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Review Article

Recent Utilization of 3D-Bioprinting Methods for Breast Cancer Models

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ABSTRACT
Animal models are the most commonly used model that helps to improve the understanding of the genetic alterations that occur in humans during the carcinogenic environment. Furthermore, these models play a pivotal role in the illustration of tumorigenesis and therapeutic strategies. With the advancement in molecular biology, the use of nanomedicine for breast cancer treatment has progressed, and more is expected to be done in the future pretrial and clinical models to achieve more success. The biocompatibility of 3D printing platforms has been reported to be adequate in terms of cell viability; however the effects on gene expression and functional aspects have received less attention. Various mechanical and visual disruptions to cells are involved depending on the type of bioprinter employed. Additional research into the mechanical and optical effects of the bioprinting process will provide more insight into the 3D printing technique' biocompatibility. To investigate the microenvironment of breast tumours and 3D bioprinting methods have also been studied. Modalities for bioprinting include extrusion-based (EBB) printing, droplet-based (DBB) printing and laser-based bioprinting. Different research has indicated that new developments of novel cancer modelling have emerged with 3D bioprinting technology. Those studies need to be properly explained and analyses in a Broadway in this review and to help in the progress of cancer research.

Key words: 3D printing, Extrusion-Based Bioprinting, Photocuring-Based Bioprinting, Droplet-based Bioprinting, Microenvironment.

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INTRODUCTION

Breast cancer is among the leading causes of high mortalities (6.9%) with an estimated 2.3 million new cases (11.7%), in women globally, today. It represents one of the most common forms of cancer.[1,2] It is one of the most invasive types of cancers and second leading cause of death after lung cancer in women. Over the years, it has received advances in nanomedicine development of treatments.[3,4] Other forms of medication, such as the pre-clinical models have failed in the clinical trial stage, however are still undergoing more trials. There are cell culture strategies that have been employed to try and address the limitations of conventional *in vitro* models.[5,6] They include patient derived cells, microfluidic systems, bioprinting and advanced 3D cell structure. 2D models are used but they have their limitations such as lack of tumor microenvironment (TME) and cellular heterogeneity which propels the career development and is treatment resistant. The effectiveness of clinical translation of nanomedicine trials requires that the preclinical models are able to identify alternatives used for treatment response and recapitulate the tumor characteristics to ascertain its efficacy and level of safety.[7,8] To conduct the extensive research, animal models are used before translating to human models as a precautionary measure. Additionally, animal models are used to improve the use of nanomedicines in the wake to develop safer and efficient alternatives for breast cancer treatments.[9] Tissue models for cancer research 3D bio-printing allow the recapitulation of the cancer micro-environment so as to study cancer pathogenesis and metastasis accurately.

MATERIALS AND METHODS

To this study, a literature review of articles published between 2016 and 2021 was carried out, mainly through the PubMed and LILACS databases. Thus, articles from systematic reviews, clinical trials, *in vitro* and *in vivo* studies were selected in English languages. We selected 90 articles related to the theme in question.

Techniques for 3D bioprinting

Extrusion method in cancer research is by far the most widely utilized by Cancer researchers favouring extrusion bioprinting as it is the only bioprinting technology that makes it possible to manufacture core-shell types of biomaterials. Manufacture in a core shell arrangement of various biomaterials enables research into particular cell-cell and cell-extracellular matrix interactions Figure 1. The major reason for such is the simplicity of options, cheap investment cost and the ability to print very viscous bioinks filled with high cell density.

Extrusion-based bioprinting

In extrusion-based bioprinting, progressing fibers are created through constant expulsion power rather than single beads. This innovation is appropriate for printing profoundly thought-cells which implies high thickness bio-inks. The ink utilized in expulsion bioprinting is appropriated by mechanical power like screw or cylinder or utilizing gas or compressed air.[10,11] Pneumatic-driven extrusion the expulsion procedure including pneumatic power uses packed air without a valve or

