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Applications of 3D Printing Technology in Angiogenesis Model for Cancer Research

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Abstract. 3D printing is a technology that has gradually advanced with the development of technology and has been used in medicine and cancer treatment. 3D printing's ability to prototype quickly allows it to quickly convert fundamental cancer research findings into therapeutic applications, which could speed up or possibly completely transform the entire medication discovery and development process. Angiogenesis is an essential process in normal development and adult physiology but is disrupted in many diseases. The review summarizes the applications of 3D printing technology in angiogenesis models for cancer research. 3D printed PLA-bio-glass composite for bone tissue engineering can be used to study bone healing and regeneration. The *in vitro* microvascular model using 3D printing technology can be applied to explore the role of angiogenesis-related proteins. A 2D or 3D histological model is sufficient to track cancer cell dynamics. A combination of 3D bio-printed blood vessel models and spheroids can be a cost-effective and efficient method for drug testing to evaluate both tumor growth and angiogenesis. In future research, it can be expected to develop technologies to support 4D printing of personalized adjustable bionic periosteum with anisotropic microstructures to accelerate angiogenesis and bone healing.

Keywords: 3D printing, angiogenesis, cancer.

1. Introduction

Cancer is a collective term for a group of diseases that are presumably characterized by malignant tumors that can be present in various parts of the body [1]. One of the most important characteristics of cancer, especially in solid tumors, is angiogenesis, which means the development of new blood vessels [1]. Angiogenesis, which is the process by which Existing capillaries or postcapillary veins develop to form new blood vessels, is an important process in the normal development of the human body but may be dysregulated in many diseases. The concept of generating targeted blood vessels to treat disease was proposed by experts more than 50 years ago, and two of the first drugs targeting vascular endothelial growth factor were bevacizumab and pegaptanib, which were approved for the treatment of cancer and neovascular ophthalmic diseases in 2004 [2].

The emergence of new technologies, especially three-dimensional (3D) bioprinting, has been used in angiogenesis models, as well as other models. Since 3D printing allows for rapid prototyping, 3D printing has the potential to expedite and potentially completely change the entire drug discovery and development process, bringing fundamental findings in oncology into the clinic [3].

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This review sheds light on the most recent uses of 3D printing in cancer research and treatment, ranging from basic science and drug discovery to clinical applications and medication development. These include customized nonbiological medical gadgets, 3D-printed cancer cell models, and anticancer pharmaceuticals. Ultimately, the difficulties associated with using 3D printing for cancer applications are discussed, and the prospects for these applications are projected.

2. Angiogenesis

Angiogenesis is an important process in normal development and adult physiology, however, in many diseases, normal growth may be disrupted. Tumor angiogenesis is a highly complex process that includes steps that include degradation of the vascular endothelial matrix, endothelial cell migration, endothelial cell proliferation, endothelial cell ductal branching to form vascular rings, and formation of a new basement membrane [2]. Due to the abnormal structure and function of this neovascularization in tumor tissues and the imperfect vascular stroma, this microvasculature is prone to leakage, and thus tumor cells directly penetrate the vasculature to enter the bloodstream and form metastases at distant sites without undergoing a complex invasive process [2]. Therefore, angiogenesis plays an important role in tumor development and metastasis, and inhibiting this process can significantly prevent the development and spread of tumor tissue.

3. 3D printing

With the development of technology, more and more new technologies are used in medical treatment, including 3D printing. 3D printing is now widely used in the medical field, including cancer treatment. 3D printing, by virtue of its ability to produce prototypes quickly, can provide specialists with customized products and can easily create prototypes.

3D printing simulates complex structures specific to the patient in a highly accurate and relatively easy way. The high precision and relative ease with which 3D printing mimics complex patient-specific structures can be used in cancer treatment as a way to print anti-cancer agents, 3d bioprinter cell models, and customized medical devices [4]. Two-dimensional models have been traditionally used in cancer research due to their affordability and simplicity, and they have contributed to the discovery and development of many drugs. However, most of the studies are not directly translated into clinical applications. This is becausel cell cultures do not recapitulate the *in vivo* tumor microenvironment in humans. Instead, animal models are expensive and species differences lead to differences in gene expression, protein expression, and soluble factors (cytokines, growth factors, etc.), which are critical for studying cancer progression [3]. While the development of 3D cell culture models has overcome these problems, it has also resulted in longer culture times, less-than-ideal reproducibility, and higher costs. Bioprinting utilizes 3D printing technology to build 3D bio-printed models that closely resemble actual tissues or organs by embedding living cells, biomaterials, and growth factors in layers on a scaffold [3]. 3D bioprinter cell models have been developed to alleviate this problem, and the benefits of this technology are cost reduction while increasing the flexibility and complexity of structural design [3].

