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Applications of 3D Printing in Plant Science-An Updated Review

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Abstract

Three-dimensional (3D) bioprinting, also known as additive manufacturing (AM), is a rapidly evolving field, with a focus on fabricating organ and tissue constructs by layering organic materials, living cells, and biochemicals according to a given digital mode. 3D printing, also called Additive Manufacturing (AM), has the potential to be a technological revolution in the manufacturing industry. Some of the applications of 3D printing technology in plant science are bioprinting of plant cells, plant tissue cultured cell production, plant tissue culture lab-ware, production of plant secondary metabolites without plants, plant derived compounds for nozzle design, plant phenomics, plant phenotyping, ecological research, as a botany teaching tool, green bioprinting, and printing laboratory equipment. Plant bio-printing may improve understanding of plant shape and morphogenesis, and could serve for the mass production of desired tissues or plants, or even the production of plant-based biomaterial for industrial uses. Bioprinting is the ultimate and the most progressive step of engineering applied to plant cell culture. However, plant bioprinting may be difficult due to rigid plant cell walls, unlike animal cells that do not have a cell wall, although plant cells have a distinct advantage, totipotency, which allows a plant cell, under strict environmental conditions, to develop a tissue scaffold that serves as the precursor for an organ, and the whole plant itself, organogenic steps that are under strict genetic control. The culture methodologies of bioprinted plant cells could be assimilated to the culture of immobilized cells. Additionally, sustainable practices, and the potential impact of factors influencing 3D printing must be considered.

Keywords: 3D printing; Prototyping; Plant tissue culture; Micropropagation; Light quality; Culture vessel design; 3D bioprinting; Bioink; Green plants

1. Introduction

Three-dimensional (3D) printing technology, also known as additive manufacturing, is a method that uses computer-aided manufacture to stack materials, layer by layer, to form a monolithic three-dimensional entity [1]. Additive manufacturing technology has the advantages of reducing the consumption of raw materials, saving production energy consumption, reducing time costs, etc [1-10]. It has flexibility with regard to product production and is not limited by product shape and structure. Globally, 3D printing technology has been widely used in many fields such as manufacturing, construction [3], electronics, biomedical, aerospace, automotive, textile, plant science research and food

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[1-82, 198-200]. In recent years, 3D printing has become a transformative technology for the printing of custom-designed objects outside of traditional manufacturing practices[1-20]. Printer technology has improved significantly with faster printing speeds, wider choice of print materials, lower machine costs, and free and open-source software [1-40]. In turn, 3D printing technology is now being adopted across many scientific disciplines for rapid prototyping with emerging utility in plant science [1-82, 198-200]. 3D Printing promises to produce complex biomedical devices according to computer design using patient-specific anatomical data [1-20]. Since its initial use as pre-surgical visualization models and tooling molds, 3D Printing has slowly evolved to create one-of-a-kind devices, implants, scaffolds for tissue engineering, diagnostic platforms, and drug delivery systems [1-60]. The ability to design and fabricate complex, 3D biomedical devices is critical in tissue engineering [1-82].

Over the last decade, the development of technologies such as 3D bioprinting has engendered paradigm shift in many scientific areas including plant biology[1-82]. For instance, 3D bioprinted constructs of plant cells or inspired by plants decipher information about single-cell, tissue, and whole plant interactions with their surrounding environment. [1-82]. 3D printing is the state-of-the-art manufacturing technique underpinning a remarkably infinite prospect of customizability, flexibility, and sustainability in the production of tailored geometries additively by depositing materials layer-by-layer [1-82, 198-200]. Different feed stocks for 3D printing have been already used, but successful substitution of plastics with a more sustainable and environmentally friendly components, including plant cell wall polysaccharides, have been recently demonstrated [1-82, 198-200]. As the three polysaccharides (pectin, hemicellulose and cellulose) in natural cell wall interact and create together mechanically tunable structure, reusing them together in the ink formulations for 3D printing and mimicking different mechanical properties of printed material is the main scientific challenge[1-82, 198, 199-200].