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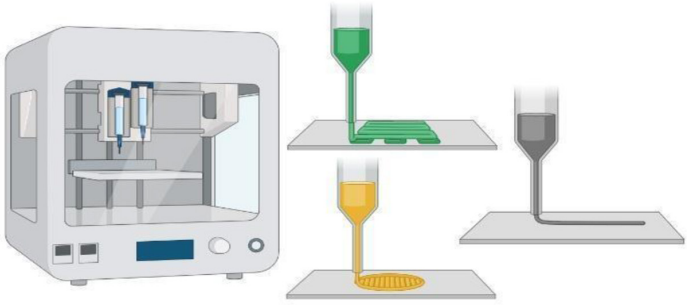

Figure 1: 3D Bioprinting Technology -Bioprinting entails the creation of a 3D structure through the computer-controlled disposition of biological.

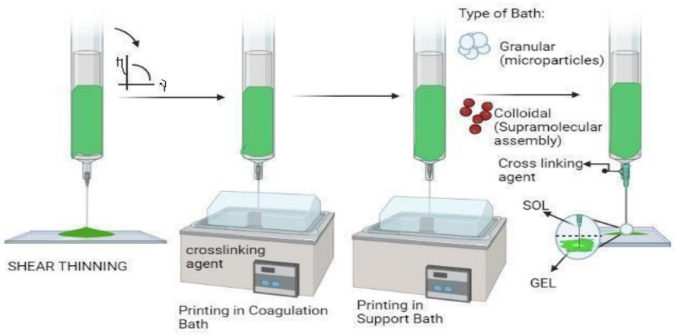

Figure 2: Extrusion-based bioprinting, progressing fibers are created through constant expulsion power rather than single beads.

valve-based arrangement Figure 2. The vacuum apparatus with cleaned air is associated with a bio-ink filled needle. Pneumatically expulsion of the bio-ink causes shear pressure, which implies just the kind of bio-inks that have shear-diminishing properties, can keep up with filamentous shape after expulsion. Valve free expulsion is moderately basic. For high-accuracy execution, nonetheless, valve-based expulsion is favored. This is quite possibly the most helpful procedure for printing cell-loaded bio-ink.[12] Mechanical micro-extrusion alludes to when the spout hole has a breadth of under 1 mm. Mechanical-driven expulsion is reasonable for exceptionally thick bio-inks, like manufactured and regular polymers. One usually utilized mechanical miniature expulsion method is the cylinder-based expulsion, which utilizes a cylinder associated with an electric engine. Turning in the engine through an electrical heartbeat drives the cylinder forward subsequently pushing bio-ink through the spout.[13,14] The screw-driven expulsion strategy gives more volumetric control and is valuable for higher consistency biomaterials. In this technique, a screw associated with the engine rather than a cylinder drives the arrival of the bio-ink. This interaction can oblige bigger pressing factor drops through the spout. Mechanical techniques give higher goals and better printability for a bigger scope of biomaterials; however, it requires a more tight resilience determination of the smash and spout. Expulsion-based bioprinting is best for high cell densities and is somewhat quick moving. As local tissues contain thickly pressed cells, printing cells at high thickness is critical for use in regenerative medication. An assortment of bio-inks can be utilized in this strategy, which is a benefit. Quite possibly the most regularly utilized bio-inks for expulsion is alginate. Bio-inks utilized for extrusion-based bioprinting must be genuinely gooey to improve the goal and stay stable in the precisely upsetting interaction. Notwithstanding, the subsequent high shear powers in pneumatic driven expulsion lead to low cell practicality. By the way, the cell practicality is as yet about 90% in cells made from extrusion-based bioprinting. The resolution is relatively low.[15,16]

