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NANOBIOMATERIALS/BIOINKS BASED SCAFFOLDS IN 3D BIOPRINTING FOR TISSUE ENGINEERING AND ARTIFICIAL HUMAN ORGANS

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Abstract. Bioinks are combinations of live cells and biomaterials that are sometimes blended with tissue factor or other biomolecules for the purpose of extrusion if the desired effect is desired. The term "bioprinting" refers to a relatively new method that involves the application or depositing of biomaterial solutions or bioinks in order to produce three-dimensional (3D) constructions that have topologies and mechanical/biological qualities that are similar to those of genuine human tissue or organs. Printed structures are widely used in tissue engineering to heal or repair damaged tissues or organs, as well as in vitro tissue modeling to test and validate newly created medications and vaccinations before being administered to patients. Supporting and directing cell development toward its native surroundings. When it comes to the formation of biological structures, one of the most important challenges is to ensure that specific physicochemical and biological signals are present in a harmonious manner in order to regulate the activity of cells. Additionally, in order to stimulate stem cell differentiation toward certain tissues, exact arrays of stimuli must be established. The introduction of bioactive material with a nanoscale can control the destiny of cells, contributing to their differentiation and enabling the biofabrication of useful structures. Using nano-composite bio-ink, it is possible to create scaffolds that are instructive for cells or cells can be high quality images onto the media. In addition, the addition of nano into 3D printed configurations may make it possible for these structures to be manipulated by a range of external physical stimuli, thereby providing an additional instrument for usage in healthcare applications. With that being said, there is an interest in the development of biological systems that have functionalities such as motion, shape alteration or sensing. In this study, we emphasize how the use of nano-biomaterials in bioprinting techniques results in strategies for tissue regeneration that show promise.

Keywords: *Nanomedicine, Drug delivery, cancer, cancer metabolism; cell signaling; drug development*

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1. Introduction

The combination of biomolecules (such as growth factors) and/or living cells can result in the creation of biomaterial solutions. These solutions can then be utilized for the printing of useable scaffolds or constructs for the purposes of cell transplantation. The process in question is referred to as bioinks. In the course of the printing process,

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two half (3D) structures are built up layer by layer by depositing biodegradable polymers or biomaterial solutions in accordance with a pattern that has been predetermined (Figure 1) (Murphy *et al*., 2014; Chen, 2019; Decante *et al*., 2021).

Printing with live cells is called bioprinting and the structures that are printed are called constructs; printing without living cells is called printing and the structures that are printed are called scaffolds. Unless otherwise noted, bioprinting and its offspring constructions are referred to in this research. Extrusion bioprinting is one of the most popular bioprinting methods now available for use in construct manufacturing. Extrusion of the biomaterial or biomaterial solution is accomplished through the utilization of mechanical forces (Murphy *et al*., 2014; Zhang *et al*., 2021). Three types of mechanical forces are used in extrusion bioprinting: screw-driven, piston-driven and pneumatic. In the poppet valve printing method, the bioink or bioactive solution is pushed out of the needle by pressured air. As a result, the pressure of the air compressor is utilized to control the amount of bioink or biomaterial solution that is deposited. Pneumatic-driven printing has become quite popular because of the benefits of its straightforward operation and low maintenance costs. Within the syringe, the bioink or biomaterial solution is mechanically extruded by means of a piston or a screw during the printing process that is driven by either a piston or a screw (Ning *et al*., 2020). Both screw-driven and piston-driven printing have the potential to provide higher mechanical forces and an increased degree of direct control over the flow of bioink when compared to pneumatic-driven printing (Ning *et al*., 2020; Chen, 2007; Chen & Kai, 2004; Chen *et al*., 2007; Zimmerling *et al*., 2021).

2. Biomaterials/bioinks

Through the use of polymers, a significant number of biomaterial solutions and/or bioinks for the purpose of bioprinting have been developed. Polymers are organic biomaterials that have long chains and high water contents. They have the ability to facilitate tissue regeneration and cell processes such as adhesion, proliferation and differentiation by generating an environment that is similar to that of a hydrated tissue (Benwood *et al*., 2021). Synthetic polymers and natural polymers are the two categories of polymers. In spite of the fact that synthetic polymers are frequently inert to biological processes, they possess powerful mechanical properties and the inherent potential to support cellular actions.