2. 3D Printing: Chuck Hull

Three-dimensional printing technology has experienced unprecedented growth and is revolutionizing the manufacturing industry [1-82]. This flexible technology provides the advantages of customization, prototyping, various fabrication techniques, and complex geometries at a low cost in a short timeframe[1-82]. Additive manufacturing technology has come a long way since its inception when Chuck Hull, co-founder of 3D Systems, developed the first 3D printer in 1983 [1-82]. In the following years, there was a growing interest in this technology, and it became more affordable and accessible [1-82]. Chuck Hull is an American inventor who is the co-founder, executive vice president and chief technology officer for regenerative medicine of 3D Systems [1-82]. He is one of the inventors of the SLA 3D printer, the first commercial rapid prototyping technology, and the widely used STL file format [1-82]. Hideo Kodama of Japan is created with the first 3D printing patent in 1981[1-30]. Chuck Hull, an American inventor, is often regarded as the “Father of 3D Printing. [1-45]. Hull developed the stereolithography fabrication system in 1984, which remains a foundational technology in 3D printing today[1-82]. The first documented iterations of 3D printing can be traced back to the early 1980s in Japan [1-82]. Charles Hull is the inventor of stereolithography, the first commercial rapid prototyping technology commonly known as 3D printing. Charles Hull is also recognized as the Father of 3D printing technology[1-82].

3. 3D Printing: Categories

According to the different material stacking principles, 3D printing technology can be divided into five categories [1-82]: (1) Extrusion-based 3D printing technology, including Fused Filament Fabrication (FFF), Direct Ink Writing (DIW), Liquid Deposition Molding (LDM); [1-82]. (2) 3D printing technology based on UV beam scanning photosensitive resin, including Stereo Lithography Appearance (SLA), Digital Light Processing (DLP), Liquid-crystal display (LCD) [1-82]. (3) 3D printing technology based on powder sintering, including Selective Laser Sintering (SLS) and Selective Laser Melting (SLM) [1-82]. (4) 3D printing technology based on lamellar materials such as Laminated Object Manufacturing (LOM) [1-82]. (5) 3D printing technology based on jet technology—3D inkjet printing is also called Three-Dimensional Printing (3DP) [1-82]. Different processes have different requirements for printing materials; 3D printing materials can be divided into four categories in terms of material types [1-82]. Organic polymer material such as nylon, plastic and epoxy resin, metal materials, stainless steel, titanium, gold and silver [1-82]. Inorganic non-metallic materials, such as glass and ceramics[1-82]. Composite materials, such as cement and wood–plastic composites [1-82]. Materials’ properties influence the printing process and final products [1]. PLA is a crispy substance and has adverse performance and high stress relaxation in deformation recovery [1-8-10]. Before 3D printing can be used routinely for the regeneration of complex tissues (e.g. bone, cartilage, muscles, vessels, nerves in the craniomaxillofacial complex), and complex organs with intricate 3D micro-architecture (e.g. liver, lymphoid organs), several technological limitations must be addressed [1-82, 198-200].

4. 3D Printing: Bioprinting

Three-dimensional (3D) bioprinting, also known as additive manufacturing (AM), is a rapidly evolving field, with a focus on fabricating organ and tissue constructs by layering organic materials, living cells, and biochemicals according to a given digital mode [1-40]. Over the past 15 years, progress in 3D printing technologies has produced novel technologies such as bioplotting, extrusion-based bioprinting, stereolithography, inkjet-based bioprinting, fused deposition modeling, and laser-based bioprinting [1-50]. However, these techniques still have limitations regarding cell viability, long-term functionality, and accurate process parameters [9,10]. In recent years, the characterization and fabrication methods concerning new bioinks have received much attention, largely because the absence of bioprintable materials has been identified as one of the most rudimentary challenges for rapid advancement in the field of three-dimensional (3D) printing [9, 10]. Bioinks for printing mammalian organs have been rapidly produced, but bioinks in the field of plant science remain sparse [9, 10]. Thus, 3D fabrication of plant parts is still in its infancy due to the lack of appropriate bioink materials, and aside from that, the difficulty in recreating sophisticated micro-architectures that accurately and safely mimic natural biological activities is a concern [9, 10-82, 198-200].