Photocuring-based bioprinting

Stereolithography uses a photocuring-based method setting photosensitive polymers to frame tissue developed under accurately controlled lighting. The bright beam is aimed at a supply of photosensitive polymers. For each layer testimony, laser filters a 2D example by going through its way point-by-point and the controlled light collaborates with the bio-ink material to polymerize it as indicated by a particular plan. After one layer is relieved, the printing stage moves upwards or downwards, away from the laser source, so that new unpolymerized ink material can stream into position for the following layer.[17,18] Digital light processing (DLP) The technique is quite similar to SLA, except for the distinctive light examining mode. Rather than point-by-point, in DLP the light is projected onto the outside of the layer without a moment's delay. This enjoys a huge benefit in handling time.[19] Photocuring-based bioprinting approaches have a quick creation time. Less reliance on mechanical powers creates higher cell practicality. What's more, complex designs of tissues can be developed utilizing these methods with the high goal.[20] In any case, the cycles require an exceptionally cautious determination of biomaterials, and photoinitiators are frequently acquainted with the bio-ink to further develop photosensitivity which can influence cell feasibility. Additionally, the combined UV openness is a disadvantage.[21] The excellent resolution and fast speed of stereolithography bioprinting offers a strong cancer research potential. In stereolithography, however, bioprints must be clear enough that light passes the material and cross-links the photopolymer without substantial dispersion. To minimize light scattering and homogenous crosslinking, cancer cell density is usually kept at a low level which is another limiting factor for this technique.,[22,23] Compared with extrusion, the stereo printing technique increases the cost of the printer device and the difficulties of its modification. The results showed a higher migratory potential than traditional 2D cell culture for breast cancer cells grown on 3D scaffolds. Moreover, there was a higher drug resistance compared to 2D models in 3D printed matrices. 3D printed scaffolds therefore offered biomimetic microenvironment for the growth of cell breast cancer and are available for the research of the behaviour and assessment of new treatments against breast cancer.[24] This inkjet bioprinting method enabled the production of high-performance, flexible, and regulated spheroids of the cellular breast cancer Figure 3.

Droplet-based bioprinting

Bead-based bioprinting utilizes drops of controlled volumes of bio link to deposit at foreordained areas. Because of its exact control of testimony, effortlessness, and adaptability, it has an enormous space of utilization including regenerative medication, transplantation, high throughput screening, oncology, and so on.[25] Inkjet bioprinting is a non-contact procedure of bioprinting in which beads of bio-inks are launched out under tension.[26] Inkjet methods utilize the actual properties of bio-inks, like thickness, surface pressure, thickness, and so on Inkjet bioprinting can be ceaseless or drop on request (DOD).[26] While consistent inkjet bioprinters ceaselessly discharge bio-inks, DOD inkjet bioprinters utilize constrain heartbeats to launch drops when required. The pressing factor beats are normally created by warm, piezoelectric, or electrostatic actuators.[27] The warm actuator in a warm inkjet bioprinter is an electric warming unit that disintegrates the bio-ink answer for the structure of a fume bubble. In the end, the fume bubble extends because of the pressing factor and quickly detonates, creating a heartbeat pressure that launches a bio-ink drop.[28] Such temperature changes don't essentially influence cell reasonability as the cycles just take a couple of microseconds Figure 4. The bio-printed cells have been evaluated to keep up with

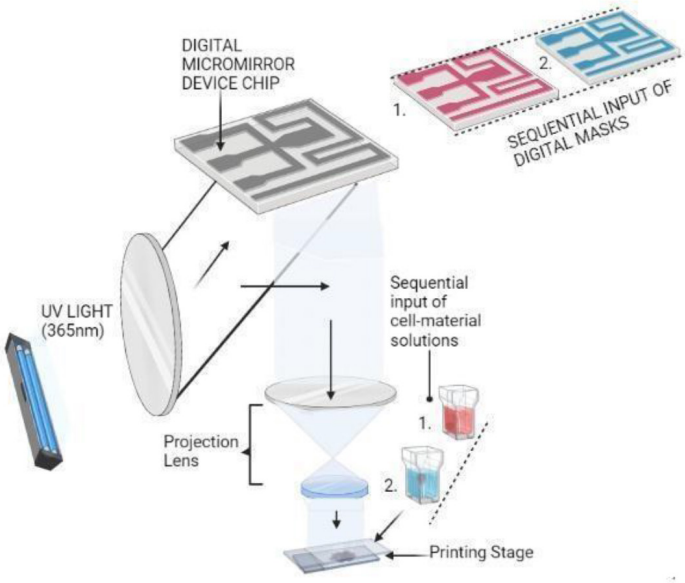

Figure 3: Stereolithography (SLA), a photocuring-based method setting photosensitive polymers to frame tissue developed under accurately controlled lighting.

