

**International Journal of Biology, Pharmacy
and Allied Sciences (IJBPAS)**

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3D BIOPRINTING: AN EYE TO FUTURE ASPECTS FOR SPACE, CELL, TISSUE ENGINEERING AND ORGAN REGENERATION

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Received 24th Sept. 2023; Revised 25th Oct. 2023; Accepted 8th Jan. 2024; Available online 1st Oct. 2024

<https://doi.org/10.31032/IJBPAS/2024/13.10.8404>

ABSTRACT

Bioprinting in three dimensional array is a rapidly evolving scientific technique having immense scope in tissue engineering that comprises of in-silico algorithms and drug screening approach to construct the biological model. Bioprinting assisted constructed organs and tissues can open new avenues in pharmaceutical sciences and research. It can construct organ along with tissue in vitro and open doors for new extremes in pharmaceutical sciences and research. The principal work outline involves the fabrication process and encapsulation along with different variations like scaffold based bioprinting, scaffold free bioprinting, extrusion-based bioprinting and laser assisted 3D bioprinting. We compile the current trend in bio-ink selection process and its applicability in development of economical and more efficient 3D printing.

Keywords: 3D Bioprinting, Bio-ink selection, Scaffold based Bioprinting, scaffold free bioprinting, extrusion-based bioprinting and encapsulation

INTRODUCTION

The technology of bioprinting in three dimensions is the fastest growing technology gaining ground in engineering tissue, organs in regenerative medicine and screening of

drugs. The main objective is adhering several types of biomaterials, cells and bio-molecule by layering with controlled spatial type of distribution [1]. The bio-ink used in 3D

printers exploit living cells, biomolecules and biomaterials to generate 3D printed tissue [2] and includes layering biomaterial one over another to construct structures like kidney, bone, and cornea. Basic infrastructure involves layering cells procured from taking consent from a patient or either taking stem cells. Recent advancement of 3D Bioprinting in veterinary sciences as well as human model researches have summarized that bioprinting utilizes acquaintance animal models and human model system to examine the safety and efficacy of bio printed tissues [3]. In the past decade, 2D cell culture technology has played an important role in

finding out novel leads for drug formulation. Inefficiency in simulating similar procedures in animal models and raised ethical concerns over testing animals has enforced the 2D culture to take a back step [4]. Therefore exploration of 3D bioprinting applications has paved a way to finding novel techniques such as to construct the chip model system [5], [6]. The biological computer is an implanted device that is primarily utilized for cellular functions such as creating therapeutic effects or monitoring the body's activity. This is composed of proteins, RNA and DNA. It can also do rudimentary math computations [7].

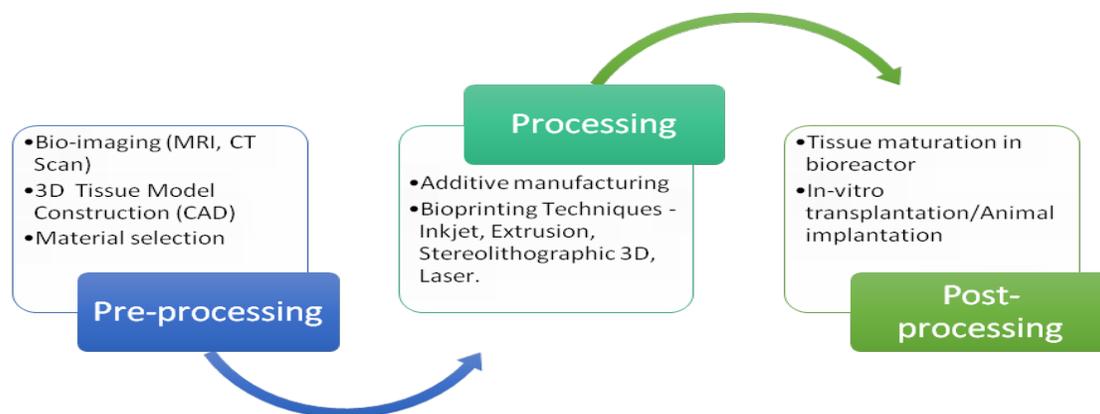


Figure 1: Processes in 3D Bioprinting

3D Bio printings construct Approach: A novel Bio-ink selection (Scaffold-Based Bioprinting, Scaffold-Free Bioprinting, Extrusion-Based Bioprinting, and Laser Assisted 3D Bioprinting)

Printable biological materials, cells, tissue and organ are used in 3D bioprinting to construct the tissue organoids. Biomaterials deliver suitable niche and structural supports for proliferation, cell adhesion, differentiation, and migration [8]. For the

generation of tissue architecture two popular methods are: scaffold-based bioprinting which deploy an exogenous scaffold to provide mechanical support to developing tissue or scaffold-free bioprinting which make the most of the inherent ability of cells for structuring tissue construct using higher viable cell density. Characteristic differences between these two approaches is variation in the number of cells used for structuring in which scaffold based technique is more scalable and economical when compared to scaffold free, whereas scaffold free techniques are less toxic comparatively [9].