5. 3D Printing: Main Stages

The main stages of object 3D printing consist of three steps [9-82]. The first step is the creation of a solid *in-silico* model in CAD software to determine the global geometry of the object [9, 10]. This model is then converted into a standard format for 3D printing [9, 10]. The standard tessellation language format (STL) is here commonly used [9, 10]. The second step consists of slicing the solid *in-silico* model [9, 10]. This step will convert the object into a machine file describing the path 3D printer has to follow to deposit or fuse the material [9]. It also allows choosing the printing parameters (infill density, layer thickness, printing speed etc.) [9, 10-30]. The third step which is the printing process is automated as 3D printer equipment can execute the instruction sequence [9]. For some materials and depending on the object's final use, a post-treatment may be necessary [9]. In the case of the 3D printing strategies applied for plant cells, post-treatment consists in cross-linking of biomaterials forming hydrogels to maintain the printed architectures over cultivation time [9, 10-25].

6. 3D Printing: Plant Science

The integration of 3D bio-printing into plant science and biotechnology is revolutionizing research and applications [1-80]. While many high-throughput techniques have advanced plant biology, replicating the complex 3D organization and cellular environments of plant tissues remains a significant challenge [1-80, 198-200]. Traditional 2D culture systems fall short of capturing the necessary spatial context for accurate studies of cell behaviour, gene expression, and tissue development [1-80]. Additionally, the lack of precise simulation of plant microenvironments limits control over cellular interactions and responses to external stimuli [1-82]. Recent advancements in 3D bioprinting address these limitations by allowing precise control over cell positioning and biomaterial arrangement, thereby better replicating natural plant environments[1-80]. This enables more accurate studies of gene expression, developmental processes, and stress responses [1-80]. The technology also enhances our ability to test genetic modifications and biotechnological interventions, advancing crop improvement, sustainable agriculture, and precision breeding [1-80]. Additionally, improving reproducibility and consistency across studies is critical as this technology is increasingly implemented, allowing for the development of a reliable and standardized knowledge base [1-82, 198-200].

Madison et al., (2025) [81] reported that there are examples of how 3D bioprinting can advance both fundamental and applied plant biology are abundant [1-81]. On the fundamental side, examples include cell–environment interactions, cell-to-cell interactions, genetic engineering and editing as well as emergent properties of cellular and tissue interactions, such as novel positional cues and non-cell-autonomous signaling [1-82]. Applied research, meanwhile, can leverage 3D bioprinting for micropropagation, metabolic engineering, molecular farming, haploid induction, trait editing, organ-on-a-chip systems, and synthetic tissue engineering [1-82]. It can also be used to screen responses to stresses, pathogens, or other biotic factors in breeding programs[1-82]. To successfully harness the potential of 3D bioprinting in plant science, significant efforts must focus on optimizing bioprinting parameters to avoid excessive disruption to cells or biological material [1-82].

Biomass materials are mainly degradable materials made from woody, herbaceous, gramineous and vine plants, as well as their processing residues and waste, through physical, chemical and biological means[1-10-82]. Biomass materials have the advantages of being green, widely sourced and cost-effective. They have broad development potential as green 3D printing consumables [1-10-82]. In recent years, as the concepts of green and low-carbon have been emphasized, the research of biomass materials in 3D printing has been gradually developed and some comprehensive review articles

have been published in this field [1-10-82, 198-200]. Following are the few examples of application of 3D printing technology in plant science research [1-10-82].