4. Applications of 3D printing in cancer treatment

3D printing has been utilized in multiple areas in cancer research. Table 1 summarizes current progress of applications of 3D printing in cancer research, especially studies related to angiogenesis.

4.1. Bone tissue engineering

Surgeons still have a great deal of difficulty when it comes to defects in massive bones that have been removed or damaged due to malignancy. There is a great chance of creating a non-cooperative treatment because these problems frequently do not cure. The gold standard for treating this problem is autologous bone grafting; however, this procedure comes with a high cost and requires follow-up surgery in addition to its negative effects. Furthermore, there is a finite quantity of content. Gold standard treatments come with risks [4].

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Numerous biocompatible, biodegradable, and mechanically stable 3D printing biopolymers, such as polylactic acid (PLA), polycaprolactone (PCP), and polylactic acid-perylene (PGLA), among others, may be appropriate for bone tissue engineering applications [4]. These materials (e.g., PLA) can be combined with bioactive materials (e.g., hydroxyapatite (HA), tricalcium phosphate (TCP), or bioglass (BG) to create composites that can be printed on demand and have high bone conductivity and osteoinductive qualities [4]. Nonetheless, specialists have developed a printable material with a 20% concentration of S53P4 BG and PLA in recent years. With ordinary Cartesian 3D printers, this material can be used to manufacture intricate, porous, and finely structured scaffolds [4]. In the first step, the porous structure of the material is described and mechanical stability is demonstrated, showing the potential of this new material [4]. We demonstrate the homogeneous distribution of BG particles in the PLA matrix and the long-term release of calcium from this material, which increases with increasing BG concentration [4]. As the complex's BG concentration rose, so did the adherence of mesenchymal stem cells (MSC) and their osteogenic and anti-inflammatory qualities [4]. As a result, protein array analysis after whole blood stimulation experiments failed to detect a discernible inflammatory potential. At least some of the effects seen were mediated by the calcium produced by this substance, which was dependent on the BG content. Therefore, the majority of the criteria outlined in the Diamond Bone Healing Concept—osteoconductive, osteogenesis, and mechanical stability—are satisfied by this 3D-printed material [4]. It is still not a perfect answer, though, and it must be determined if it satisfies the requirements for angiogenesis as well [4]. A crucial component of the complex process of fracture healing is angiogenesis. Both tissue regeneration and bone healing are dependent on the vascularization of the implanted biomaterial.

4.2. In vitro microvascular models

The emergence of organ-on-a-chip technologies has attracted a great deal of attention. The goal of these technologies is to use cultivated cells, which are often treated with microfluidic procedures, to create bioactive artificial tissues. Specialized cells, such as human cells that mimic human tissues, are used to model 3D tissues [5]. Artificial microvessels have a lot of promise in this area for clarifying vascular function [5]. For instance, single-vessel models for permeability measurements and vascular networks within microfluidic channels have been devised to enable the observation of angiogenesis. These methods enable multilateral investigation of the vasculature, which has promise not just for basic biology studies but also for drug screening [5].

Experts have developed an EGFL7-knockdown *in vitro* microvascular model, and with this 3D microvascular model, they have identified a novel role for EGFL7 endothelial function in the process of germinal angiogenesis [5]. In adults, germinating angiogenesis, the formation of new blood vessels from pre-existing vessels, occurs primarily during tissue growth and repair. Germinating angiogenesis also occurs in patients with cancer, and in these cases, angiogenesis leads to disorganization of the vascular network and vascular dysfunction [5].

Using an *in vitro* EGFL7-knockdown microvascular model, researchers have discovered a new function for EGFL7 endothelial function during the process of germinal angiogenesis [5]. In adulthood, tissue development and repair are the main processes that lead to seedling angiogenesis, or the creation of new blood vessels from pre-existing ones. Patients afflicted with cancer may also have germinal angiogenesis, which can result in vascular malfunction and network disarray [5].

