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Biofabrication

TOPICAL REVIEW

Bioink properties before, during and after 3D bioprinting

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Table 3. Overview of commercially available bioinks.

Company	Bioink	Material	Features
Bioink Solutions, Inc.	Gel4Cell®	Gelatin-based	UV-crosslinkable Cell viability >90%
	Gel4Cell®-BMP Gel4Cell®-VEGF Gel4Cell®-TGF	Conjugated with different growth factors	Osteoinductive Angiogenic Chondrogenic
CELLINK	CELLINK	Nano-cellulose/alginate mixture	Shear thinning Fast crosslinking For soft tissue engineering
RegenHU	BioInk®	PEG/gelatin/hyaluronic acid-based	Good cell adhesion properties Biodegradable Mimics the natural ECM Possible combination with Osteoink™
	Osteoink™	Calcium phosphate paste	Osteoconductive Chemical composition similar to human bone For hard tissue engineering
Biobot	Bio127	Pluronic F127-based	Gels at room temperature Dissolves when cooled
	BioGel	Gelatin Methacrylate based	When combined with GelKey it Covalently crosslinks when exposed to light

Gelation methods including physical [27, 34], chemical and photo-crosslinking [35] are used to ensure the stability of bioprinted constructs. Gelation of the bioink should occur *in situ* after the material exits the nozzle and simultaneously with the printing process (e.g. by photopolymerization) [32], because when it already takes place inside the printing head, blockages are created in the nozzle [23]. When hydrogel formation does not occur rapidly *in situ* the bioprinted construct might be compromised due to possible spreading of non crosslinked bioink solution. Further-

can be found (table 3). For example Bioink Solutions, Inc. offers gelatin-based bioinks containing growth factors that are specific for the printing of different tissue types.

Rheological properties of these bioinks are mostly not indicated by the manufacturer. Therefore, they might only be suitable in combination with the companies own bioprinter and would have to be adapted for other printing techniques.

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Review

Recent Advances in Biomaterials for 3D Printing and Tissue Engineering

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Abstract: Three-dimensional printing has significant potential as a fabrication method in creating scaffolds for tissue engineering. The applications of 3D printing in the field of regenerative medicine and tissue engineering are limited by the variety of biomaterials that can be used in this technology. Many researchers have developed novel biomaterials and compositions to enable their use in 3D printing methods. The advantages of fabricating scaffolds using 3D printing are numerous, including the ability to create complex geometries, porosities, co-culture of multiple cells, and incorporate growth factors. In this review, recently-developed biomaterials for different tissues are discussed. Biomaterials used in 3D printing are categorized into ceramics, polymers, and composites. Due to the nature of 3D printing methods, most of the ceramics are combined with polymers to enhance their printability. Polymer-based biomaterials are 3D printed mostly using extrusion-based printing and have a broader range of applications in regenerative medicine. The goal of tissue engineering is to fabricate functional and viable organs and, to achieve this, multiple biomaterials and fabrication methods need to be researched.

Keywords: three-dimensional printing; additive manufacturing; bioprinting; biomaterials; bioinks; ceramics; polymers; composites; tissue engineering

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The bioinks which are mostly hydrogels can be crosslinked using physical, chemical, and enzymatic methods. During the crosslinking step, the sol-gel transition occurs, and this defines the speed of printing, fidelity of the bioprinting process, and resolution. Hydrogels crosslinked using physical agents rely on non-covalent bonds for crosslinking and are generally weak. Physically-crosslinked hydrogels rely on temperature, ionic and hydrogen bonding interactions [31]. On the other hand, chemically-crosslinked hydrogels yield mechanically stable objects using 3D printing. There are many injectable hydrogels available, but for them to be used in 3D printing, they need to be fine-tuned to adjust the kinetics of crosslinking. In most of the chemically-crosslinked hydrogels, a photosensitive initiator is added to the hydrogel that forms reactive species upon exposure to ionizing radiation is used. To promote mechanical stability of printed objects, researchers have used pre- and post-fabrication crosslinking [30]. There are commercially available bioinks that offer reproducible results, such as Gel4Cell®, CellInk®, BioInk®, OsteoInk®, Bio127®, and BioGel® [32].