7. 3D Printing: Plant Phenomics

According to Griffiths (2020) [11], there are four underlying themes for the use of 3D printing in plant science research were apparent: (1) development of novel plant phenotyping tools, (2) development of novel plant growth systems, (3) printing of physical plant models for validation of analysis techniques, and (4) printing plant models as an outreach and teaching aid [11-82]. The fourth theme is considered a notable use of 3D printing in plant science outside of the peer-reviewed research survey [11]. According to Griffiths (2020) [11], the majority of 3D prints however, were for organ-specific uses with only two studies being for the whole plant which were for plant growth systems [11-20]. Therefore, 3D printing so far is largely being used to alleviate specific research problems which are likely routine or repetitive tasks and/or benefit from equipment standardization minimizing error [11-20]. A small majority of 3D print papers focused on plant roots [11], which likely reflects the challenge of phenotyping roots and the need for custom solutions [11]. On the basis of literature survey by Griffiths (2020) [11], there is clear early adoption of 3D printing technology in plant science [11-20-82]. The results showed that the applications of 3D printing in plant research are wide and will likely continue to grow [11-20-82]. However, early utility in plant research, the 3D printed work is difficult as no standardization of 3D printing practices or file sharing has yet been established[11]. Griffiths (2020) [11] also reported that community curations such as Quantitative-Plant.org have instead proven to be useful for plant image analysis software dissemination and serve as a searchable resource for users [1-20]. No such repository existed with a focus on 3D printing in plant science[11]. A 3D printing online repository was therefore, established at 3Dplantphenomics.org to fulfill this community need [11-20]. Furthermore, Griffiths (2020) [11] also reported that 3Dplantphenomics. org will remain up dated and relevant to plant scientists as new peer-reviewed research is published through periodic online literature searches[11-20]. The website currently features four curated lists of 3D models: (1) phenotyping tools, (2) plant growth systems, (3) modeling/analysis, and (4) outreach [11]. These categories will be updated as new functions of 3D printing in plant science arise[11-20] Griffiths (2020) [11] also reported that a major strength of 3D printing in plant science is that novel methods or analyses are highly reproducible by anyone with a 3D printer or access to a 3D printing online service[11]. For greater scientific transparency and reproducibility, 3Dplantphenomics.org sets recommendations for making 3D model files downloadable if not already available as supplementary data in a research paper or in an online repository [11-20]. As 3D printer technology becomes more affordable and accessible worldwide, there is an opportunity to use this to facilitate research coordination and scientific reproducibility[11-20]. Griffiths (2020) [11] is of the opinion that with development of more protocols that utilize 3D printed objects, the costs to replicate research will fall and the scientific impact of such work will be greater as a wider audience will benefit from the model file availability [11-20]. Griffiths (2020) [11] also mentioned that 3D printer provides will speed up innovations in plant science with new phenotyping tools and plant growth systems[11-20]. Designs that are useful to plant scientists are no longer required to be commercially viable for a vendor to manufacture, as 3D printers can instead print single units out cheaply and quickly[11-20]. As 3D printing technology in plant science matures, an open-source approach will be important for collective improvement of models and for adapting useful models for different plant systems [11]. To facilitate the adoption and use of 3D printing technology in plant science, an online repository has been set up at 3Dplantphenomics.org [11]. Griffiths (2020) [11] also reported non-destructive digitization of plants is an expanding research area with 3D image capture of roots, shoots, and canopies being used to visualize and understand plant developmental processes and how plants interface with the environment [11-20]. 3D printing of plants is an important means of providing ground truth validation to analysis models and is also proving to be a great STEM (science, technology, engineering, and mathematics) teaching and outreach aid [1-20].

8. 3D Printing: Plant Phenotyping

In the past decades, 3-dimensional (3D) plant phenotyping was utilized for assessing geometric properties of plants [21-23, 203]. Advances in passive and active optical sensors, coupled with 3D reconstruction algorithms, provide the basis for precise high-throughput and high-resolution 3D analysis of above-ground plant structures, advancing 3D shoot phenotyping research [1-21-33, 203]. This research work by Bömer et al., (2024) [21] reported the intricate 3D structure of plants by measuring various morphological traits and gather data about a crop's growth status, ultimately resulting in an enhanced comprehension of plant growth [1-21-33, 203]. Additionally, information about the 3D structure of a plant is essential for crop modeling, and can even be used to correct other optical sensor data [21-33, 203]. Combining 3D data acquisition with advanced analysis techniques enables measurement of diverse morphological growth parameters across various scales, ranging from the canopy to single plant and organ level [21-33, 203].

