deposited layer by layer on a platform at a rate of approximately 40,000 mm/s while maintaining a high cell viability rate of up to 90%. The liquid photosensitive polymer is solidified by being exposed to light during the process. SLA is a promising technology for various applications in tissue engineering and regenerative medicine due to its ability to process a wide range of materials and the high accuracy of the printing process. The most extensively researched bioprinting techniques are laser-assisted Bioprinting, stereolithography, and extrusion bioprinting.

The demand for organ transplantation far outnumbers the supply of available organs. Bioprinting, which allows researchers to create functional artificial organs that can be transplanted into patients, could provide a solution. Although this technology is still in its early stages, significant progress has been made. Tel Aviv University researchers successfully 3D printed a heart using human cells and materials compatible with the patient's immune system. While the heart is not yet functional, this breakthrough represents a significant advancement in the development of artificial organs.

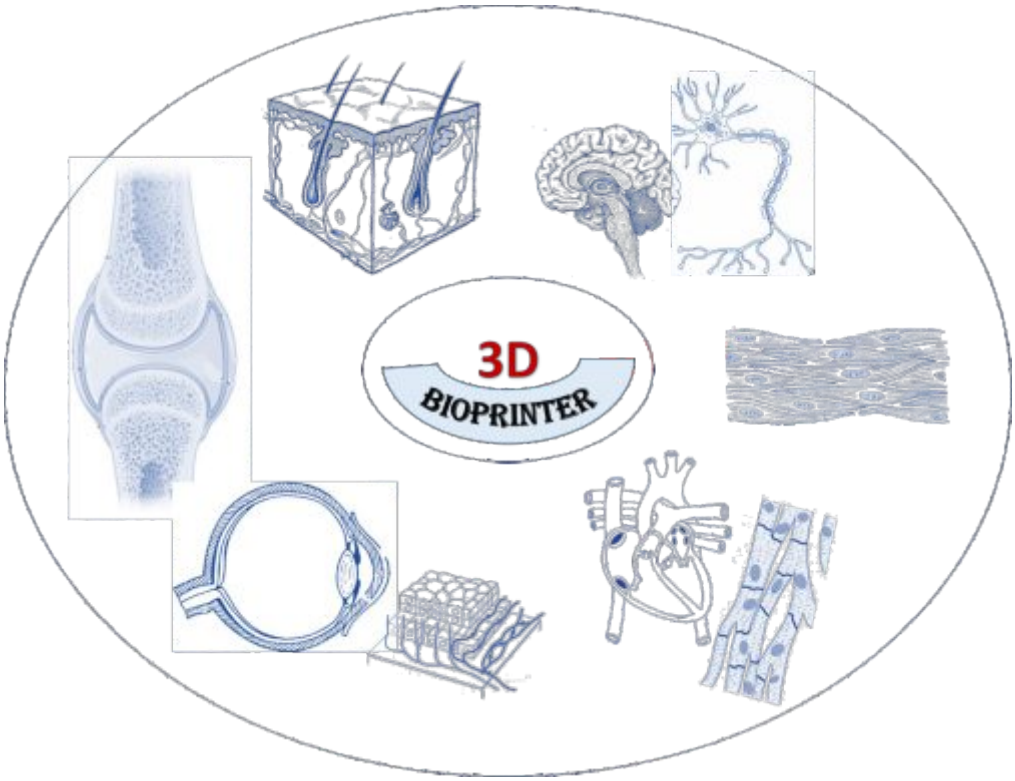

Figure 1: Applications of 3D Bioprinting Technologies in Tissue Engineering

The selection of appropriate scaffold fabrication technique plays an important role in developing structures that can support cell growth as well as promotes tissue regeneration. Traditional conventional techniques like salt-leaching, solvent casting, and gas foaming are simple and economical; however variable pore size and interconnectivity are major challenges. On the other hand, advanced techniques like 3D bioprinting and electrospinning can resolve the above issues with greater precision in scaffold design and cell placement; however, it lacks scalability due to the complex and time-consuming approaches. Hence, the appropriate selection of fabrication techniques needs a lot of consideration. Traditional approaches like salt-leaching, solvent casting, and thermally induced phase separation have proven moderate scalability in scaffold fabrication by increasing batch sizes; whereas Lyophilization being a well-established industrial technique can offer higher scalability. However, 3D bioprinting, electrospinning, and electrohydrodynamic jetting are yet more in research exploration by scientists and large-scale tissue engineering applications may be expected after developing proof-of-concept. Biological variations like donor variability, tissue-specific differences, and patient-