REVIEW ARTICLE

The arrival of commercial bioprinters – Towards 3D bioprinting revolution!

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3Dynamic Systems (3DS), a UK-based 3D Bioprinting company offers two 3D bioprinters called 3Dynamic Alpha (Figure 4F) and Omega, with the focus of constructing 3D transplantable bone and complex tissue constructs for injured patients[67]. 3DS have partnered with Bioink Solutions to offer a new gelatin-based bioink (Gel4Cell®)[68].

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Title of thesis 3D bioprinting inks based on cellulose nanofibrils and colloidal lignin particles

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Bioink materials should also be easily manufactured and processed, affordable, and commercially available, and bioprinted constructs composed of bioinks are expected to keep their designed shape, structural strength and integrity, easily engraft with the host, and degrade over time *in vivo* (Ozbolat et al., 2017). Currently, there are commercially available bioinks that offer reproducible results, such as Gel4Cell®, CellInk®, BioInk®, OsteoInk®, Bio127®, BioGel® (Tappa & Jammalamadaka, 2018), and GrowDex©. The main requirements for the bioinks and the bioink requirements in different bioprinting technologies are listed in the Figure 6 below.

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Survival Rates of Various Autologous Chondrocyte Grafts and Concomitant Procedures. A Prospective Single-Center Study over 18 Years

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the time of treatment. A total of 45 patients were operated on by classical periosteum-ACI; 80 patients received a fibrin-collagen patch (Tachocomb, Nycomed Pharma, Linz, Austria) seeded by autologous chondrocytes during cultivation and fixed either by periosteum ($n = 59$), collagen membrane by (Chondrogide, Geistlich Pharma AG, Wolhusen, Switzerland) ($n = 15$), or fibrin glue ($n = 6$); 14 patients were treated with alginate-agarose hydrogel (Gel4Cell, TBF, Lyon, France) seeded with autologous chondrocytes during cultivation; 12 patients were treated with a three-layered collagen-hydroxyapatite biomimetic scaffold (Maioregen, Finceramica, Italy) injected with autologous chondrocyte suspension directly upon implantation.

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RESEARCH ARTICLE

Structure establishment of three-dimensional (3D) cell culture printing model for bladder cancer

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MD, USA) in a humidified incubator with 5% CO_2 at 37˚C. Rapamycin (inhibitor of mTOR) was purchased from Sigma-Aldrich (St, Louis, MO, USA). *Mycobacterium bovis* BCG was obtained as a commercial lyophilized preparation (Onco-Tice, NJ, USA). BCG was resuspended in phosphate buffered saline (PBS; Hyclone, Logan, UT, USA) and aliquots with a multiplicity of infection (MOI) of 100 (1×10^7 cells/ml) were prepared and stored at -80˚C until use. A GelMA prepolymer solution was used (Gel4Cell; Bioink Solutions, Daegu, Korea).

Cell culture and construct fabrication

The 2D cell culture samples were seeded (1×10^6 5637 or T24 cells) on 60 mm plates. A 3D cell printer (In vivo; Rokit, Seoul, Korea) was used to fabricate 3D cell cultures. 5637 and T24 cells at a density of 1×10^6 cells/ml were collected by centrifuging at 1300 rpm for 3 min and suspended in GelMA polymer solution for Gel4Cell. The mixtures were gently stirred to ensure that the cells were evenly distributed, and 1 ml was drawn from the mixture into a sterilized syringe with a 25 gauge needle. The user-created branched constructs design was loaded into the computer, and the mixtures were extruded from the syringe needles into the low temperature chamber. The temperature of the nozzle fixer was maintained at 4˚C and the plate bed at 10˚C. They were controlled by a computer program design model (Creator K) by moving the nozzles in the X and Y directions and the platform in the Z direction.