Bio-Inks for 3D Bioprinting: A Scientific Analysis of Two Decades of Progress, Challenges and Future Prospects

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Abstract

The overall interest for organ replacement and tissue regeneration is gradually expanding consistently with time. The advancement in tissue engineering and regenerative drug production utilizing 3D bioprinting have succeeded to regenerate broken organs and tissues into perfect ones. The essential material for this type of printing is Bio Ink, that is a must for creating practical organs and tissue structures. However, the bio inks thus used in 3D printing technology need to satisfy certain properties that are critical for their use in this regime. Combination of various techniques and enhancement in properties of these bio inks are essential for developing more and more efficient bio inks in future as per demand. Present review consists of a thorough study on various modern state-of-art polymer-based bio inks, commonly utilized in 3D bioprinting sector. Several types of bio ink formulations starting from cell-biomaterial based bio inks to cell-based bio inks like tissue spheroids and cell aggregates so far have been used for tissue engineering and regenerative drug production. Additionally tunable bio inks, that are compatible with printable live cells, and sufficiently stable are now-a-days also evolving using chemically composed biomaterials with proper mixing of cells and hydrogels. Hope the current review would succeed to highlight large potential of the most modern bio inks, that are desired for more advanced tissue/organ structure generation using 3D bioprinting in near future.

Keywords: Bioprinting; Bio ink; Tissue; Cell; Organ; Biomaterial, Scaffold; Hydrogel

1. Introduction:

Bio fabrication is a rising field that includes formulations of tissue structures with a definite planning. Typical bio fabrication techniques include various steps like desiccation, electrospinning, micro-engineering etc. Though various fabrication techniques are available there in generating three-dimensional (3D) structures with a variety of biomaterials (Gungor-Ozkerim PS, 2018), they often possess restricted flexibility in the fabrication methodology. Modernist definition of bio fabrication is creation of helpful biological products like micro-tissues, hybrid cell-materials etc. via bio assembly, bioprinting, and consequent tissue maturation processes in a computerized way using living cells, cell aggregates, and variety of bioactive molecules. As of late, this 3D bioprinting has arisen as a distinctive bio fabrication methodology with significantly improved reproducibility powered by the machine-controlled deposition methods. Fundamentally, bioprinting method licenses for the manufacture of 3D tissue constructs having pre-customized designs and geometries using biomaterials or potential living cells as bio ink by contemporizing its deposition through mechanized maneuvers. Laser-assisted bioprinting, inkjet/droplet bioprinting, and extrusion-based bioprinting are the three most popular 3D bioprinting methods available. Coincidental or consequent printing of multiple materials are also possible by using modern multi-head deposition system. For bioprinting, at the very outset, 3D constructs (to be manufactured) are first programmed with a computer-aided design/computer-aided manufacturing (CAD/CAM) system and then the requisite structure is actually made using proper bio inks. These bio inks are vital elements in 3D bioprinting that actually get cross-linked and stabilized by the bioprinting process to produce the desired tissue structure. The right choice of bio ink depends primarily on the target tissue structure and also on the type of bioprinter used. Despite enormous potential, applications of bio-printing technology are till date restricted for lack of acceptable bio inks, required for meeting minimum requirements of bioprinting to own correct bioactivity of various cells. A perfect bio ink got to own some desired mechanical, physical, chemical, and biological properties leading to: (i) adequately mechanically strong tissue structures, (ii) gelation and stabilization processes that are adjustable for bioprinting of flexible structures, (iii) biocompatibility and biodegradability that is capable of mimicking natural microenvironment of the tissues, (iv) chemical modifications to satisfy tissue-specific needs, and (v) large-scale production with minimum batch-to-batch variations. However, all possible bioprinting applications desperately need standardized procedures for bio ink formulation. The main motto of this review is to gift all the readers an in-depth summary of current scenario of existing bio inks. It would also include both natural and artificial biomaterials used either alone or together. The studies in which biopolymers are printed with bio inks made of embedded cells (Mandrycky C, 2016) would also be discussed here. The review would initially describe natural bio inks, then gradually move towards artificial bio inks and cellular spheroids, followed by some commercial bio inks, their potential uses and flaws. A brief discussion would also be there on various new bio ink formulation techniques and related challenges.

2. 3D Bioprinting:

In the field of regenerative medicine 3D bioprinting is an emerging field that can produce cell-laden 3D structures having the capacity of mimicking body tissues playing a crucial role in drug delivery and cancer studies apart from tissue engineering. Bioprinting can generate case-specific spatial geometry with controllable microstructure, and proper positioning of different cells for fabrication of

tissue engineering. In this review, the diverse manufacture procedures would be delineated first and would be discussed shortly along with their benefits and challenges. Then Bio inks would be discussed in detail just as ebb and flow of research in this field. We can find nozzle-based techniques such as inkjet printing, extrusion printing, and laser-based ones, all have the capacity of generating the desired bio printed scaffolds using appropriate bio inks.