specific factors are the most critical factors that may significantly influence scaffold design and tissue engineering due to variable cell behavior and tissue integration. Standardizing cell sourcing, appropriate processing, tailoring scaffold designs as per specific tissue requirements, and customizing treatment plans to address individual patient characteristics are the need of an hour. Preclinical testing must establish the impact of biological variables on clinical outcomes, as well as validate safety and efficacy of the product. Thorough quality checkpoints should be employed during the fabrication process and focused pharmacovigilance for further enhancement of product performance may offer a detailed understanding and management of biological variations in tissue engineering approaches.

CARTILAGE AND BONE TISSUE

Recent advances in 3D Bioprinting have demonstrated enormous potential for transforming treatment approaches for bone and cartilage injuries. Bioprinted constructs have emerged as a promising solution for tissue engineering by allowing for the rapid and long-term regeneration of damaged tissue. Bionics principles are being used by researchers to create ideal scaffolds that closely resemble native tissues. Furthermore, bioinks such as polylactic acid are being used to improve the mechanical viability and cell growth of printed constructs. 3D Bioprinting's future appears to be even brighter, with the potential to transform surgical procedures, imaging techniques, and drug delivery technologies and thus improving the quality of the treatment and life of the patients. [1,2]

Inkjet-based bioprinting techniques are also studied for cartilage and bone tissues engineering. Xu et al. utilised rabbit articular chondrocytes dispersed in a hydrogel and printed them on a polycaprolactone matrix, which resulted in a scaffold with improved mechanical properties. The printed cells showed good viability, and the scaffolds developed dense, well-organised collagen after implantation in immunodeficient mice. [3]

The use of extrusion bioprinting techniques for bone tissue regeneration has seen significant progress in recent years. Fedorovich et al. printed a complex porous bone like tissue construct using this technique.[4] Hung et al. conducted a study on composite scaffolds for craniofacial regeneration using polycaprolactone. The incorporation of hydroxyapatite in the composite scaffold exhibited superior bone regeneration.[5] Schuurman et al. and Kundu et al. have conducted studies showcasing the development of scaffolds through the utilisation of diverse biomaterials and cell types to promote cartilage tissue regeneration. The structural support to the scaffold was provided by gelatin methacryloyl (GelMA) implanted with chondrocytes and polycaprolactone. Kundu et al. employed a multi-head deposition system for the development of polycaprolactone -alginate gel scaffolds incorporated with chondrocytes. After that, the scaffolds were introduced into the

dorsal subcutaneous area of mice resulting into a generation of cartilaginous tissues after four weeks with a noteworthy 85% cell survival rate. [6,7]

The studies of Dhariwala et al., Lu et al., and Zhou et al. demonstrated potentials of stereolithography bioprinting technique for bone and cartilage tissue engineering. Dhariwala et al. fabricated scaffolds with live CHO cells by utilising poly(ethylene oxide) as a biomaterial and 3D printing technology with a print resolution of 250 µm. Despite the limited mechanical strength observed in the produced scaffolds, the study demonstrated promise for further investigations with the incorporation of another polymer to enhance its strength.[8] Lu et al. conducted a study in which the scaffold exhibited significant mineralisation, after a period of 2 and 4 weeks, indicating that the seeded cells had undergone osteogenic differentiation.[9] Zhou et al. utilised a stereolithographic printer commonly used in small-scale production to fabricate a 3D structure loaded with cells for the purpose of studying breast cancer in bone tissue. Their findings revealed that scaffolds with lower concentrations of GelMA exhibited greater cell viability, implying that higher GelMA concentrations may impede nutrient transportation. Furthermore, the study demonstrated the feasibility of constructing intricate biological systems for investigating disease interactions at a tissue level.[10] Kim et al. accomplished the reconstruction of maxillary bone through the utilisation of a PCL/β-TCP scaffold manufactured by micro-extrusion-based 3D Bioprinting. After an 8-month postoperative period, the study observed a successful reconstruction, marking the first instance of successful maxillary bone defect restoration through a 3D-printed structure.[11]