Many of the 3D bioprinting processes are derived from traditional additive or layered manufacturing techniques. However, the direct use of biological living elements in the creation process is what makes 3D bioprinting approaches far more difficult than AM-based scaffold building techniques. There are now a number of businesses producing 3D bioprinters that can produce tissues and organs with dimensions and shapes that are therapeutically meaningful (Figure 1). Extruded plastic or drop of water, sensor or vat-based polymerization bioprinting are the three classes of scaffold-based 3D bioprinting methodologies that are the most widely used (Murphy *et al*., 2014; Hospodiuk & Dey, 2017; Ng *et al*., 2020; Gu *et al*., 2020). This is using a variety of different technical approachesusing a variety of different technical approachesusing a variety of different technical approaches and bioprinting materials.

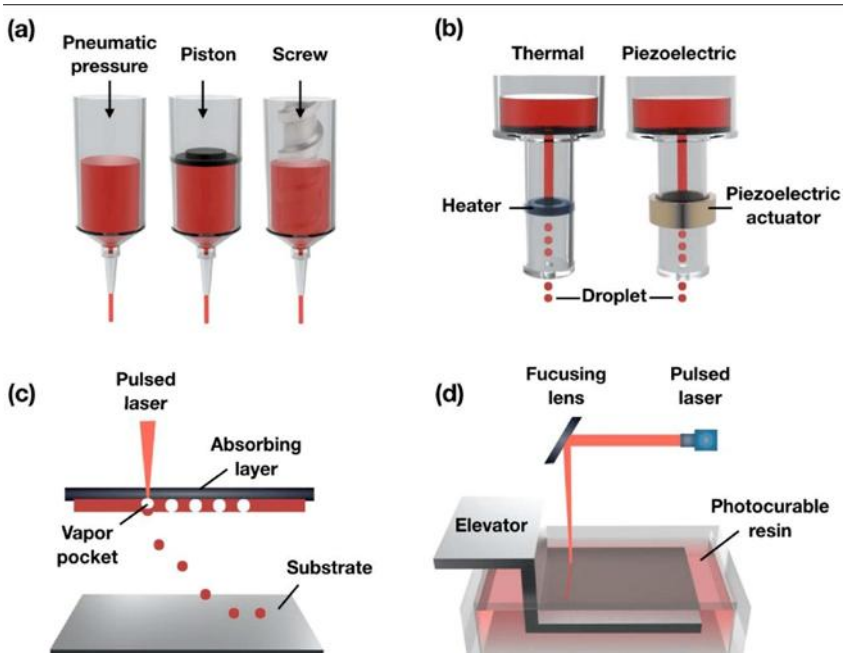

Figure 1. The following is a summary of typical scaffold-based three-dimensional bioprinting techniques: Extrusion-based, inkjet-based, laser-assisted and vat polymerization-based manufacturing techniques are all examples of bioprinting techniques (a, b, c) (Bae *et al*., 2018).

3. Nanobio Composite based-inks

In order for stem cells to differentiate, chemical cues are necessary; more specifically, specific stimuli are required in order to introduce distinction in tissues like cartilage or bone. Because growth factors are responsible for the activities of cells in both space and time, they are frequently utilized in the field of tissue engineering. Regretfully, despite being administered at large doses, these molecules undergo rapid removal and destruction in the absence of protection (Chen *et al*., 2010). This becomes even more important when it is used in conjunction with a bio-ink because it has the potential to further reduce the security of proteins that are already fragile. An innovative method was introduced by Zhu and colleagues, which allows for the direct incorporation of nano-carriers of insulin - like growth factor beta 1 (TGF-β1) into the bioprinted cartilage construct. This allows for the sustained release of TGF-β1, resulting in a markedly improved MSC chondrogenesis differentiation. The 3D-Bloodprinting (3DBP) technique, which is based on stereolithography, was utilized in this work to create the cartilage construct. Through the utilization of a moving head that was endowed with a UV source, the rectangular shape cartilage construct was crosslinked.

The bioink was composed of a hydrogel that was composed of gelatin methacrylate (GelMA) and was combined with nano-spheres loaded with TGF-β1. These nano-spheres had an average size of 120 nm and were created through the use of a co-axial electrosurgical technique (Zhu *et al*., 2015). Poly(lactic-co-glycolic acid) (PLGA), which is the material that surrounds the nano-carrier, gradually degrades while maintaining the discharge of its contents (Danhier *et al*., 2012). The quantitative polymerase chain reaction (qPCR) study demonstrated that the expression levels of collagen II and request of a party in mesenchymal stem cells (MSCs) loaded into the

hydrogel continued to increase when the bioink was augmented with TGF-β1 nano-carriers (Zhu *et al*., 2018).