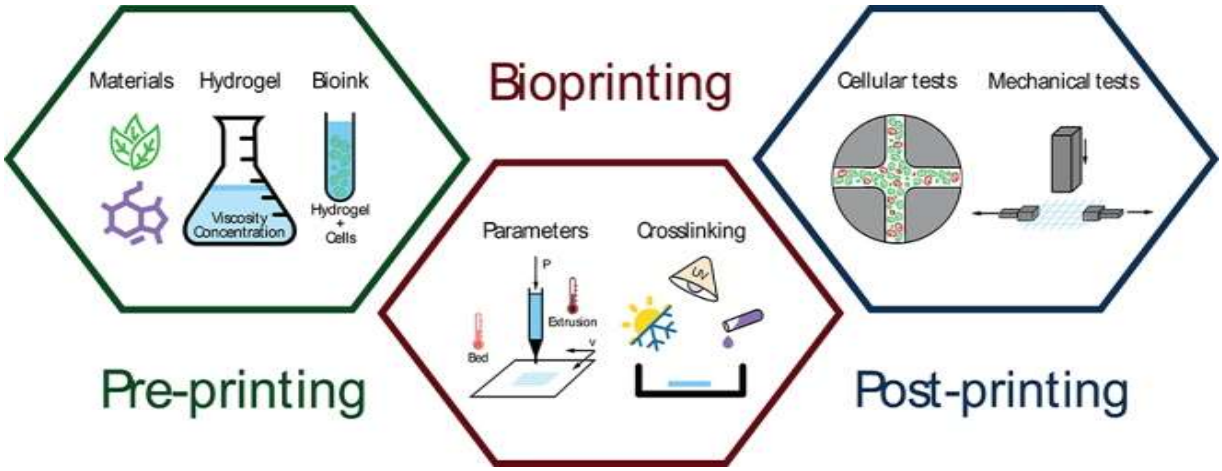

Figure 2.1: Process of Bioprinting

All the formulation techniques were found to effectively affect the cell viability, structural resolution, and speed cum precision of printing. In addition, material choice and their respective concentrations were also found to affect the quality of printing (Hong N, 2018). Every single procedure has its own discrete individual benefit and pitfall and hence we should combine multiple techniques to consolidate the upsides of every strategy. Bioprinting employs the first principle of 3D printing for generating medical specialty elements with employment of biocompatible materials. Such materials are called **Bio ink.** They should in addition contain bioactive components like live cells and growth factors. Developing novel bio inks is one amongst the nice and cozy topics of current day research. 3D bioprinting being triple crown of printing sensible tissues and organs can also create nice influence on regenerative drugs. This bioprinting is done through quite a range of techniques like inkjet, extrusion, and laser/light varieties. Inkjet bioprinters can manufacture notably high-resolution patterns (Vijayavenkataraman S, 2018) by generating little ink droplets on a substrate, that typically needs liquid bio inks. Similarly, laser-based bioprinting also needs liquid-like bio inks. Whereas extrusion-based bioprinting needs bio inks with certain viscousness as this method uses a mechanical force to drive the material via a nozzle in an exceedingly managed manner to construct 3D structures. Inkjet, extrusion, and laser-assisted bioprinting are all used for 3D bioprinting. Different other techniques of bioprinting, like Freedom Reversible Embedding of Suspended Hydrogels (FRESH) are also utilized intelligently for tissue regeneration. Stereolithography is another method that has been adopted for bioprinting. A lightweight stereolithography-based low-cost 3D bioprinter can perform point-by-point curing process that can print mixed cells explicitly with good biocompatibility. It can safely be used for screening of drugs, studying various pathological mechanisms, and preparing biological disease models. Lightweight bioprinting is that vital drive like the laser-assisted one which by choice solidifies a bio ink layer wise additively to build up the desired objects. “Continuous liquid interface production” developed by DeSimone is the most modern technique developed in this field that can astonishingly

improve the printing quality and time. Although that methodology is rarely used except for printing some really difficult and complicated tissue architectures. In this review, we would mainly concentrate on **bio inks** used in various techniques of bio printing, like Pressure-Assisted, Thermal-Assisted, Inkjet, Extrusion based, Magnetic-Assisted, Light-Assisted and Micro-fluidics Bioprinting.

3. Bio-inks:

The main constraints of bioprinting always involve choice of right biomaterials to be utilized as bio inks. Biomaterials whether natural or artificial play an integral role in drug designing (Sun W, 2020) and are utilized effectively in medical applications to support, enhance, or replace broken tissue or a biological system.