Recently one of the study by Bömer et al., (2024) [21] in Germany addresses the importance of precise referencing in 3-dimensional (3D) plant phenotyping, which is crucial for advancing plant breeding and improving crop production [21-23]. Traditionally, reference data in plant phenotyping rely on invasive methods [21-23]. Recent advancements in 3D sensing technologies offer the possibility to collect parameters that cannot be referenced by manual measurements[21-23]. This work by Bömer et al., (2024) [21] focuses on evaluating a 3D printed sugar beet plant model as a referencing tool [21-23, 203]. According to the study conducted by Bömer et al., (2024) [21], fused deposition modeling has turned out to be a suitable 3D printing technique for creating reference objects in 3D plant phenotyping [21-23]. Production deviations of the created reference model were in a low and acceptable range [21-23]. Bömer et al., (2024) [21], were able to achieve deviations ranging from −10 mm to +5 mm[21-23]. In parallel, Bömer et al., (2024) [21] demonstrated a high-dimensional stability of the reference model, reaching only ±4 mm deformation over the course of 1 year[21-23]. Detailed print files, assembly descriptions, and benchmark parameters are provided, facilitating replication and benefiting the research community[21-23]. According to the study conducted by Bömer et al., (2024), consumer-grade 3D printing was utilized to create a stable and reproducible 3D reference model of a sugar beet plant, addressing challenges in referencing morphological parameters in 3D plant phenotyping [21-23]. The reference model is applicable in 3 demonstrated use cases: evaluating and comparing 3D sensor systems, investigating the potential accuracy of parameter extraction algorithms, and continuously monitoring these algorithms in practical experiments in greenhouse and field experiments [21-23]. Using this approach, it is possible to monitor the extraction of a non-verifiable parameter and create reference data[21-23]. The process serves as a model for developing reference models for other agricultural crops [21-23]. Bömer et al., (2024) [21] reported that consumer-grade FDM 3D printing enables to produce highly accurate and stable reference models for application in 3D plant phenotyping, contributing to solving the issue of referencing morphological parameter extractions[21-23]. The proposed reference model is accurately reproducible and stable over a longer period, indicating that FDM 3D printing is a suitable production technique for the suggested applications [21-23]. The introduced process of creating a 3D reference model for sugar beets can serve as an example for developing similar reference models for other widely used arable or horticultural crops [21-23, 203]. Through the experiments conducted, it was determined that 3D reference models can serve a wide range of applications in 3D plant phenotyping [21-23]. Besides representing a standardized approach for comparing 3D sensor systems based on their accuracy and reconstruction completeness of plants, an evaluation of the precision of parameter extraction algorithms designed for high-throughput phenotyping under ideal conditions was demonstrated using Bömer et al., (2024) [21] reference model [21-23]. Additionally, the reference model is used to monitor the extraction of morphological parameters under practical conditions [21-23]. Therefore, it is possible to provide verification data for 3D morphological parameters that cannot be referenced using traditional referencing methods used in plant phenotyping [21-23, 203]. Bömer et al., (2024) [21] also mentioned that, this work will provide files and a detailed description for reprinting the model, along with precise manual and automated benchmark parameters for plant and single-leaf parameters, enabling the research community to replicate and benefit from this research [21-23, 203].

