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GELMA BASED 3D BIOPRINTING IN REGENERATIVE MEDICINE - A REVIEW

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Abstract
Over the past few years, research and progress in 3D printing have become evident. The process of bioprinting involves the use of a bioink composed of human cells or tissue. For example, 3D printing in organ transplantation aims to develop an organ that can synchronize with other physiologic components. In the past ten years, bioprinting has made a substantial leap. It has been used in the fabrication of living tissues for its application in various areas. Moreover, this technology has also been commercialized, resulting in its significant interest from the research fraternity. Thus, this review provides a brief on the development of the field from its foundation to the current commercialization with respect to the polymer Gelatin Methacrylate.
Keywords: 3D Bioprinting, Stem cells, GelMA, Regenerative Medicine

Introduction

Bioprinting, a 21st-century manufacturing paradigm, has found its main application in regenerative medicine for the transplantation of artificially generated tissues and organs. Though this technology seems to have developed in the recent past, its evolution began in 1984. In 1986, Charles Hull patented stereolithography, a device for printing tangible 3D objects from data on a computer [1]. Then, a selective laser sintering (SLS) printing process was developed by Carl Deckard [2]. Together, these two techniques led to the birth of 3D bioprinting [1]. In 1999, scaffold molding techniques were used by Anthony Atala's team to produce a synthetic human bladder at the Wake Forest Institute for Regenerative Medicine. In 2003, a bioprinting technique based on inkjet technology was introduced by Tom Boland [3], and in the same year, multicellular spheroids for 3D printing were developed by Garbor Forgacs and his team at the University of Missouri. This technique emerged as the first step towards scaffold-free printing of cells [4]. The following year laser technology to print bio-inks and mammalian cells into three-dimensional structures was introduced by Douglas Chisey's team at the Naval Research Laboratory [5]. The next major step for the industry occurred in 2009 when Organovo and Invetech [6] created the first commercial bioprinter printed skin construct. Some considered this to be the bioprinting endeavors closest to being functional tissue replacements

developed by Anthony Atala at Wake Forest University [7]. Thereafter, ear-shaped constructs [8] and a heart valve model were developed using bioprinting [9]. Finally, in 2014, the first commercially available liver tissue model was produced based on Organovo applied bioprinting techniques [6]. Complex 3D multicellular constructs bloomed by the use of these advanced technologies. Nowadays, organ printing has started to reach its vogue, which is evident through the 3D printed ovaries [10]. Several techniques have been developed with advances in patterning of polymer using micro-contact printing, inkjet fabrication, robotic deposition, dip-pen lithography, and nanoimprinting lithography. Research on 3D printing is growing every year [Figure 1], and 3D bioprinting can become the fortune of medicine.

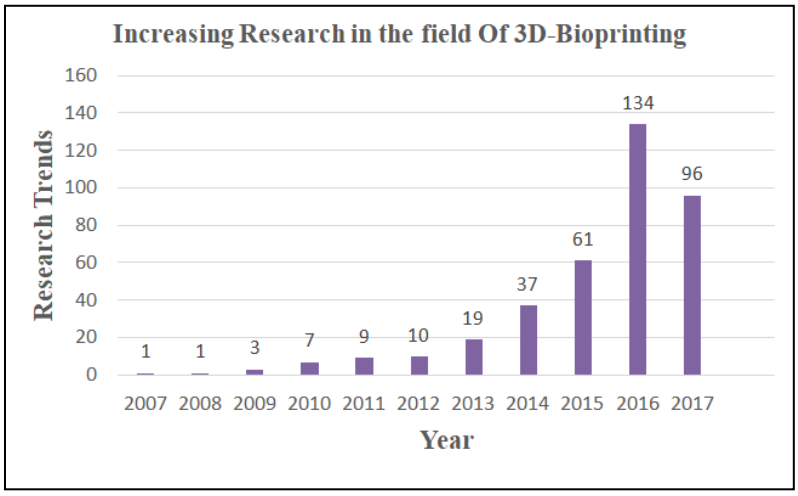

Figure 1: Research growth in 3D bioprinting

3D BIOPRINTING

Bioprinting is a 3D fabrication technology used to generate artificial tissues and create complex 3D structures of biological organs. Since 1984, research for engineered skin tissue has been in the act. The goal of printing human tissues has necessitated the development of cytocompatible bioinks formulated from biomaterials [11] like hydrogels [12]. In this, stem cells have emerged as the most crucial tool for tissue engineering and regenerative medicine due to their distinctive properties of self-renewal and pluripotency. According to their origin, stem cells can be classified as embryonic stem cells (ESCs) or adult stem cells, or the recently discovered induced pluripotent stem cells (iPSCs) [13].