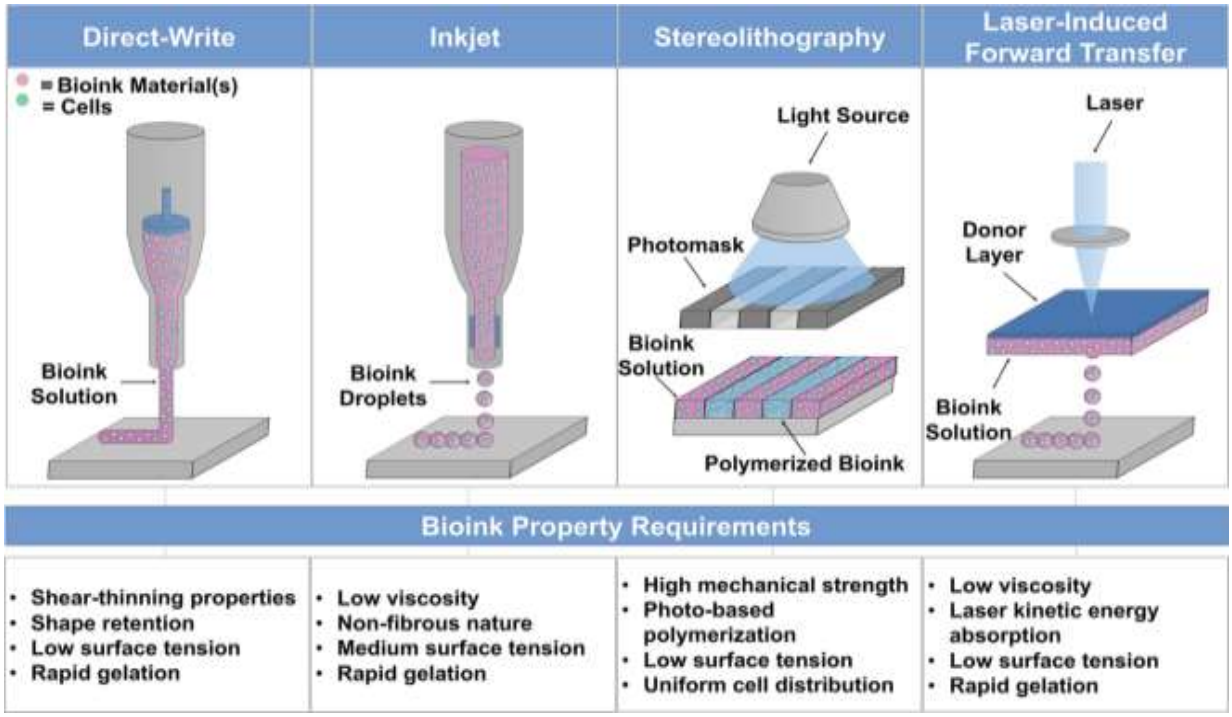

Figure 3.1 Different Bio Inks

Biomaterials are generally composed of cells only, however in some cases, an extra carrier material encapsulating the cells is additionally present. This carrier is typically a biopolymeric gel, acting as a 3D molecular scaffold. The cells present get attached to this gel that allows them to unfold, grow and proliferate. Hydrogels are the mostly used bio inks. Pre-fabrication crosslinking of the precursor hydrogel solutions to lift it to a state of higher viscosity, followed by post-fabrication cross-linking is most desired. Bio inks can embody cells in various forms like single cells, aggregated cell spheroids, cellular rods, mini-tissues or organoids, silk coated cells, cells microcarriers seeded with cells, or encapsulated cells in mixed microenvironment. Here, in this short review, we would try to briefly summarize some of the most used natural and artificial polymers that can be efficiently used as bio inks.

(a) Thermoplastic Polymers: A portion of the conventionally utilized biomaterials are **thermoplastic polymers** in view of their innate flexibility, adaptability, and adjustable habitual properties. Resorbable thermoplastics are one amongst the primary sort of materials that were developed for medical applications. This category of materials has found use in several applications like surgical anchors, screws, pins, and implants. Polycapro-lactone (PCL) (Murphy SV, 2020) could be a perishable polyester that has found wide application in the medical field. PCL is hydrophobic and semi crystalline in nature. This thermoplastic has excellent flexibility in drug delivery and as a system material for bio printed constructs. Polylactic acid (PLA) based thermoplastics are ordinarily used within biomaterial field being approved by Food and Drug Administration (FDA) (Hospodiuk M, 2017) because of its degradability, processability, and biocompatibility. Explicit application of thermoplastics as a support material for protecting softer hydrogel-based materials inside the body demands proper blending of PLA with materials like nanohydroxyapatite and carbon nanotubes. Once incorporated into a block co-polymer with PLA, the degradation mechanics, property, crystallinity, and stiffness are simply tailored. This category of materials has found their usage in drug delivery and tissue engineering in low mechanical stress environment.

(b) Hydrogels: Hydrogel is a network of crosslinked compound chains that are deliquescent, typically found as a colloid in which water is the dispersion phase. The cross links that bond the polymers of a hydrogel can be of two broad categories (Hölzl K, 2016): physical and chemical. Physical cross links contain gas bonds, hydrophobic interaction, and chain entanglements. Thanks to these cross links and structural integrity, colloidal gel network doesn't dissolve much in water, rather they are extremely absorbent. Employment of hydrogels in imprinting has appeared as an active domain of analysis in the last half decade. Although they are ideal materials for cell culture and encapsulation, nonetheless their and hydrous states throw challenges to sound reproduction of planned constructs. In general agarose or albuminoids are employed as the "3D papers" or support and polyethylene glycol diacrylate (PEGDA) is used to print cell encapsulated gels. Induced gelation of alginate with photopolymerizable gels like Gelatin Methacrylate (GelMA) and PEGDA (Gillispie G, 2020) conjointly has now a days become one of the most popular bio inks. Currently, varied bio printable hydrogels have arrived each with specific targeted aims and options. Natural polymers like alginate, chitosan, hyaluronic acid (HA) and gelatin after slight modification can also show smart printability, despite restricted tailoring ability. Artificial hydrogels like polyglicidol and polyethylene glycol (PEG) have shown wonderful chemical properties and mechanical tailoring ability. Chemical modification of natural polymers like HA, alginate, gelatin etc. tailored by chemists to formulate hydrogels has tried to break the demarcation between artificial and natural polymers. Current trend of making multicomponent inks (combos of synthetic/natural/modified materials) can be very useful in near future. Hydrogels usually need a cross-linking step post or throughout bioprinting and might involve light chemical processes and ionic cross-linking for that and this cross-linking of hydrogels via novel methods has really become a promising field recently. Self-healing and shear-thinning hydrogels however, now a days have come into picture that generally do not require any further cross-linking steps. All these approaches have succeeded to form new hydrogels with definite edges in tailor ability and functionality over the natural materials.