9. 3D Printing : Printable Laboratory Equipment

Recent technical advancements, affordability, and accessibility of 3D printers have allowed resource-restricted researchers to become makers, manufacturing the low-cost hardware needed for their specific research workflows [26]. McNair et al., (2024) [26] are of the opinion that numerous do-it-yourself (DIY) scientific equipment designs are openly available on the internet, including pipettes, microscopes, centrifuges, and 3D scanners [26]. For the biological sciences, 3D printing can be used to create new equipment, replicate equipment that would otherwise be prohibitively expensive, or customize existing models [26]. Although there may be a perception that 3D printing has high barriers to entry such as cost and the requirement for specialized knowledge, these obstacles can be overcome with minimal time, effort, and a basic understanding of 3D printing[26]. Scientists can evaluate the potential of 3D printing for their own lab equipment by working with public maker spaces and printing services[1-26-41]. These groups often hold workshops and training sessions that can help guide researchers through the process of purchasing and using their own printer[26-41]. Because of these perceived barriers, the adoption of 3D printing technology has been slow, but within the plant sciences, multiple projects already implement 3D printing as part of their workflow [26-41]. From entire systems enabling non-destructive root and shoot imaging; tissue culture vessels; models of roots, shoots, and pollen; and bio-printed functional plant cells, these 3Dprinted projects are wide reaching [1-26-41]. They have applications in hydroponics and tissue culture, root system architecture phenomics, education and outreach, and more [1-26-41]. Low-cost, repairable lab equipment is rare within the biological sciences[1-26-41]. By lowering the costs of entry using 3D printing and open-source hardware, McNair et al., (2024) [26] developed a modular system of 3D-printable designs called COBLE (Collection of Bespoke Laboratory Equipment), including novel and remixed 3D- printable lab equipment that can be inexpensively printed, assembled, and repaired for a fraction of the cost of retail equivalents [1-26-41]. McNair et al., (2024) [26] presented a novel tools that utilize 3D printing to enable a wide range of scientific

experiments[1-26-41]. McNair et al., (2024) [26] include additional resources for scientists and labs that are interested in utilizing 3D printing for their research [26-41].

10. 3D Printing: Green Bioprinting

Bioinks are basically biomaterial solutions containing living cells and are essential components in bioprinting [1-10]. Bioprintability, non-toxicity, insolubility in cell culture medium, visco-elasticity, high mechanical integrity and stability, the ability to stimulate cell adhesion, and biodegradability at a steady rate are all necessary features of biomaterials for enabling high-quality tissue regeneration [1-26-41-82, 198-200]. In 2003, the term "bioink" and the phrase "biopaper" were first used in the context of organ printing [1-82]. The original plan was to create (or possibly print) a biopaper (hydrogel) and then bioprint it using living cells or tissue spheroids as the bioink [9, 10]. Bioink was first used to describe a biological component that was inserted in 3D on or within a hydrogel. Nevertheless, plant-based biomaterials and printing plant parts are slowly making advances in the area of bioprinting [1- 9, 10]. Although, most plant cells are totipotent, that is, they have a strong potential to develop a tissue scaffold that acts as the precursor for an organ [9, 10-82, 198-200]. From that organ, an entire plant can be generated, even if the environmental conditions are unfavorable [18]. Despite cell totipotency, new plant cell-related discoveries in the field of bioprinting research are currently lacking [9, 10-82]. Moreover, the primary challenge to successful plant tissue printing is the lack of safe and intricate micro-architectures designed to mimic natural biological function [9, 10-82].

Recently, the green bioprinting concept was established following the effective use of an extrusion-based bioprinting methodology developed for printing human cells for manufacturing photosynthetic microalgae-laden hydrogel scaffolds [1-24-26-82, 198-200]. Green bioprinting refers to printing plant cells or other plant-based fabrication[1-24-26-82]. Primarily, green bioprinting is a promising emerging immobilization technology for plant cells that can allow the development of new bioprocesses and monitoring systems for secondary metabolite production [1-24-26-82]. The use of the bioprinting approach, that is, cell encapsulation in hydrogels through computer aided design based 3D printing, can advance the progress toward the success of a structured plant cell-laden 3D scaffold [1-24-26-82]. In addition, novel methods or analysis can be easily replicated using a 3D printer, which is a key advantage of 3D printing in plant research [1-24-26-82, 198-200].