Several studies have been explored to study the use of Laser-assisted bioprinting (LAB) technique in bone tissue engineering, including the development of biopapers to enhance cell viability. Galbraith et al. conducted a study where they fabricated a self-assembling biopaper composed of osseous tissue using hASCs that had been induced to differentiate into osteogenic cells. This biopaper showed increased levels of ALP activity and matrix mineralisation compared to biopapers made from stromal cells, implying that hASCs may have advantages over stromal cells for specific applications.[12] In their study, Kawecki et al. employed laser-assisted methods to accurately position human umbilical vein endothelial cells (HUVECs) on biopapers, resulting in the production of viable cells and tube-shaped endothelial structures after seven days. The results imply that laser-assisted techniques can augment scaffold regeneration by aiding the formation of a suitable capillary network in the implanted scaffold.[13] Catros et al. explored the consequences of 3D cell positioning within polycaprolactone (PCL) biopapers, demonstrating that scaffolds with internal cell placement exhibited markedly increased proliferation rates

relative to those with cells deposited solely on the scaffold surface.[14]

SKIN TISSUE

Skin bio-printing is an innovative technology with potential applications in various fields. Developing functional skin that has appropriate vascularity, innervation, and can perform functions like touch sensation and perception is the primary obstacle. However, skin bio-printing has the potential to address the increasing demand for skin bio-fabrication for clinical and research applications, such as regenerative medicine, modeling physiological/pathological conditions, and the cosmetic/pharmaceutical industry. Furthermore, the use of skin bioprinting technology may assist in creating tissue that is physiologically relevant, resulting in improved and consistent functional outcomes for burn victims, as well as contributing to faster healing, less scarring, and improved aesthetic results. In conclusion, skin bio-printing technologies have the potential to significantly enhance wound healing and patient outcomes.

Skin tissue production using inkjet-based bioprinting methods has been studied by Lee et al., Rimann et al., and Skardal et al. Adult human dermal fibroblasts and epidermal keratinocytes were grown inside of a collagen gel scaffold by Lee et al. using a microvalve-based bioprinting technique. The printed scaffolds were dimensionally stable, keeping their original shape and allowing three compact layers of keratinocytes to develop on the scaffold's surface. After being exposed to an air-liquid contact, keratinocytes formed a thick epidermal layer.[15] The bioprinted scaffold used in Rimann et al.'s alternative polyethylene glycol based (PEG) skin model showed vascularisation and the highest histological similarity to native skin.[16] In-situ printing was employed by Skardal et al. to apply mesenchymal stem cells (MSCs) and amniotic fluid stem cells (AFS) to complete skin lesions. The results show that inkjet-based bioprinting techniques are promising for producing skin tissue for mending wounds.[17]

Extrusion bioprinting approaches for skin tissue have generated excellent results in recent study. Printing multilayered skin-like tissue constructs with superior shape stability and cell survival was shown by Lee et al. and Kim et al.[15,18] Using a combination of extrusion and inkjet printing technologies, Lee et al. prepared a multilayered scaffold with fibroblasts and keratinocytes suspended in a culture medium. For mechanical stability, the cells were printed between crosslinked layers of a hydrogel. The printed cells showed high vitality after an 8-day culture period, with fibroblasts having a higher cell density than keratinocytes. Kim et al. used an extrusion-based bioprinting method, and samples revealed biological characteristics comparable to native human epidermis after 7 days of culture.

Koch et al. evaluated the possibility of laser-assisted Bioprinting (LAB) for creating skin tissue from a range of skin cells and discovered that LAB resulted in

good cell viability with no change in phenotypic, indicating that the transfer process did not compromise the cells' integrity.[19]

NEURAL TISSUE

Bioprinting has shown promise in the field of neural tissue engineering, allowing for the quick fabrication of physiologically appropriate brain constructions for cell therapy and drug testing. Controlling brain area development, vascularisation, and optimising printing parameters for cell viability are significant challenges. The combination of Bioprinting and organoid technologies could result in complex neurological networks. This method may reduce the requirement for animal models, hence boosting biomedical research in the field of neurological disorders.