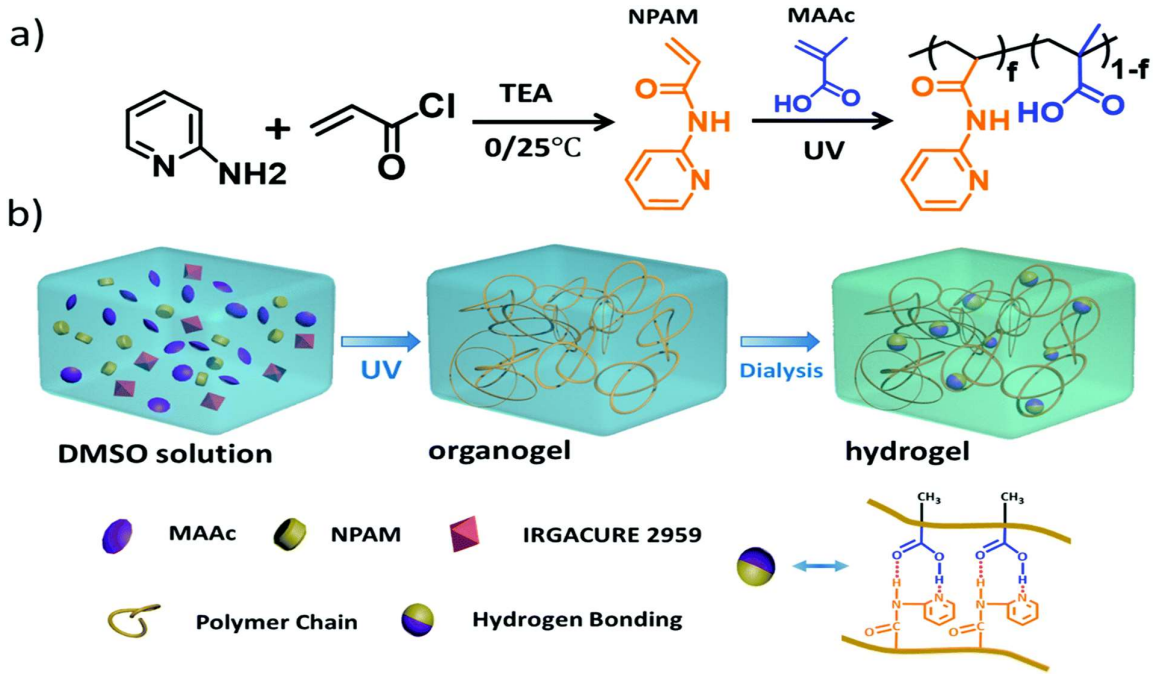

Figure 1.2 Hydrogel Formation

(c) Resins: They are usually viscous substances that can be converted into rigid polymers through specific procedures. Though resins are naturally occurring substances, they can be created synthetically too. Some of these synthetic artificial resins have properties like natural plant resins while many of them are completely different. Resins belong to a special category of photo-cross linked biomaterial inks using various techniques like stereolithography (SLA), digital light processing (DLP) and 2-photon polymerization (2PP) (Ribeiro A, 2017). These cross linkable resins (light assisted) are extensively used in bio fabrication, spanning from thermoset-like materials to soft and hydrated hydrogels. Upon irradiation with UV light, these resins form 3D network. Methacrylate resins as well as GelMA and PEGMA are mostly used for printing of hydrogels and polyesteracrylates for printing of thermosets. DLP method enables massive scale fabrication (Wang C, 2016) of light evoked thiolene resins, polyester acrylates, plastic fumarate resins etc. Cross-linkable resins prepared with ultraviolet light have an oversized variation of properties, depending on the type of the monomers used, density of structural network, light exposure, and secondary cross-linking steps, starting from hydrous hydrogels to useful resins. However, using lower energy light sources, modern techniques allow fabrication of bio ink resins minimizing harmful effects of cytotoxic and mutagenic UV light. Proteome studies envisage that UV cross linkable materials, cells show no discernible injury to their proteome.

4. Biomaterials for Bio Inks and their Properties:

As discussed earlier, bio inks employed for 3D bioprinting ought to possess some vital qualities like smooth printability, characteristic mechanical properties, modifiable functionality, controlled biodegradability, and non-toxicity to target cells. Proper bio ink ought to be selected to regenerate desired tissue structure or organs. Various biomaterials so far have been reported as bio inks (Mirski R, 2019) and natural biomaterials obtained from natural resources have variable benefits over artificial

ones. However, artificial polymers also possess their own unique properties that are absent in natural polymers like tunable mechanical stability, photo crosslinking capacity and so forth.