This approach to integrating biomolecules into bioprinted structures is legitimate. Through the utilization of this method, it is possible to recreate the dynamic presence of biomolecules in the extracellular matrix (ECM). To achieve the desired release profile, it may be necessary to adjust the biomolecule release, which may require a significant amount of time for optimization. This top-down methods of production, on the other hand, results in the loss of a sizeable quantity of material, which is not ideal in situations where the material is considered valuable or where there is a limited supply. Donor biological material is frequently a limitation that must be taken into consideration when contemplating therapeutic applications. In addition, the amount of time required for production could be reduced if the exposure to ultraviolet light could be volumetric rather than punctual, as is the case with a laser source that emits ultraviolet light.

4. Techniques involving cells that are based on nanobiocomposite inks that are operated remotely

It is possible to create nano-composite bio-inks that actively engage with the cells housed inside the bioprinted structure. In fact, when combined with 3DBP, the ability to manipulate nanoparticles through a variety of external physical stimuli offers an additional tool. An external magnetic field may be used to organize and manipulate superparamagnetic nanoparticles (NPs), enabling further patterning possibilities beyond the first pattern produced by 3DBP. As demonstrated in the work of Buyukhatipoglu et al. (2010) it is feasible to locally change a bioprinted structure by combining these methods. They looked at how cell viability and bioprinting parameters are affected when iron oxide nanoparticles are added to alginate solutions.

Whether the non-enriched or nano-functionalized bioink was bioprinted, the manufacturing parameters remained same. The vitality of cells was not impacted by iron oxide nanoparticles, particularly when the NPs were injected into the hydrogel at a low concentration as opposed to when the NPs were allowed to be absorbed by the cells. They demonstrated how the hydrogel's viscosity rarely affects the manipulation of nanoparticles after bioprinting by using a magnet that will move and accumulation the nanoparticles after the bioprinting process (Buyukhatipoglu *et al*., 2009; 2010). They describe the ability to shift biological elements within the bioprinted construct from one location to another as an application of these synergistic technologies. If NPs were moved across the construct, it could have an effect the viability of the cells, despite the strategy appearing promising due to following the cells' absorption of NPs, its stated ability to start moving the cells. Furthermore, following magnetic manipulation, the faithfulness of the printed form may alter. In the end, non-targeted biomaterials may also be displaced by the magnetic field's drag force.

5. Nanobioinks for artificial Tissues

Nanomaterials' extraordinary qualities and powers have propelled them to the forefront of tumor therapies research. Numerous nanomaterials are demonstrating their potential to revolutionize cancer therapy, such as liposomes, Quantum dots (QDs), carbon nanotubes (CNTs), polymeric micelles (PMs), dendrimers, mesoporous silica

nanoparticles (MSNs) and quantum dots (QDs) are all examples of nanomaterials. These technologies have the potential to be utilized in the production of complex biological structures that possess active properties. This would involve the investigation of the synergistic coupling of three-dimensional bioprinting and nanotechnology. There has been evidence of biological structures to possess the ability to sense, move and change form. By bioprinting nano-enriched bio-ink, it is possible to merge organic tissue with functional electrical components. For instance, in a human heart model, hydrogels loaded with cells and nanoelectronic components have been placed together. With this knowledge, cyborg tissues—a three-dimensional hybrid of synthetic tissue and electronics—can be produced.

The bioprinted structure in the bionic hear example was able to both receive and broadcast RF noises. A proof of concept was presented that demonstrated the integration of electrical circuits made from nano-elements with three-dimensional biological constructs. It is superior to the planar flexible electronic devices and sensors that were previously utilized, despite the fact that the conductive components are now discrete rather than continuous (Mannoor *et al*., 2013).

A tissue that is bionic has to perform similarly to the original organ, either by enhancing or substituting a lost bodily function. Using additive printing in conjunction with designed nano-biomaterial surfaces has demonstrated promise in the development of bioactive devices. One example is the use of natural red blood cell membranes that have been wrapped onto polylactic acid nanoparticles in order to replicate real red blood cells (RBCs) with a final aspect of 133 nanometers (RGB-NPs). RBC-NPs are able to bind cytolytic toxins through a non-specific bonding process by utilizing the inherent properties of the human red blood cell membrane. By doing this, they may be included as an active part of a detoxification bioprinted device, which was created by encapsulating RBC-NPs in a hydrogel to provide biomimetic detoxifying characteristics that are similar to those of the liver.