In vitro plant substrate growth can better control cellular composition, thereby improving the product's material characteristics and chemical make-up tunability [1-24-26-82]. Furthermore, with the controlled deposition of gel-scaffold material, the macroscopic substrate architecture may change to better fit application-specific needs, thereby allowing the creation of structures in a near-final form through casting or 3D bioprinting and minimizing waste further [1-24-26-82, 198-200]. In addition, novel methods or analysis can be easily replicated using a 3D printer, which is a key advantage of 3D printing in plant research[1-24-26-82]. This suggests that bioprinted plant constructs could be useful replacements for complex living systems[1-24-26-82]. These constructs may aid in the resolution of unanswered biological questions, with potential for use as learning tools in plant research[1-24-26-82]. Cellular components, such as the CW and mitochondria, are dynamic entities[19], hence, understanding these dynamics is essential to determine the morphophysiological response of cells to various growth conditions[1-24-26-82]. This can be easily accomplished using 3D bioprinting, while the layer-by-layer printing method can be useful for studying subcellular and molecular dynamics within plant cells [1-24-26-82, 198-200].

Bioprinted root-based 3D models or biorobots may help clarify the geometrical and mechanical properties of root analogs[1-24-26-82]. Decellularized spinach leaves, for example, can help explain leaf venation, while the chemical analysis of decellularized leaves can be useful for learning about the components present in plant leaves[1-24-26-82]. Decellularization processes are used to create pre-vascularized, a cellular tissue engineering scaffolds from a wide range of plant tissues. The rapid development of different plant species and their abundance provides several could be used to form effective and sustainable bioinks [9-24-26-82, 198-200]. Whole organ perfusion decellularization procedures can be modified for the synthesis of diverse plant tissues [9-24-26-82]. For the preparation of spinach (*Spinacia oleracea*) leaf-based decellularized extracellular matrix (dECM) scaffolds, leaves are first cannulated through the petiole, while parsley (*Petroselinum crispum*) stems are cannulated through the basipetal end of the stem [9-24-26-82]. A series of hexane treatments (98%, mixed isomers) and 1 × phosphate-buffered saline (PBS) solution are then used to remove the cuticles surrounding the leaf epithelial tissues on the plants [9-24-26-82, 198-200]. Spinach leaves have been used as a model biomaterial for dECM-based natural scaffolds due to their availability, intricate vascular network pattern, density, and petiole with a large diameter, which simplifies the cannulation process[9-24-26-82]. The use of this type of process as a bioink preparation medium remains uncommon for plant printing but has attracted attention in the field of green bioprinting, as a result researchers are attempting to develop several methodologies to understand plant cellular dynamics, cell to cell translocation, leaf venation system, plant–environment interactions, and the plant

immune system[9-24-26-82]. New bioink processes for printing plant cells on a large scale in a simple and quick manner are also being developed [9-24-26-82, 198-200].

Bioinks are classified into two main categories: scaffold-based bioinks and scaffold-free bioinks [9-24-26-82]. In bioprinting, scaffolds are fibrous, porous, or permeable three dimensional (3D) biomaterials that allow biological liquids and gases to pass through while facilitating cell interaction, viability, and extracellular matrix (ECM) deposition with minimal inflammation and toxicity while biodegrading at a controlled rate[9-24-26-82]. Natural bioinks are polymers derived as biomaterial from naturally occurring resources [9-24-26-82]. Several natural biomaterials are commonly used as bioinks in 3D printing, including agarose, alginate, cellulose, chitosan, collagen, dECM, and silk [9-24-26-82].

Natural biomaterials will continue to be used for tissue engineering, with the goal of discovering and creating more complex morphologies, vasculature, and functioning tissue architectures that are commonly found in plant tissues [9-24-26-82, 198-200]. This goal can be accomplished by improving the mechanical, rheological, and biological properties of the biomaterial mixture employed in the production of bioinks [15]. dECM-based bioinks laden with living cells show the promises in the production of 3D printed functional organs or tissues [9-24-26-82]. However, this method can only be used in the production of a limited number of tissues and organs, as it requires large number of particular cells for these bioinks [9-24-26-82]. Many other innovative biomaterials with stimuli-responsive hydrogels, supramolecular functionality, and reversible crosslinking polymers have recently emerged, which is promising in terms of bioink selection and usage[9-24-26-82]. Although fabrication has significant potential in plant science research, the printing process of plant tissue[9-24-26-82, 198-200]. As 3D printing technology becomes more affordable and available, it will improve research coordination and scientific methodology[31], and advance plant science and growth systems [9-24-26-82]. Designs that are useful to plant scientists could be easily obtained using 3D printing without the need to be made commercially available [9-24-26-82, 198-200]. As this technology evolves in plant science, an opensource approach may be necessary for collective model improvement and adapting useful models to different plant systems [9-24-26-82]. Thus, it is evident that in the field of bioprinting, encapsulating plant cells in biomaterials to create a bioink formulation could be effective in treating a wide range of human and other animal illnesses[9-24-26-82]. In addition, bioprinted plant cells can facilitate the understanding of plant cell paracrine effects in in vitro models of human and animal diseases [9-24-26-82]. The development and improvement of various bioinks is one of the primary paths for the advancement of green bioprinting. A greater diversity of bioinks will lead to a larger scope for green bioprinting [9-24-26-82, 198-200].