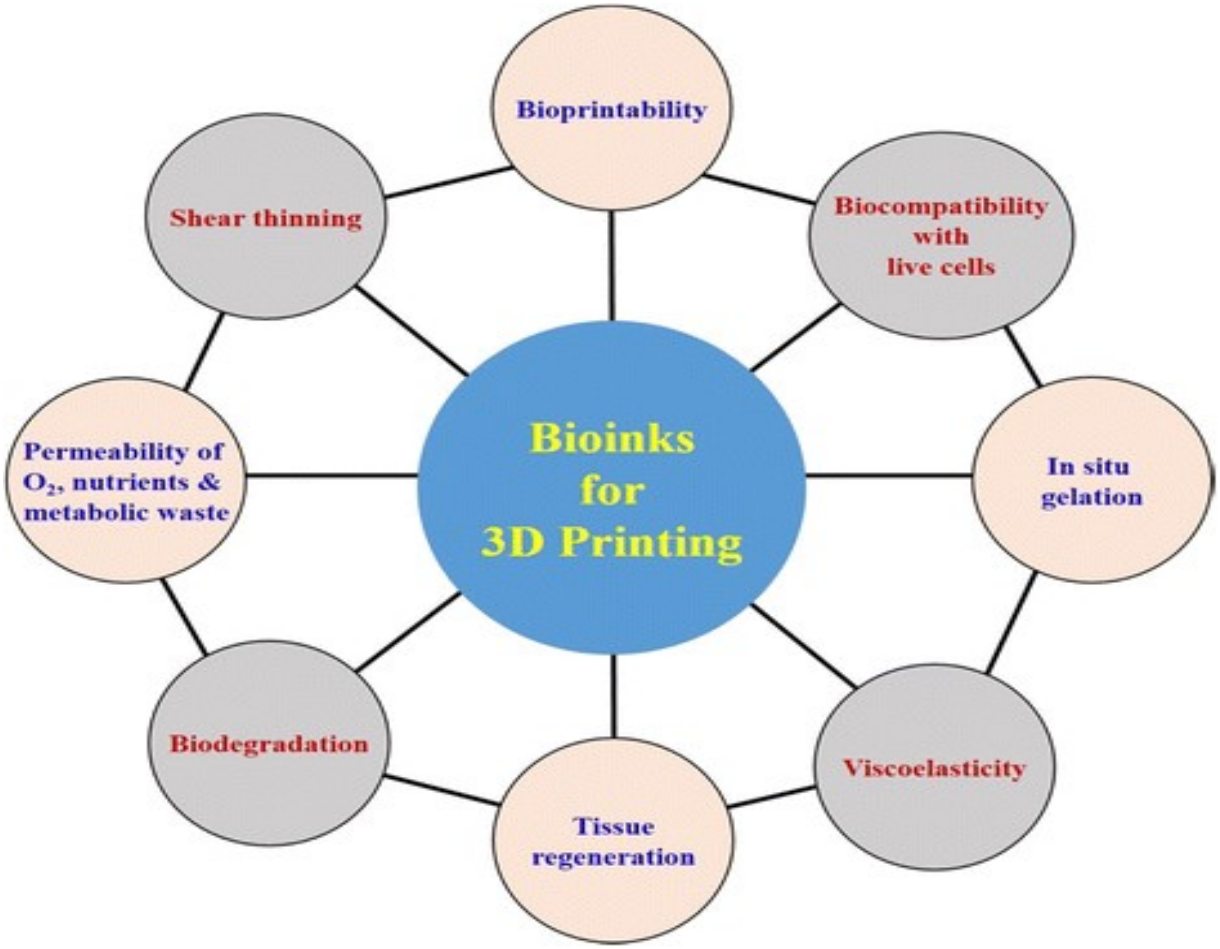

Figure 2.1 Properties of Bio inks

(i) Natural Bio Inks

(a) Agarose-based bio inks: The marine saccharide Agarose, prepared from algae, is one of the most popular biopolymers due to its amazing gel forming ability. Agarose is a linear chain compound with repetitive agaro-biose units with a backbone made up of disaccharides like D-galactose and 3,6-anhydro-L-galactopyranose (Wang C, 2016). However, its capacity to help in cell development is limited, albeit its impressive gelation, mechanical and biocompatibility properties. Hence, blends of purposeful biomaterials together with the agarose gel became quite popular. In 2017 Kreimendahl and his team separately reported employment of agarose-based bio ink consisting of both collagen and fibrinogen. Flexibility of these agarose-based mixed biomaterials to create stable 3D structures and support epithelial tissue and fibroblast cell growth was meticulously reported by them. Yang's group in the same year used agarose/collagen in conjunction with Na alginate for bio ink production for animal tissue engineering applications. Biomaterials thus printed showed increased mechanical properties along with their superior gelling capability. In 2016 Gu and his team succeeded in

developing useful neurons and nerve cells from pluripotent stem cells utilizing agarose along with alginate and carboxymethyl-chitosan (CMC). Chemically modified agarose like carboxylated agarose was also reported to be used as bio inks for formulating automatically tunable 3D tissue constructs. Human Mesenchymal Stem Cells (hMSCs) were also used in some studies yielding terribly high cell viability compared to native agarose gel. Degree of carboxylation could be altered (Huang Q, 2017) to get different gels with varied mechanical properties as per the demand. Ozler and his team reported use of cellular aggregates for direct cell 3D printing with sleek muscle, epithelial tissue, and fibroblast cells. Flexibility of those constructs to fuse with each other to produce higher cell viability was also demonstrated by them. Although these type of bio inks show smart gelation properties, chemical modifications and appropriate mixing are often needed for supporting the resultant 3D printed structures and for boosting a lot of cellular functions as well. In 2017 Park and his team successfully pointed out that a suitable combination of agaroses having both high and low relative molecular masses can actually enhance its ability to make 3D structures as support towards live cells. Fibroblast cell usage in in-vitro studies proved 2:1 (high: low) quantitative mixture to be most sensible and suitable for bioprinting in soft tissue engineering.