According to the study's authors, their method showed how to effectively address particular to the patient's location functions related to medicinefunctions related to medicine (Chen *et al*., 2017). Their study is now limited by the fact that the device's effectiveness in a dynamic flow arrangement has not been shown. To tell you the truth, the device was only pushed to the limits in a deterministic experimental situation, where it was immersed in a way to solve and used to purify the content of the solution.

The human body's tissues and organs may move, which affects their physiology and capacity to regenerate. Actuators included into biological constructions via the biofabrication process might thereby replicate the natural tissue's movement capabilities. The term “4D bioprinting” refers to the capacity of 3D bioprinted structures to evolve over time. A number of extensively reviewed publications have been published in recent years in this field (Gao *et al*., 2016; Yang *et al*., 2020). Because cardiomyocytes can self-act, they are natural muscle actuators and have served as an inspiration for the development of a new class of soft-engineered, active-motion devices.

Indeed, bioinspired soft robots have been created via bioprinting. Shin et al. (2018) developed a soft robot that resembled a batoid fish and could move autonomously in a swimming motion. CNTs were used into the bioink formulation to provide the GelMA substrate with mechanical guiding and conduction capacity. They could, in fact, create a supporting framework that resembled an internal skeleton, which would allow the cardiomyocytes to connect with one another and indigenously bend a

component of the substrate in order to generate swimming motions. This would be achieved by implementing the anisotropy that is caused by the carbon nanotubes (Shin *et al*., 2018). The bionic construct cannot be controlled wirelessly since it requires electrical stimulation in order to drive the cell beating.

6. Conclusion

In recent years, three-dimensional printing has emerged as a promising new technology that researchers in the field of bioengineering could employ to create three-dimensional biological structures that are unprecedented in their level of complexity. Many of the tissue engineering needs needed to construct biofabrication systems have been met by 3D printing. The several approaches used by the 3D printing technology to create scaffolds for tissue regeneration are covered in this review article. Sintered scaffolds were found to improve the mechanical properties of the biomaterials and did not exhibit any cytotoxicity, according to a number of studies that were conducted on the generation of 3D printed scaffolds for bone formation using CS (calcium silicate) and β-TCP (tricalcium phosphate).

When it came to the generation of 3D printed scaffolds, the polymeric biomaterials that were utilized the most frequently were PCL and PLGA architectures. The biomaterials that are used to make the 3D printed constructions, on the other hand, have been suggested to have a high degree of heat stability and improved cell viability for the production of new bone. This possibility has been put forward. To create intricate architectures for the production of tissues, we will be able to modify certain biodegradable polymeric materials in the future. These materials include polyurethane, polylactic acid, polyanhydrides, polyglycolide and others. Electrospinning, as opposed to fused deposition modeling, is a technique that can be used to create a framework for tissue regeneration. This could be an effective method for tissue regeneration.

It was unearthed that the Ultra violet 3D printed scaffolds had stronger cell viability than the shirtless scaffolds when photopolymerization was implemented to the creation cell-laden scaffolds following the application of photopolymerization. Through the process of fusing bio-ink particles prior to post-printing the cell suspension onto biopaper, a few research studies were able to create tissue spheroids, achieve smooth deposition of cellular aggregates and achieve cell motility for applications related to tissue regeneration. The scaffolds that were created through the use of inkjet and extrusion-based 3D printing techniques had a significant impact on the adhesion, proliferation and differentiation of new bone tissues, according to research that was carried out both in vitro and in vivo respectively.

The growth factor-infused 3D printed samples have improved collagen and extracellular matrix (ECM) and produced high glycogen levels that support the development of new tissue and cell proliferation. Owing to some shortcomings in the creation of three-dimensional scaffolds for tissue regeneration, three-dimensional printing methods such as laser printing, inkjet and extrusion were extensively employed. Combining bioprinters with various operating principles might be a good way to get around the problems. It is necessary to have a multidisciplinary team consisting of medical specialists, engineers, physicists, chemists and attorneys in order to improve the techniques that are currently being used for 3D printing. This indicates that the public and companies that specialize in 3D printing will need to invest more heavily in order to successfully construct tissue-engineered organs ranging from small to large in size.

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