Hydrogels are polymer networks ideal for cellular support and tissue regeneration due to their high water content, biocompatibility, biodegradability, and tunable mechanical properties [14]. Furthermore, due to the structural and compositional resemblances to the ECM, the extensive framework for supporting cellular proliferation, and the convenience for delivery in a minimally invasive manner, hydrogels have been widely used in tissue engineering [15, 16]. Hydrogels with various compositions and forms have been employed as 3D constructs for organizing cells and as scaffolds for delivering biomolecules [17]. In 2011, Schuurman et al. used a mixture of PCL (polycaprolactone), a thermoplastic component in addition to cell-laden hydrogel (alginate) and human chondrocyte cell line (C20A4 cells) as bioink for the development of hybrid tissue constructs using a bio-scaffolder dispensing system. Constructs of different shapes and sizes were developed, and their mechanical properties and cell viability were assessed. Thus, they created a novel approach for generating organized living grafts with improved mechanical stability [18]. Bioinks are different from other biomaterials in that they have the unique ability to be deposited as

filaments during bioprinting [19, 20]. In 2015, Kajsa Markstedt et al. formulated a bioink that combined the fast cross-linking ability of alginate and the shear-thinning properties of nanofibrillated cellulose (NFC) for the 3D bioprinting of living soft tissue with cells. When printability was evaluated, this showed the development of a 2D grid-like structure and 3D constructs (due to the shear-thinning behavior of the bioink).

Furthermore, using MRI and CT images as blueprints, anatomically shaped cartilage structures, such as a human ear and sheep meniscus, were 3D printed. Cell viability of 73% (after 1-day) and 86% (after 7-days) 3D culture was exhibited with human chondrocytes bioprinted in the non-cytotoxic, nanocellulose-based bioink. Based on these results, it was concluded that nanocellulose-based bioink served as a suitable hydrogel for 3D bioprinting with living cells. Thus demonstrating the potential use of nanocellulose for 3D bioprinting of living tissues and organs [21].

GELATIN METHACRYLOYL BASED BIOINKS

Gelatin methacryloyl (GelMA) is an attractive photocurable material that was prepared from a mixture of chemically modified gelatin and methacrylic anhydride (MAA) in the year 2000 [22]. Pure gelatin is water-soluble, which forms thermo-reversible transparent hydrogels. However, it is not stable at body temperature and could not be tuned by researchers. To preclude these drawbacks, gelatin had been chemically functionalized with unsaturated methacryloyl groups to result in gelatin methacryloyl (GelMA). GelMA forms covalently cross-linked hydrogels by photo-initiated polymerization and enables cell encapsulation with high viability [12]. It is considered an important bioink material due to biocompatibility, good mechanical properties, ability to be photopolymerized in situ, and printability. GelMA can be classified into type A GelMA (a product from acid treatment) and type B GelMA (a product from alkali treatment). Type A GelMA has proved to have more efficiency when compared to type B GelMA. Type A GelMA is more similar to collagen in terms of amino acid components, iso-electric point, the capability of helix structure formation. It also has better resolution with tunable physicochemical properties, better physical gelation properties at room temperature, good cell viability (about 75%) [23,24].

Thus, GelMA possessed many relevant characteristics to serve as tissue engineering scaffolds. In addition, the photocrosslinkable feature of GelMA enables flexibility for microengineering by different microfabrication [5]. As a result of this versatility, GelMA hydrogels could be used to produce tissues for clinical, diagnostic, or pharmaceutical research purposes.

Table 1: Application of GelMA In 3D bioprinting

Polymers	Cells	Growth factors	Applications	Technique	References
GelMA + nHA	MSCs +BrCa cells +Bone Stromal cells	–	Breast Cancer Study	Stereo lithography	[25]
GelMa +CS-AEMA +HAMA	BM-MSCs	rh FGFb	Neo cartilage formation	3D Bio-plotter	[26]
GelMA	MG 63 & NHOst Cells	–	Bone Tissue Engineering		[27]
GelMA	MC-3T3 Cells	–		Unibody 3D microfluidic chip printing	[28]
GelMA + PEGMA	BM-MSCs	TGF-ß3	Cartilaginous tissues for musculoskeletal	3D Bio-plotter	[29]