(b) Alginate-based bio inks: Alginate, the charged polysaccharide is also a natural biopolymer obtained from algae, often named as alginic acid or algin. The Alginate compounds (Norouzi M, 2016) are mostly made up of have two monomers: (1–4)-β-D-mannuronic acid and α-L-guluronic acid. The first one helps in gel formation, while the second one and their mixtures help in inducing flexibility to the fabric. These biopolymers can effectively entrap water and different other molecules due to capillary action enabling them to diffuse inside out and thereby making them most efficient bio-inks. Thus, in 2013 Zhang with his group used these alginate-based bio inks along with animal tissue cells for printing hollow constructs, in which the special vessel-like microfluidic channels can effectively transport chemical element, nutrients, biomolecules etc. ultimately support cell growth. In a similar study, Yu and his group also tried alginates along with animal tissue cells as bio inks for developing cannular constructs with a triaxial nozzle assembly. Gao and his team next discovered a co-axial system of alginate-based colloidal gel material that might print strong 3D constructs having micro-channels for effective nutrient delivery. In 2015 Christensen and his team reported a metal alginate bio ink using mouse fibroblast cells for construction of vascular-like structures using salt crosslinker (Wolf MT, 2012). Similarly, various polymers were homogenized with alginate, like PCL, poloxamer, hydroxyapatite, gelatin etc. to fabricate numerous desired 3D constructs. Alginates are best utilized to foster 3D neural tissues. Using alginate-based bio inks, human embryonic stem cells could be bio printed for the first time. Zhao with his group succeeded to construct a 3D tissue model of cervical tumor using Hela cells and a mix of bio inks consisted of gelatin, alginate, and fibrinogen for in vitro study. In 2017 Ahlfeld and his team mixed artificial nano silicate clay with alginate and CMC to formulate two different bio inks. This nano silicate clay incorporation further enhanced the power of the alginates and CMC samples (Ono M., 2017) to unleash loaded medicine in a slowly paced sustained manner. Alginate-based and hyaluronate-based nano fibrillated polysaccharide composite bio inks were compared for formulating animal tissue constructs using pluripotent stem cells in another study. A separate study reported the use of alginate in combination with different artificial polymers like Poly (Ethylene Glycol)-Tetra-Acrylate (PEGTA) and GelMA for developing the desired 3D bio printed materials. Addition of PEGTA helped significantly in bioprinting of advanced multilayer hollow 3D systems like stable tube-shaped structures. In future this method might help in getting more and more

sophisticated vascularized tissue constructs for various tissue engineering applications. Thus, all these studies emphasized on varied benefits of alginate-based bio inks over the traditional hydrogels.

(c) Collagen-based bio inks: Collagen obtained from natural biomaterials is the main element of ECM. It is used as a bio ink material either alone or in a combined state due to its amazing biocompatible properties. They can be crosslinked by modifying temperature or pH and by using vitamin B. Collagen crosslinking always provides redoubled strength and visco-elastic properties compared to the non-crosslinked scleroprotein. Direct usage of these scleroproteins (So WH, 2020) in 3D printing is quite difficult and hence use of different alternative gelation materials become essential. In 2017 Yang and his group recommended the use of a mixture of scleroprotein and alginate for cartilage tissue regeneration. In a similar work, combination of scleroprotein with gelatin was exploited utilizing Drop on demand (DOD) inject technique to co-culture of human epithelium cells and hMSCs. The power of usage of mixed bio inks for constructing stable 3D constructs with high biological activity and rheologic properties was clearly established. A new collagen-based bio ink was also reported by Yeo et al. Their results showed redoubled mechanical and biological properties (López-Marcial GR, 2018) with introduction of scleroprotein. They used scleroprotein as the core biomaterial and alginate as sheath biomaterial along with human stem cells in their study. A similar work demonstrated employment of scleroprotein with alginates crosslinked by polyphenol. Bio inks consisting of human fat stem cells showcased higher cell viability and proliferation compared to alginate-based bio inks after printing. A recent work has proved the power of transglutaminase-crosslinked gelatin-based bio-printed 3D constructs in developing vascularized structures. These structures are generally useful in constructing complicated tumor models and in various fields of tissue engineering.

(d) Hyaluronic acid-based bio inks: The natural ECM Hyaluronic acid (HA) abundantly seen in cartilages and connective tissues are often employed in 3D bioprinting for formulating 3D structures. Various types of HA-based bio inks have been reported till date. An important study used photo-crosslinked HA (Mirdamadi E, 2019) as bio inks to get enhanced physical properties. Like all natural polymers, HA also has low mechanical properties and slow gelatinous behavior, compared to all artificial hydrogels. Very recently Ouyang and his team using secondary crosslinking methodology has successfully created HA-based stable 3D printed constructs that resulted in sensible cellular adhesion properties that could be further enhanced by addition of oligopeptides within the hydrogels. Poldervaart and his team has shown how HA-based hydrogels by reacting with methacrylate can show high osteogenic properties (Fan R, 2019). Methacrylate cluster here intelligently uses the photo-crosslinking mechanism to enable the hydrogels to get cross-linked. Some recent studies revealed that combination of HA with different artificial polymers help in generating sufficiently stable structures with considerable cell viability. Chemical and photo-crosslinking are employed only to boost the useful properties of these bio inks. Various mixtures of different artificial polymers (Axpe E, 2016) and their efficacy as injectable gels were sincerely investigated. Varieties of cells could be bio printed by employing them all. In 2017 Sakai and his group reported HA-gelatin based bio inks that may be polymerized even using visible radiation with the aid of Ruthenium-based complexes. In a separate work, extremely tunable HA-CMC combination gels were formulated. Variation of mechanical properties and cell viability with varying concentrations suggest that higher concentration might yield higher cell viability and impart sufficient stability to the printed 3D constructs. All such studies as a whole manifest the benefits of HA as bio inks in 3D bioprinting technology.