Scaffold-Based Bioprinting in 3D bioprinting- Usage of support biomaterials for organized structuring of cells comes under this particular segment of bioprinting techniques. Various simulations are made using factors and artificially manufactured domains to mimic the *in-situ* tissue

microenvironment. Scaffold-Based Bioprinting is amendable in terms of enumeration of cell count. However, decreased cell-cell interactions and commune elevates cell toxicity [10]. Hydro-gels are the favored class of bio-ink materials used in tissue engineering. Hydro-gels (derived naturally or synthetically) is used to encapsulate cells during the bioprinting process [11], [12], [13]. Hydro gels are widely used as it allows cells to grow under a water adsorbent environment enabling a degree of flexibility while culturing (**Figure 2**). These biomaterials aid in *in-vivo* biomimicking of the extracellular matrix environment (ECM). Moreover, it is abundant and affordable too [14], [15]. Several hydro-gels have been engineered for bioprinting techniques and include alginate, collagen, gelatin methacrylate, gelatin, hyaluronic acid etc. [16].

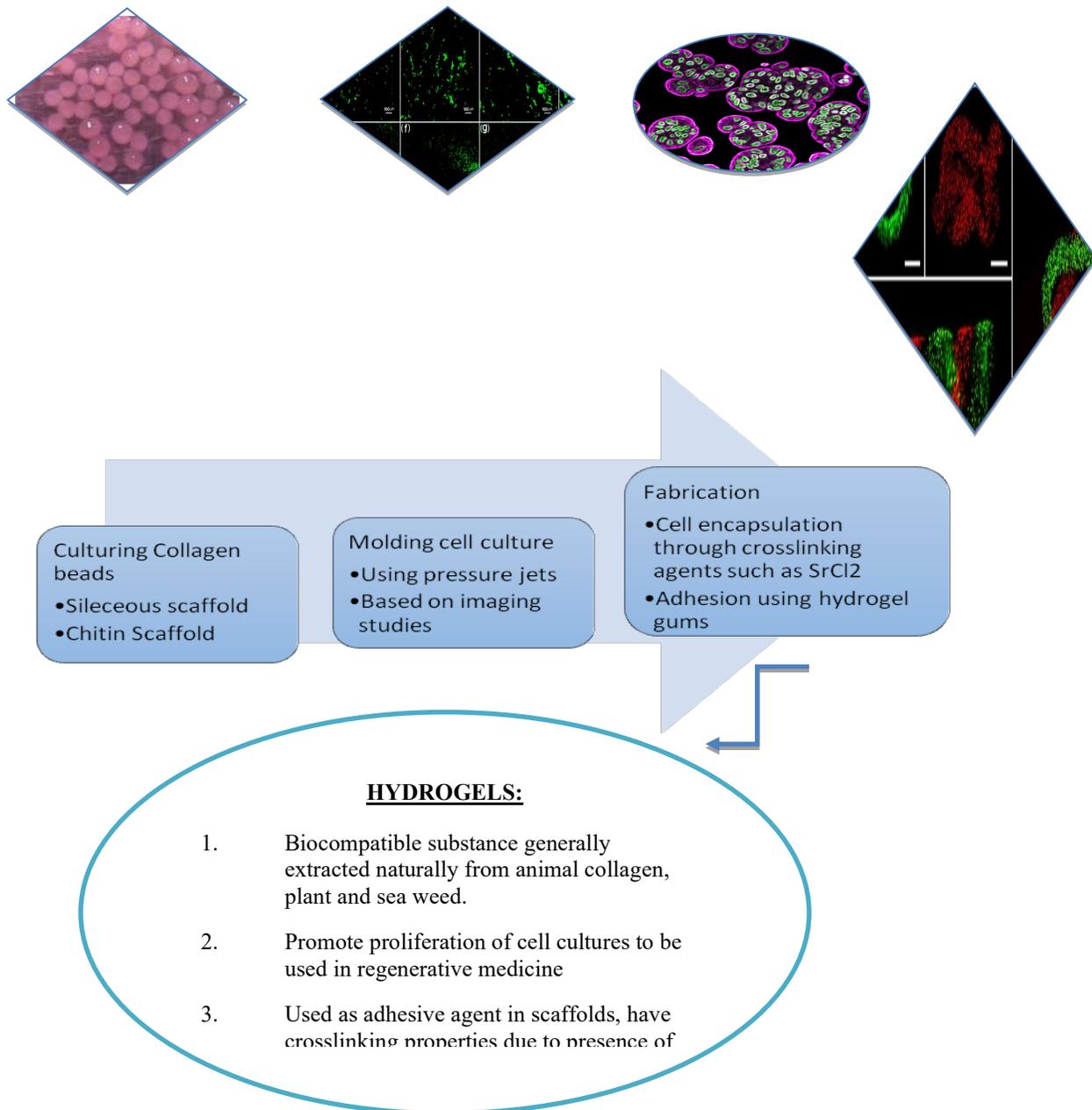


Figure 2: Hydrogel encapsulation during bioprinting process

Scaffold-Free Bioprinting

Scaffold free approach involves self-assembly of cell factors and components to generate a mechanistic model of the human system. This approach prefabricates cell plates and spheroids or tissue strands to

create 3D bio-constructs [9]. It reduces the cytotoxicity of the exogenous materials and reduces the maturation time after bio-printing as compared to scaffold-based bioprinting [10]. Newly-formed tissue (Neo) tissue represents the scaffold free bioprinting and

generates the high number of cells to support the semi-permeable structure which supports the gas exchange. This makes them to be printed directly after generation, easily scalable, and also put forth efficient way of action for future scaffold-based bioprinting approaches [17].

Extrusion-Based Bioprinting

Extrusion based bioprinting is based on pressure-driven technology. The bio-ink is collected and funneled onto a nozzle and driven by mechanical pressure. Extrusion based bioprinting technology have ability to accommodate higher cell density as compared to other bioprinting technology. However the resolution is very limited and poor with the size of resolution under 100 micro meters [18]. A highly viscous hydro-gel is used in this bioprinting technique to be utilized in fabrication process and it is not interfered with any kind of chemical additives generally added to cure the printed structure. These hydro-gels are non-Newtonian fluids undertaken in account for extrusion-based bioprinting which resort to the rheological behavior of the hydro-gel ink for methodical operation [19]. To enable plug-like flow for maneuvering the extrusion process with impact print quality, assistance