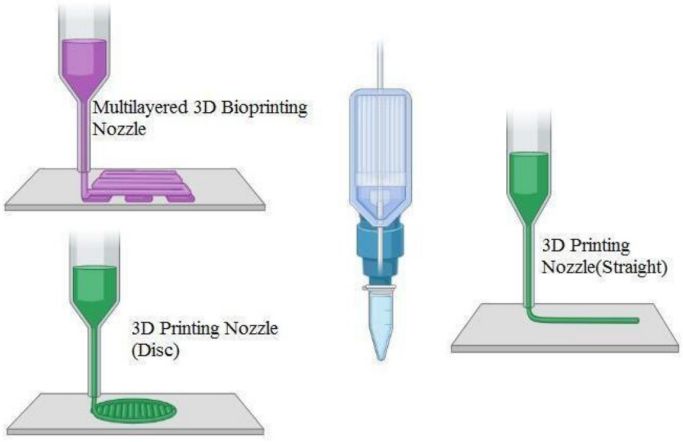

Figure 4: Droplet-based bioprinting based- Inkjet bioprinting is a non-contact procedure of bioprinting in which beads of bio-inks are launched out undertension.

their multiplication limit, genotype, aggregate, and capacity. On the other hand, in a piezoelectric inkjet bioprinter, a voltage beat makes the piezoelectric actuator change its shape. The abrupt change in the volume of the liquid chamber containing bio-ink in this way causes the arrival of a bead. There was no issue in supporting the cell feasibility even get-togethers measure. Electrostatic actuators work also. At the point when a voltage beat is applied between a pressing factor plate and an anode, the pressing factor plate avoids. There was around an 80 to 95% yield in the cells printed from warm, piezoelectric, and electrostatic inkjet bioprinting strategy, affirming high cell reasonability.[29-31] Expulsion of the voltage beat takes the pressing factor plate back to its unique shape shooting a bio-ink bead. Inkjet bioprinting procedures show guarantee as they give high-goal printing because of its fine command over the launch of beads and pico-liter estimated ink drops.[32] EHDJ bioprinting procedures utilizes an electrical field to drive the bio-ink drops. The bio-ink arrangement is siphoned through a needle associated with a high voltage generator.[33] High-goal printed tissue can be accomplished, initially because the spouts are a lot more modest in breadth than inkjet printers. This permits the drops to be substantially more engaged and exact. Furthermore, electrohydrodynamics produces beads that can be fundamentally more modest than this breadth. The size of the drop is likewise impacted by the voltage applied-high voltage bringing more modest drops. In conclusion, horizontal varieties are insignificant in drop arrangement because of the engaged circulation of electric field lines.[34] EHDJ printing is a relatively intricate interaction. Cautious determination, control, and streamlining of bio-ink are fundamental for this procedure as it isn't just subject to the consistency, surface pressure, and thickness, yet additionally the electrical conductivity and vanishing pace of the bio-ink.[35] Cell reasonability relies upon the applied voltage, bio-ink stream rate, and bio-ink properties. Acoustic bioprinting the acoustic bioprinting strategy keeps the biomaterials liberated from adverse pressure like warmth, high voltage, high pressing factor, and any type of shear pressure. Beads are shot out utilizing a delicate acoustic field through a spout. In any case, delicate acoustic fields are not equipped for shooting drops of bio-inks that are thick or have high cell fixation. Studies on this method are very restricted.[36] Micro-valve bioprinting Micro-valve bioprinting utilizes an electromechanical valve to control the launch of drops. The spout opening of the gadget is gated by the valve which is unlatched by an attractive field made by a voltage beat. The pressing factor in the liquid chamber containing bio-inks beats the surface pressure bringing about the age of a drop.[37] Cells are less inclined to harm through this interaction than piezoelectric bioprinting because of the low scope of pneumatic pressing factor utilized. Drop-based bioprinting offers an astounding spatial goal which makes it alluring for application in tissue designing and regenerative medication. These strategies likewise give a decent goal and generally high cell suitability at a lower cost. However, bead-based techniques additionally have their downsides. The most noticeable issue is the obstructing of the spout when the bio-ink is excessively thick.[38] The production of uniform spheroids on high-speed microchips is very likely to lead to the development of pathology and cancer medicines, because these cancer spheroids are a three-dimensional model for cancer *in vivo* imitation of the carcinogenic environment.[39] Increased bioprinting leads to relatively high cell survival, notably for piezoelectric actuation systems, in addition to high-throughput and high-resolution advantages. The primaCustom inkjet bioprinting method that employs hydrogel cell-embedded arrays deposited in the modelling of breast cancer by means of a drop-on-demand approach on polyethylene glycol methacrylate chips tory restriction in inkjet printing is that the demand for bioink is low in viscosity (~0.1 Pa).[40] Laser-assisted bioprinting with comparative systems as inkjet printers, laser-helped bioprinting utilizes laser heartbeats to actuate microbubbles. Backing containing bio-ink as a slender sheet or film is connected to laser-retaining metal or metal oxides, generally gold or titanium. A laser bar is struck at the interface of the objective substrate and the absorptive layer, causing warm volatilization and consequently the development of a microbubble. Bio ink drop is shot out through the extension of the microbubble.[41] Beating the restrictions of other drop-based strategies; laser-helped bioprinting upholds bio-inks with higher viscosities. The obstructing issue is missing on account of the laser-assisted method as it is without spout. The non-contact, nozzles-free measure additionally shields cell parts from shear pressure bringing about higher cell practicality.[42] However, the phone's reasonableness likewise gets somewhat decreased because of the beat laser innovation engaged with this instrument.[43] The laser-helped bioprinting additionally gives high-goal printing. However, it is profoundly costly and mind-boggling, prompting a few functional issues.[44] Even though the cycle is quick, bead size restricts the general volume testimony over the long run.[45]