(ii) Artificial Bio Inks

Apart from naturally available biomaterials, numerous types of artificial bio inks based on fibrin, cellulose, silk, ECM, cell aggregates, cell spheroids, etc. are also used in 3D printing.

a) Fibrin-based bio inks: Fibrin, generally seen inside blood helps in coagulation. Fibrin colloids are generally created out of fibrinogen due to catalytic treatment of thrombin possessing fantastic biocompatibility and biodegradability despite weak mechanical properties. Hence these bio inks are not much effective.

b) Cellulose-based bio inks: Carboxy Methyl Cellulose (CMC), the semi flexible saccharide can be obtained from cellulose. Environment-friendly colloidal gels can be obtained from this CMC by fixing its concentration, mass, type of salts, and the number of methyl groups attached. A CMC solution was found to form gels below 37°C and CMC based bioactive glasses were used to regenerate 3D bone structures. Production of a nanocellulose-alginate-based bio ink for its effective use in tissue engineering was reported by Markstedt and his group. In another work nanocellulose hydrogels (Piras CC, 2017) could formulate patient-specific auricular gristle tissues that manifested wonderful retention of form, size and cell viability. Polysaccharide nanofibrils and cross-linkable xylan-based bio inks also showed high mechanical integrity and amazing printing properties. In some studies, alginate based nanocellulose showed higher cell viability compared to their HA analogues.

(c) Silk-based bio inks: Silk fibroin, the natural macromolecule is obtained from silkworm. These silk-based scaffolds are mostly utilized in the field of regenerative drugs and tissue engineering due to some of their special properties. In 2015 Das and his group prepared a silk-gelatin based bio ink using sonication and enzymatic crosslinking. A different work successfully showed how silk and gelatin as bio inks can typically enhance biocompatibility, cell permeability and tissue integration in soft tissue reconstruction, using glycerin as the physical cross linker. Alginate based silk fibroin macromolecules were also used as bio inks in Inkjet printing, cross-linking the tyrosine residues with peroxidase. Xiong and his team showed efficaciousness of primarily gelatin-silk based inks to regenerate skin tissues. In 2018 Zheng and his team reported silk-based bio inks made up of PEG exhibiting excellent high-resolution printability (Dzobo K, 2019). Recently, spider silk is also additionally receiving a lot of attention attributable to its amazing mechanical properties. DeSimone used recombinant spider silk proteins in a separate study for preparing bio inks. Even if printed constructs showed lesser cell viability using spider silk-based bio inks, once gelatin was added, the results were promising. For improving and enhancing these cell viability, biocompatible materials were added to silk to enhance the standard of printed materials.

(d) Extracellular matrix (ECM)-based bio inks: ECM is a mixed framework consisting of various parts like collagen, glycosaminoglycan, chondroitin sulfate, elastin, etc. along with cells. Decellularized ECMs (dECM) can be obtained from specific tissues wherefrom cells are removed by a stepwise procedure keeping the ECM intact that can be ultimately crushed to create a powder-like state and dissolved in appropriate solvents to prepare bio inks. Printability of these dECM-based bio inks (Shin M, 2019) can be enhanced by addition of different hydrogels like PCL obtained from different tissue varieties. Researchers have developed a system which might exactly manage the

temperature and pH of the bio inks to create gels even at 37°C while printing. It was shown by them that precise stacking of such cells by the system cannot succeed to impact the cell viability even with heating, to incite any harmful impact to the printed cells. 3D printing with dCEM along with dual stem cells was reported for internal organ patch development (Ali M, 2019). Although dECM was found to provide sensible cell viability and functionality, isolation and quantification of DNA and ECM constituents from specified tissues, they are too expensive compared to other alternate gel-bio ink formulations used in 3D bioprinting