of the shear thinning property of these non-Newtonian fluids are used. Fabricated Gel MA (Gelatin methacrylol)-based scaffolds via visible light-based SLA (stereolithography apparatus) is a typical example in which the gel is mixed with eosin Y and NIH-3T3 fibroblasts are analyzed by a scaffold wavelength of 522 nm [20]. After the scaffold is mixed with camphorquinone, fluorescein or riboflavin monitoring of morphology and cytotoxicity is must. These compounds are blue light-absorbing initiators utilized for extrusion process [21].

Laser-Assisted 3D Bioprinting

Laser-assisted bioprinting (LAB) is done using laser as an energy resource for layering biomaterials on a support. This technique consist of three instrumentation subsets: a pulsed laser source, a ribbon coated with liquid biological material which is to be layered on the metal film and a substrate [22]. Advantages involve a) high micro-scale resolution, b) faster deposition and c) no shear stress (due to nozzle pressure) to preserves cell shape. However the only disadvantage is a) very high cost and possible strain due to photo-induced stress to maintain a shape.

Table 1: Role of Bio-inks polymer used for 3D bioprinting

Bio-ink type	Bio-ink	Advantages	Disadvantages	Application	References
Naturally derived hydrogels	Collagen HA alginate, fibrin, gelatin agarose chitosan	Naturally cell-adherent can provide native ECM-like microenvironment	Generally low mechanical strengths and difficult to modify	Liver on a chip, cartilage, skin vascular tissue etc	33
Synthetically derived hydrogel	PEG, GelMA, PLURONIC F-127	Mechanical properties manipulated like temperature, sensitivity and photo cross link ability	High bioactivity and process ability	Vascular network, liver on a chip	24
Cell spheroids and tissue strands	MSCs, L6 rat myo-blasts	High cell density	High cost, time consuming, difficult in handling	Nerve graft, vascular network	29
Melt-cure polymers	PLA(Polylactic acid), PU, PCL(Polycaprolactone)	Easy handling, mechanically robust	High cost, high temperature and low cyto-compatibility	Heart on a chip, liver on a chip	20
Differentiated Extracellular matrix	Alginate	Retain ECM(Extracellular Matrix) components that induce tissue formation	inferior post-printing shape fidelity	Muscle, bone muscle	4