CHALLENGES AND PROSPECTS FOR THE FUTURE

Biomaterials and living cells can be precisely positioned in 3D bioprinting technology to reconstruct complex structures that can be utilised for disease modeling and medication screening. Researchers have employed this technology to create tissue models with organ-specific activities, drug testing applications, and transplantation potentials in the domains of liver, heart, vascular structure, and cancer. Despite recent advances in this research, obstacles remain in terms of the printing platform, cells, and materials utilized to construct tissue models, including limitations in fully replicating the cellular organization and structural complexity of native tissues. Increased resolution, printing speed, biocompatibility, and scaling-up are among the technological hurdles facing 3D printing platforms. Microscale resolution is currently only possible with light-assisted bioprinters, which is also dependent on the type of material utilized and the cell concentration in the printing mixture. To manufacture complicated single-cell structures such as capillary networks and the blastocyst cavity, higher printing resolution is still in high demand. For printing organ level structure, higher printing speed remains a key problem. As printing time goes on, the viability of cells in the printing fluid decreases, especially for metabolically active cell types like liver and muscle cells. The biocompatibility of 3D printing platforms has been reported to be adequate in terms of cell viability, however the effects on gene expression and functional aspects have received less attention. Various mechanical and visual disruptions to cells are involved depending on the type of bioprinter employed. Additional research into the mechanical and optical effects of the bioprinting process will provide more insight into the 3D printing technique' biocompatibility. Lastly, there are still hurdles to the scale up of bioprinted tissue constructions. The majority of the applications that have been described thus far have been based on small sample sizes.

CONCLUSION

Future work is needed to standardize printers, cells, materials, and the printing process in order to consistently manufacture huge amounts of tissue models for clinical and commercial uses. To fully fulfill the potential of 3D bioprinting in building advanced *in vitro* disease models and precision medicine, advances in both science and technology in the domains of medicine, engineering, and biology will be required.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

DIW: Direct ink writing; **FDM:** Fused deposition modeling; **LIFT:** Laser induced forward transfer; **TERM:** Tissue engineering and regenerative medicine; **DLP:** Digital light processing; **SLA:** Stereolithography; **EHDJ:** Electrohydrodynamic Jetting.

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