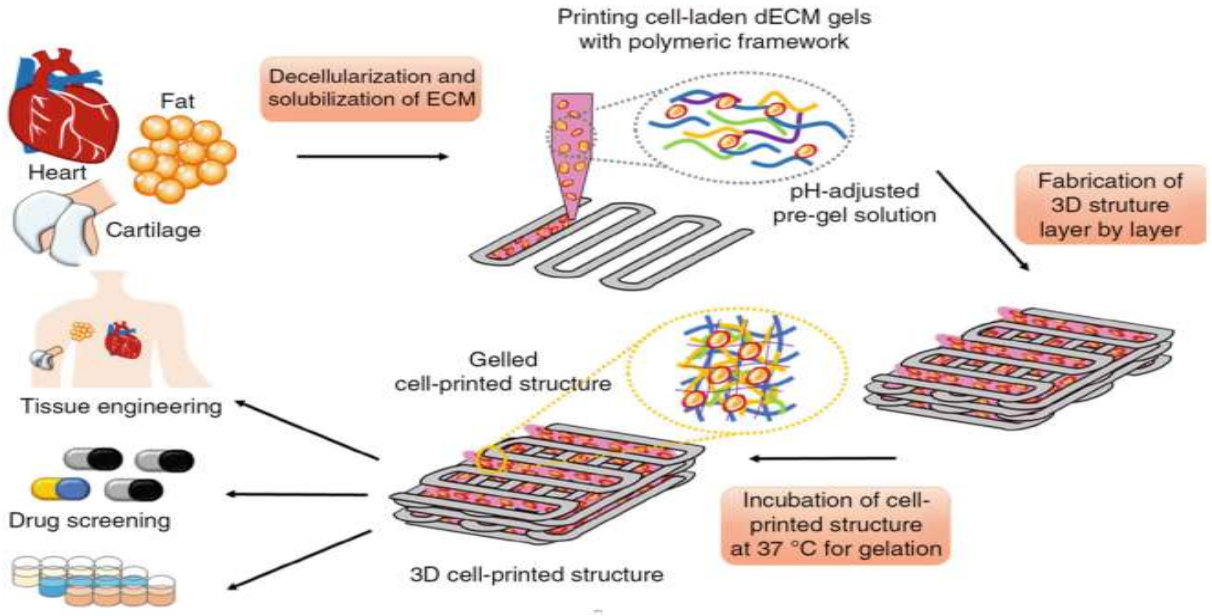

Figure 3.2 (ECM)-based bio inks

(e) Cell aggregates as bio inks: Bio inks consisting of spherical cell aggregates (spheroid) containing thousands of cells are generally required to develop 3D printed constructs. Such spheroids are serially distributed by self-assembly method into biocompatible scaffolds. Yu and his group showed a completely unique bio ink made up of tissue spheroids for developing 3D constructs not hampering any scaffold. Tissue strands up to eight cm long could be developed by speedy fusion of cells utilizing self-assembly method (Bakirci E, 2017) despite using any harsh chemicals as crosslinker or support material. Cell aggregates or cell sheets could also be formed employing some thermo-sensitive compound gels like Poly (N-isopropyl acrylamide) as substrate. When spontaneous cell growth starts onto the substrate, the cell sheets can be made detached by application of gentle heat not distressing the arrangement of the cell-matrix. Those detached cell sheets after getting completely separated can safely be used as potential bio inks for 3D bioprinting. Bio inks of this type displayed better effectiveness than the traditional cell aggregates for carefully preserving the ECM flawlessness.

5. Challenges and Future Perspectives:

Although 3D bio-printing has solid capacities to create the desired tissue and organ structures effortlessly, it desperately needs enhancement of various properties of bio inks and inevitably effective

commercialization of the 3D printed products. This technique can promote the scope of development of more advanced case-specific 3D structures for medical emergencies. Among various other available techniques, the cell-laden hydrogels are most popular used for developing these 3D constructs. Choice of proper bio inks varied accessible bio inks and their properties have been discussed throughout this review. Though ideal bio ink preparation is yet to achieve, because of its gigantic commitments in various fields, it can possibly be utilized in industrial applications too over the long haul. Despite the fact that cell laden bio inks are normally utilized, ECM-based bio inks, decellularized bio inks, cell aggregates or spheroids likewise show promising outcomes towards manufacture of useful tissues or organs utilizing this 3D bioprinting innovation. Nonetheless, this load of strategies needs enormous number of explicit cells that typically restricts use of this 3D bio imprinting in different cases. Advent of advanced high resolution and low cost bioprinters may help to solve that problem enhancing the prospects of this specific area. Numerous novel polymeric biomaterials with supramolecular reasonableness, reversible crosslinking and stimuli responsive hydrogels have been accounted for as of late. Eventual fate of bio inks and 3D bioprinting is hence exceptionally encouraging, prompting manufacture of cutting-edge case-explicit tissue/organs and gadgets over the long haul.

Conclusion

Bioprinting is no doubt a rapidly emerging, quickly developing and promising technology to generate 3D tissue constructs with exactly outlined structural geometries utilizing living cells and/or biomaterials as potential bio inks. These bio inks are an important part of bioprinting and generally include biomaterials (such as hydrogels), cells, or cell aggregates, or their combos. Many natural (e.g., alginate and gelatin) and artificial (e.g., PCL, PEG, Pluronic) polymers are commonly used as bio inks. Though there are various efforts on advancement of this technology, development of acceptable bio inks that satisfactorily meet bioprinting necessities regarding mechanical, rheological, and biological properties are limited so far. Thus, the advent of latest bio ink materials and engineering of novel bio ink formulations are presently major areas of interest. Additionally, a lot of work is needed in making models and standards to check and judge the properties of various bio ink materials. To the current end, new metrics must be compelled to be developed for evaluating these bio inks and bioprinting processes, that are important to standardize their uses. Additionally, the development of latest computational models is another space of interest to totally analyze the printability and behavior of the bio inks before experimental optimization. Keeping in mind the preceding topics, this review has given top to bottom insights about these bio inks, and it is normal that this might benefit the broad audience in the interesting field of bioprinting and tissue designing in general.

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List of Abbreviations:

Three-Dimensional (3D)
Computer-aided design/computer-aided manufacturing (CAD/CAM)
Freedom reversible embedding of suspended hydrogel (FRESH)
Polycaprolactone (PCL)
Poly lactic acid (PLA)
Food and drug administration (FDA)
Polyethylene glycol diacrylate (PEGDA)
Gelatin methacrylate (GelMA
Hyaluronic acid (HA)
Polyethylene glycol (PEG)
Stereolithography (SLA)
Digital light processing (DLP)
2-Photon polymerization (2PP)
Carboxymethyl-chitosan (CMC)
Carboxymethyl-cellulose (CMC)
Human mesenchymal stem cell (hMSC)
Poly (Ethylene Glycol)-Tetra-Acrylate (PEGTA)
Drop on demand (DOD
Extracellular matrix (ECM)
Decellularized ECM (dECM)

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