Cell, tissue and Organ constructs (Additive processes using Bioprinting)- 3D

Bioprinting facilitates technological intervention in exploring disease specific pathways and its manipulation using bioactive compounds for holistic toxicological studies and diagnosis oriented treatment [23]. Aiming at the development of whole organ for *in-vivo* studies, 3D bioprinting can possibly be an alternative for animal based clinical studies. 3D bio printed structures could possibly help in treating non functional organs, alleviating issues that occur in searching suitable test organs from unethical sources viz animal based tissues for human organ regeneration and reduce immune complications and/or structural incompatibilities that emerge from allogenic transplant. 3D

bioprinting comes up with challenges; one is the usage of magnetic fields during additive manufacturing and related acoustic aberrations. One more complication comes up as 3D bioprinting allows the homogenous circulation which switches off the micro-architectural behavior of tissues. This behavior is responsible for multi-potentiality of tissue construct by undergoing various cellular orientations within culture. It can be solved by using multi-unit bioprinting approaches. A typical example may be Ultrasound bioprinting in which culture consist of structured and aligned MG63 cells, which gives high yields of interferon after inducing with polyinosinic acid-polycytidilic acid, cyclohexamide and actinomycin in humans, within the alginate multi-layered

extrusion-based construct [24]. Various applications of Bioprinting include using scaffolds for drug delivery, studying disease mechanisms, or development of personalized medicines. A specific example can be a study on the treatment of osteo-myelitis using 3D printed analysis of rifampicin loaded PCL scaffold [25]. Extensive researches are focused on making the technique more feasible which implies cheaper synthesis of

biomaterials and by using various simple layering and coating mechanism to be beneficial in the repair of cardiovascular, nervous system and optical tissues. A recent study has reported creation of canine bilateral carotid and femoral arteries, 3D bioprinted vascular grafts, homologous mesenchymal stem cells printed onto conventional artificial arterial grafts [26].

Table 2: Challenges in 3D bio-printing of tissues and organs

Tissue/organ	Challenges	References
Cartilage and bone	mechanical strength; growth factor for improved functions	21
Liver-on-a-chip	integrated fabrication approach	34
Heart-on-a-chip	cardiac tissues Formation	20
Body-on-a-chip	different tissues Integration; high throughput achievement	36
Muscle	muscle cells pre-alignment; vascular structure formation and innervations	15
Vasculature	multilayer vessel fabrication; 3D vascular structure	29
Skin	vascularization; involve secondary and adnexal structures	16

Tissue Regeneration for cardiovascular diseases in India- In the developed countries, cardiovascular diseases (CVDs) are one of the major causes of mortality. Other ailments such as stenosis affect heart valves; stroke and arrhythmia patterns are also among worsened heart conditions [27]. Due to lack of repair or auto-regeneration process a vital problem that arises with all these heart related conditions is the loss of irreplaceable cardiomyocytes. The lost cardiomyocytes are superimposed by non-functional scar tissue that can result in acute cardiomyopathy a condition that makes it

hard for the heart to deliver blood to the body which in turn can lead to heart failure. Bypass surgery of coronary artery, cell based therapy, left ventricular assist device etc. are certain remedies to these conditions and worst cases report to heart transplantation [28]. The next major problem is finding donors for transplant and the problems associated with the transplantation viz immune rejections which has feeblish success rates [29].

3D Bio printing: Application in Biomedical field

In regeneration process: New therapeutic treatments are designed to combat neuronal pathogenesis, initiation and connections via 3D bioprinting of neuronal cells into structure as major focus on Alzheimer and Parkinson networks are still on back foot owing to complexity and multilayering of brain [30].

Skin- Bioprinted skin can be used for several applications such as: evolution of contemporary drugs, wound healing studies, and dermal toxicology research. The fibroblast and keratinocytes are printed on a stabilizing support called matrigel to construct skin substitutes. A particular microenvironment is set up for cells and similar to that exist in human skin. In recently amniotic fluid stem cells were researched and printed for skin regeneration and wound healing [31, 32].

Drug discovery- Drug discovery studies are very important *in vitro* development of model of tissue system. It's a holistic approach to target the tissue and organs. Artificial organs play a sophisticated role in 3D bioprinting. It's insufficient in the 2D monolayer and multilayer system. Reduced 3D tissue models are used for big experimental studies, drug discovery approach, and development in clinical studies of new model generation drugs. During the

period of drug discovery process, one of the five selected candidates from thousands of compound development in clinical studies gets displayed from 24 candidates. Meanwhile medical development mainly gives the 55%-60% of the entire cost by means of the bulk of the drug discovery approach. The attention rate is reducing drug candidates in the drug discovery process and clinical development (mainly Phase II and Phase III). In recent time the main challenge and opportunity are for clinical research and development (R&D) [33], [34]. No research validates the technique if they lack application part. So, 3D printing applicability in discovery of drug provide expanded horizon in medical product and drug customization and personalization. Many platforms are used in drug discovery process screening studies for example cell spheroids and development of the cellular structure which is although plummeting the number of animals operated for drug development studies [35].