			applications		
GelMA+ HASH	HMSCs	−	Stem Cell Differentiation	Microprinting	[30]
GelMA +Silicate Nanoplatelets	HUVECs+ hMSCs	VEGF	Bone Tissue Engineering	Extrusion based bioprinting	[24]
GelMA + PEG	PDLSCs	−	Periodontal tissue engineering	Pressure assisted value-based bioprinting system	[31]
GelMA + HAMA	Human Adipose Stem Cells	−	Bone Tissue Engineering	A newly developed handheld device based on extrusion-based bioprinting	[32]
GelMA + Sodium alginate + PEGTA	HUVECs	TGF-ß1		Vascular tissue engineering	[33]
GelMA	HepG2 & NIH3T3	−	Tissue Engineering, Organ printing, 3D Drug delivery platforms	Direct write bioprinting	[34]
PEGDA	MSCs	TGF-ß3	Cartilage tissue engineering	Thermal ink jet printing	[35]
GelMA (GPG Bioinks)	HUVECs	−	Tissue engineering	Extrusion based bioprinting	[36]
GelMA + Graphene nanoplatelets	Mouse neural stem cells & Rat (PC-12)	−	Neural tissue engineering	Stereolithography	[37]
Alginate + Methylcellulose	Mouse fibroblast L929	−	not mentioned	Regen-HU printer	[38]
GelMA-PEG	MSCs	TGF-ß		Bone and cartilage tissue engineering	[39]
PEGDA + GELMA	NIH 3T3 fibroblasts	−	Tissue engineering	High-resolution stereolithography	[40]

Commercialization Aspects

With the help of robotic machines and biomaterials, organ printing forms the building blocks of 3D functional organs. It is a highly automated process that offers scalable, reproducible mass production of engineered living organs. The process includes the involvement of multiple cell types positioned precisely to mimic their natural counterparts. Besides, making any functional organ requires three components: cell technology, a biomanufacturing method, and in vivo methods of integration. In cell technology, the function of the cell for clinical application is taken into consideration, while for biomanufacturing technology, the cells and the biomaterial combine to form a functional 3D configuration. Furthermore, in vivo integration deals with the immunological safety related to in vivo studies. Additionally, the hurdle is to get tissue-specific or organ-specific cells which are currently not possible with the available isolation and differentiation technologies. Once such a control has been achieved, post-transplantation rejection by the recipient can be minimized. Another challenge ailing 3D bioprinting is the integration of vascular networks. It is challenging to engineer 3D tissue or organs without vascularization as cells cannot exchange gas, nutrients, and waste products. This further result in low cell viability and faulty organ. Here, the cells should be supplied with proper nutrients, growth factors, and oxygen. For this, an appropriate system has to

be developed. Currently, multi-scale tissue fabrication is not available. Instead of fabricating, a vascular tree printable semipermeable microfluidic channel can be made to mimic vascular environments, which will supplies oxygen and other essential nutrients.

Future Aspects

In the United States, for every 15 minutes, one name is added to the organ transplant waiting list [41]. In 2013, the situation proved that only less than one-third of waiting patients could receive matched organs from donors [42]. However, this growing deficit is unlikely to be met by a supply of transplantable organs that has stagnated over the last decade [43]. One of the techniques to alleviate this organ crisis is tissue engineering and regenerative medicine [44]. To meet this increasing demand for organs, 3D bioprinting could be used, which is anticipated to dominate the global market in terms of market share by 2022 [11].

Conclusion

The great hope of 3D bioprinting for producing reproducible biological constructs lies in creating structures that can be studied for implantation, in-vitro drug delivery, and toxicity in pilot studies [11]. In addition, the 3D cell-laden constructs (GelMA hydrogels) could be designed to mimic the structure of native tissues. This promises its applications in tissue engineering and regenerative medicine.

Another important field is to create hybrid hydrogels by mixing GelMA with other materials, such as inorganic particles, carbon materials, biopolymers, and synthetic polymers. This approach could generate hybrid materials that combine the advantageous properties of the other components, such as superior mechanical properties and conductivity, with the bioactivity of GelMA. Moreover, GelMA based biomaterials will continue to serve as promising candidates in many other biomedical applications that remain explored. In the near future, novel microfabrication techniques will further expand the spectrum of applications of GelMA derived scaffolds. With these emerging technologies and goals, 3D bioprinting will be the realm of future medicine.

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Authors' Contributions:

Ms. Dhakshanya Predheepan and Mr. Subhajit Hazra have made equal contributions to this manuscript.

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