The use of inkjet-based bioprinting techniques for neural tissue engineering is a fast-expanding field with huge potential for the development of new therapeutics for a wide range of neurological illnesses. Lorber et al. conducted a study that demonstrated that shear pressures did not induce significant cell distortion or damage during the droplet ejection process. Additionally, the printed cells showed comparable survival rates.[20] In another study, Tse et al. printed a combination of neuronal and supportive glial cells, which exhibited high cell viability and faster neurite growth compared to non-printed cells. These findings hold great promise for the potential use of Bioprinting in the treatment of extensive, full-thickness skin burns.[21]

The use of extrusion bioprinting technique for neural tissue engineering has been shown to be a promising alternative to traditional autologous grafts. Hsieh et al. conducted a study on zebrafish traumatic brain injury model and demonstrated the effectiveness of polyurethane (PU) scaffolds embedded with neural stem cells (NSCs) in repairing neural tissue.[22] Meanwhile, Owens et al. developed nerve grafts made up of mouse bone marrow stem cells and Schwann cells using a multichannel extrusion-based bioprinter. These nerve grafts were implanted in rats and resulted in the restoration of motor and sensory functions at 40 weeks. The use of extrusion bioprinting allowed for the fabrication of precise and complex structures that closely resemble autologous grafts, which are currently the best option for nerve rehabilitation.[23] The research of Hsieh et al. and Owens et al. demonstrates the potential of extrusion bioprinting for neural tissue engineering and could lead to new treatment options for neural injuries.

Zhu et al. employed the Stereolithography bioprinting to transform neural stem cells into neurons. The study discovered that low doses of GelMA were efficient in increasing hNSC proliferation and encouraging neuronal differentiation. After 14 days of culture, GFAP and -tubulin III expression, as well as neurite elongation, suggested effective neural differentiation. The use of nanoplatelets of graphene to induce hNSC differentiation into neurons

is a groundbreaking and promising strategy in the field of tissue engineering.[24]

CARDIAC AND VASCULAR TISSUE

The potential of 3D bioprinting technology in managing myocardial infarction and its complications by creating clinically viable cardiac constructs is evident. The injection of hydrogels derived from decellularised extracellular matrix into damaged cardiac tissue has proven to be effective in enhancing function after ischemia. The use of bioprinting technology presents promising prospects for scaffold micropatterning and scaffold-free systems to reproduce the histological makeup of native cardiac tissue. Numerous bioink formulations based on various biomaterials and composite bioinks have been created for 3D printing of heart tissue engineering, such as heart patches, tissue-engineered cardiac muscle, and other bionic structures.

Inkjet-based Bioprinting has shown great promise in the printing of cardiac and vascular tissue. Xu et al. have successfully fabricated a heart-like structure with two ventricles using primary feline adult and human H1 cardiomyocytes encapsulated in alginate hydrogel. The printed structure possessed porosities that facilitated cell viability, while electrical stimulation triggered rhythmic beating of the entire construct.[25] In a similar vein, Boland et al. introduced a novel technique for producing microscopic porosity in alginate/gelatin gel by printing a crosslinking agent into the solution. This approach allowed the formation of different types of tubes, which served as a scaffold for endothelial cells to grow.[26]

Extrusion bioprinting has proven to be a promising technique in the fabrication of cardiac tissue constructs for regenerative medicine, as demonstrated by Duan et al. In their study, human aortic valvular interstitial cells were encapsulated in a hybrid hydrogel that was 3D bioprinted. According to their findings, it is possible to develop functional cardiac tissue using this technique because the cells have a high level of viability and the capacity to remodel the hydrogel and contract spontaneously.[27] Similarly, Gao et al. used extrusion bioprinting to create a bio-blood-vessel (BBV) by combining atorvastatin-loaded PLGA microspheres and decellularized extracellular matrix derived from vascular tissue. In their study. They found that this technique significantly increased the formation of capillaries and arterioles.[28]