Bio-preservation- Cryopreservation methods are used in bulk volumes that hamper the best cooling and producing the detrimental variation of tissue and cells during bio preservation. 3D bio-printing technology can be used to transform bulk sample of cells into nano-liter precipitations

can be used to tackle the issues related with conventional cryopreservation (e.g., ice crystal formation) [36]. Recently, a bio

printing in combination with innovative bio-inspired cry-inks was employed to vitrify red blood cells (RBCs) [37].

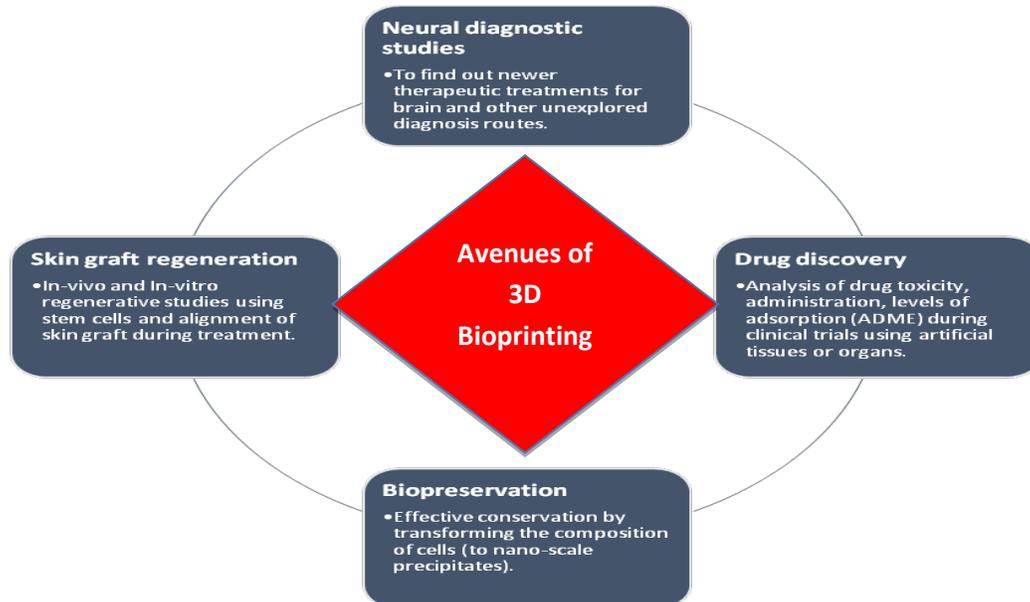


Figure 3: Avenues of 3D Bioprinting

A proposal was published by the European space agency ESA in 2017 to put insight into developing 3D printing of living tissue purely for space exploitation motive. Two of the institute one TUD University Hospital (Dresden and other OHB System AG (Bremen, Germany) come to corroborate to initiate development of modified recipe by adding methyl-cellulose and alginate to increase the viscosity of the blood plasma which in turn shows fluid inconsistencies when used in altered gravitational conditions. These additional substances were obtained by researchers from plants and algae respectively which proved to be a feasible

solution for a self-contained space expedition. This project aims at making the skin grafts of astronaut compatible for mars environment [38].

Futuristic prospects- This review imparts knowledge about recent advances in 3D bioprinting strategies and bio-inks in cell or tissue engineering. However, despite various advancement that have been achieved in the field there are still many concerns such as biocompatibility and proper integration of the printed organ with the body. Overall, 3D bioprinting techniques is a viable and high-throughput tissue printing which offer optimum spatial control and defined

patterning of cells when compared to conventional methods of tissue culture. Upcoming ameliorations are now focusing on combining of 3D methods to work in an efficient workflow and to standardize the process of creating cell and tissue structures formation. Novel biotechnological and nanotechnological applications are emerging to be possible solutions. The biological computer is used in analyzing intercellular activity and gene mutation [39], [40]. Future research focuses on bioprinting can also overcome vascularization i.e. nutrient diffusivity in vessels, which is among the most important factors limiting bioprinting applications in tissue engineering [41-49]. Further the usage of simulation models and system biology approaches along with machine learning algorithms for predictions can be advantageous in-order to streamline complex processes during grafting, drug compatibility studies, diagnostic researches etc.

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