The study illustrated the applicability of this strategy for figuring out the molecular mechanisms behind vessel emergence using micro-vessels on a chip. We examined the impact of EGFL7 loss on 3D sprouting angiogenesis and endothelial barrier function using an *in vitro* human microvessel model. One of the main characteristics of many chronic conditions, including osteoarthritis, age-related macular degeneration, and cancer, is pathologic angiogenesis (OA).

Implant rejection and cartilage deterioration can result from OA, thanks to factors such vascular endothelial growth factor (VEGF)-mediated pathological angiogenesis. Bevacizumab (BVZ), an anti-VEGF medication, has been demonstrated to promote cartilage regeneration and stop the advancement of osteoarthritis (OA) [5]. The study illustrated the applicability of this strategy for figuring out the

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molecular mechanisms behind vessel emergence using micro-vessels on a chip. We examined the impact of EGFL7 loss on 3D sprouting angiogenesis and endothelial barrier function using an *in vitro* human microvessel model. One of the main characteristics of many chronic conditions, including osteoarthritis, age-related macular degeneration, and cancer, is pathologic angiogenesis (OA). Implant rejection and cartilage deterioration can result from OA, thanks to factors such vascular endothelial growth factor (VEGF)-mediated pathological angiogenesis. Bevacizumab (BVZ), an anti-VEGF medication, has been demonstrated to promote cartilage regeneration and stop the advancement of osteoarthritis (OA) [5]. The discovery that patients with osteoarthritis have blood vessels in the articular cartilage and inner meniscus, which are typically avascular tissues, led to the formation of the connection between pathologic angiogenesis and osteoarthritis [5]. Among other things, members of the vascular endothelial growth factor (VEGF) family are important in OA, AMD, and cancer. This angiogenic mechanism is linked to tissue deterioration and the gradual degradation of the extracellular matrix (ECM) in osteoarthritis. In fact, one of VEGF's functions is to block tissue inhibitors of matrix metalloproteinases (TIMPs) and promote the degrading enzymes matrix metalloproteinases (MMPs) [5]. Additionally, by monocyte chemoattraction, VEGF-mediated cell invasion may cause implanted cartilage scaffolds to resorb prematurely *in vivo* [5]. These findings highlight how crucial it is to control VEGF activity during OA and while looking for scaffold-mediated fibrocartilage and avascular regeneration [5].

4.3. Cancer cell dynamics

Physiologically responsive *in vitro* models capable of tracking cancer cells in a tissue-like environment are severely lacking in cancer research. Although a variety of cell types can be found in current models, such as 2D or 3D *in vitro* cultures, they are unable to capture the intricacy of an entire microvascular network [6]. This work attempted to show that bioprinting cancer cells on excised animal tissue was a feasible way to build a tumor microvascular model. 4T1 murine cells, which were procured from the American Type Culture Collection (Manassas, VA), were the breast cancer cell lines utilized in these investigations. Cell culture and labeling was the initial stage [6]. When the cells achieved 75% confluence, they were subcultured [6]. After printing on day 0 and cultured on day 5, printed DiI+ 4T1 cells were still alive. Cell migration and proliferation were tracked over a 5-day period using time-lapse imaging. Both the number and area of cells grew dramatically over time. Following cultivation, angiogenic microvessels and cancer cell clusters co-localized [6]. Tissues containing bio-imprinted cancer cells had a higher frequency of vascular islands, which are isolated segments of endothelium cells. This suggests that the presence of cancer cells affects the early phases of angiogenesis [6]. The approach for exploring tumor cells with microenvironmental alterations was validated using bioprinting of 4T1 cancer cells that were knockdown for catechin L on wild-type tissues or non-targeted 4T1 cells on NG2 knockdown tissues. These findings demonstrate the possibility for studying cancer by bioprinting cancer cells onto real mouse tissues.

4.4. Drug testing

With 3D-bioprinted vascular system, tumor microenvironment can be minimized, which can be used for drug testing. A screening system was built *in vitro*. The bio-printed human vascular endothelial cells and lung fibroblasts built the vascular layer. Then multicellular tumor spheroids were seeded. After culture for 4 days, blood vessels migrated into spheroids, and angiogenesis occurred [7]. With the *in vitro* 3D bio-printed model, researchers treated the model with anti-cancer drugs and tested tumor microenvironment, angiogenesis, and cancer development. The results showed that a combination of temozolomide (TMZ) and sunitinib, which is an angiogenic inhibitor, effectively inhibited angiogenesis and tumor growth [7].