MUSCULAR TISSUE

Musculoskeletal tissue engineering has seen many applications for three-dimensional bioprinting. One important use of bioprinting is the capacity to produce implantable tissues that can restore or replace tissue that has been lost or damaged as a result of an illness, an injury, or aging-related degeneration. Bioprinting stimulates tissue regeneration by creating scaffolds with exact control over their architecture, mechanical characteristics, and cell distribution. For instance, the use of bio-printed muscle

tissue in the treatment of musculoskeletal injuries or degenerative diseases like muscular dystrophy has great potential. Creating complex models of musculoskeletal tissues, like the bone-muscle-tendon interface, using bioprinting enables more precise drug testing and disease modeling. Bioprinting enables the creation of interface tissues that are essential for the proper functioning of musculoskeletal systems. With Bioprinting, the precise control of material and cell placement necessary for the fabrication of interface tissues is achievable. Examples of interface tissues include the bone-cartilage interface and the tendon-bone interface, both of which are implicated in injuries and diseases such as osteoarthritis and rotator cuff tears.

Miri et al. devised a novel method to create intricate hierarchical structures that resemble muscular tissues. They have employed a hybrid approach, utilising microfluidic chips that have been fabricated through pneumatic extrusion and crosslinked with UV light. Various bioinks, such as PEGDA and GelMA, that contain different types of cells, have been printed into specific patterns. After seven days, the researchers observed sufficient proliferation rate. Remarkably, the printed structures maintained their patterns and interfaces even after being implanted in rats for 30 days. The researchers deduced that an optimal concentration of 10% GelMA for the prints is consistent with the studies conducted by Zhou et al.[29] In a summary, this research work showcases the immense potential of extrusion bioprinting for creating complex and hierarchical structures that bear a striking resemblance to muscular tissue. The study offers valuable insights into the use of multiple bioinks and their effects on cell proliferation rates and print patterns, opening up new avenues for in vitro and in vivo studies.

Bajaj et al. conducted a study that sheds light on applications of stereolithography bioprinting for muscular tissue generation. They employed dielectrophoresis and SLA in PEGDA hydrogels to pattern and encapsulate the cells.[30]

CORNEAL TISSUE

The use of bio-printing technology holds significant promise in the field of ophthalmology. One of the most exciting potential applications is the creation of corneal tissue through tissue engineering. This could help address the shortage of donor corneas available for transplant and reduce the risk of rejection by creating personalised corneas. Further, the pace of drug development process could be increased by using bio-printed corneal models for controlled drug testing. This would eliminate the need for animal testing. Moreover, this will help to understand the ophthalmic disease model and the underlying mechanisms in a better way, which in turn will help to develop new methods of treatment. Last but not least, printing of retinal tissues is also made possible, which can help patients with degenerative retinal diseases to regain

their vision.

In order to overcome the shortcomings of corneal transplantation, Isaacson et al. created a cornea made of collagen with corneal keratinocytes using extrusion-based technology. It was observed that this can be a workable substitute for the conventional Penetrating Keratoplasty procedure. In their study, they found 90% viability rate on the second day and 83% after a week.[31]

Sorkio et al. studied laser-assisted bioprinting for repairing corneal tissue. In the study, human adipose derived stem cells and limbal epithelial stem cells were printed on three different scaffolds and adequate cell proliferation was observed.[32]

CONCLUSION

Regeneration of cartilage and bone, skin, neural, cardiac, vascular, muscular, and corneal tissue has been successfully studied by numerous scientists using a variety of bioprinting techniques and promising results are obtained. These studies offer a strong foundation for the advancement of tissue engineering and 3D printing of artificial organs. The advancement in this area will facilitate the improvement in regenerative medicine and drug discovery process and reduce the need for animal testing. These bioprinted models can be an ideal substitute for researching drug efficacy and toxicity because they can mimic the mechanical and biochemical characteristics of native tissues. These models can also be used to research diseases and personalized medicines. After the success of 3D bioprinting, a growing trend toward 4D bioprinting has been observed in recent years. 4D bioprinting adds a time factor in 3D printing to build a base structure that can be reshaped or reconfigured over time to take on the desired form. Various hydrogels, shape-memory polymers, and other responsive materials are being studied in combination with 4D bioprinting to develop multifunctional tissues and organs with ability to respond to environmental changes.

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