5. Future technologies

In order to speed up angiogenesis and bone repair, it appears as though technology will enable the 4D printing of customized, changeable bionic periosteum with anisotropic microstructures in the future. Ideally, a perfect biomimetic periosteum will wrap various bone surfaces to provide the optimal milieu

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for bone repair [8]. This entails luring osteoblasts, promoting local vascularization, and mineralizing the extracellular matrix (ECM). In order to mimic the natural periosteum's role in promoting bone regeneration, aligned cell sheets are inlaid into shape-shifting hydrogel for the first time using a 4D printing process that incorporates biophysical signals and spatially changeable physical qualities. The exterior hydrogel layer allows the biomimetic periosteum to digitally coordinate its 3D geometry to match the specific macroscopic bone shape, hence maintaining a milieu favourable to bone repair. Superior osteogenic differentiation properties are exhibited by the inner aligned layer of human mesenchymal stem cells (hMSCs), which also promotes co-cultured cell motility and angiogenesis [8]. *In vivo* experiments showed that the aligned biomimetic periosteum not only transforms pre-set forms into physical barriers, but can also actively encourage local angiogenesis and early-stage osteogenesis.

Table 1. Applications of 3D printing in oncological angiogenesis research

Study purpose	Methods	Conclusions	Ref(s)
3D-printed composite material made from polylactic acid (PLA) and bioactive glass (S53P4) for bone tissue engineering, addressing the challenges of treating large bone defects.	A 3D-printed composite of PLA and 20% S53P4 bioactive glass, analyzing its mechanical stability, porous structure, calcium release, and interactions with mesenchymal stem cells to assess its potential for bone tissue engineering.	The 3D-printed PLA-bioactive glass composite exhibits favorable properties for bone tissue engineering, including osteoconductivity and mechanical stability, but further research is necessary to evaluate its ability to promote angiogenesis, essential for effective bone healing and regeneration.	[4]
The role of EGFL7 in endothelial function and germinal angiogenesis, particularly in pathological conditions like cancer and osteoarthritis (OA). It highlights the implications of EGFL7 in regulating angiogenesis and vascular integrity.	An *in vitro* 3D microvascular model using EGFL7-knockdown human primary endothelial cells to analyze the effects of EGFL7 on VEGF-A-induced sprouting angiogenesis, vascular permeability, and endothelial junction formation. The study also functionalized medical-grade collagen with bevacizumab (BVZ) encapsulated in PLGA particles to target pathological angiogenesis.	EGFL7 is crucial for maintaining endothelial integrity and proper response to angiogenic signals. Its knockdown disrupts endothelial junctions and increases permeability, emphasizing the need to regulate VEGF activity in conditions like cancer and OA.	[5]
The physiologically responsive *in vitro* models to track cancer cells in a tissue-like environment by developing a tumor microvascular model using bioprinted cancer cells on excised mouse tissue.	Breast cancer cell lines (4T1) were cultured, labeled, and bioprinted onto mouse tissues. Time-lapse imaging tracked cell migration and proliferation over five days, while clusters of cancer cells were monitored alongside angiogenic microvessels.	Bioprinting cancer cells on living tissues can effectively study microvascular dynamics in relevant environments, revealing that the presence of cancer cells influences early stages of angiogenesis.	[6]
In vitro model for drug testing	3D bio-printed vessel layer, seeded by multicellular tumor spheroids	An effective model to do drug testing for both angiogenesis and tumor growth.	[7]

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6. Conclusion

3D printing technology has been widely used today in the treatment of cancer research, for example, angiogenesis. The convenience and benefits of this emerging technology compared to past technologies. 3D-printed polylactic acid-biogas composite can be used for bone tissue engineering. *In vitro* microvascular models using 3D printing technology is effective for testing angiogenesis-related genes and proteins. Tissue culture with 3D printing technology can be used to investigate cancer cell dynamics. The combination of vessels and tumor spheroids as an innovative model can be used for drug testing, considering both tumor growth and angiogenesis. Although this technology is not perfectly and skilfully adapted to the treatment of cancer today, future research may optimize the 3D printing and even 4D printing technologies and apply it in cancer research and treatment.

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