11. 3D Printing: Plant Tissue Culture

Bioprinting is the ultimate and most progressive step of engineering applied to cell culture [9-24-26-82]. Unlike animal systems, the application of bioprinting in simple plant tissue cells is still in a nascent phase and has yet to be studied [9-24-26-82, 198-200]. One major advantage of plants is that all living parts are reprogrammable in the form of totipotent cells [9-24-26-82, 198-200]. Plant bioprinting may improve scientists' understanding of plant shape and morphogenesis, and could serve for the mass production of desired tissues or plants, or even the production of plant-based biomaterial for industrial uses[9-24-26-82, 198-200]. 3D bioprinting is needed to accurately deposit cells, biomaterials and biomolecules layer by layer from compute-aided equipment and software, which have been possibly built by integrating modern scientific and technological knowledge, including cell biology, engineering, science of materials and information technology[9-24-26-82, 198-200]. 3D bioprinting is emerging as a promising technology for fabricating complex tissue constructs with tailored biological components and mechanical properties[9-24-26-82].

Plant bioprinting may be difficult due to rigid plant cell walls, unlike animal cells that do not have a cell wall, although plant cells have a distinct advantage, totipotency, which allows a plant cell, under strict environmental conditions, to develop a tissue scaffold that serves as the precursor for an organ, and then the whole plant itself, organogenic steps that are under strict genetic control [9-24-26-82, 198-200]. A second possible problem might be the efficiency of cell and tissue regeneration once a scaffold has been printed [9-24-26-82]. In principle, bioprinting would be required to shape plant cells into a scaffold, which would serve as a building block for engineering plant tissues for partial organogenesis to produce specific products rather than a whole plant [9-24-26-82, 198-200]. Other than bioprinting involving animal cells and tissues, the application of bioprinting to other complex multicellular organisms, especially plants, has not yet been tested or studied [9-24-26-82]. According to WICAKSONO and TEIXEIRA DA SILVA (2015) [75], unlike its direct function in biomedical sciences, plants might be considered less interesting as a bioprinting subject, also because tissue culture and micropropagation already provide a suitable and robust system for producing plant cells, tissues or organs in a sterile *in vitro* environment [9-24-26-82]. Furthermore, the printer cartridge could overlay different building blocks of different cellular origins onto media or substrates containing different inducers such as plant growth regulators (PGRs) [9-24-26-82, 198-200].

A plant cell or tissue can be made to survive, grow and develop artificially *in vitro* when placed on a suitable medium that contains macro- and micronutrients, carbohydrates, vitamins, and PGRs. The most commonly used basal medium is Murashige and Skoog [75]. To obtain plant cell aggregates that could form multiple cell clusters and eventually a tissue, cell suspension cultures in liquid media might serve as the optimal printing form when placed in the printer cartridge rather than the use of "dry" cells, which can die easily due to oxidation[75]. Growth scaffold as a template for shaping the product: importance of basal medium, medium additives and plant growth regulators [75]. A scaffold is essential in organic tissue printing as a base to direct tissue growth[75]. Gel material for plant tissue can be calcium alginate, agar, agarose, polyacrylamide, gelatin, or even synthetic material like polyurethane [9-75-82]. Other cheaper soft material like starch from various source like sago, cassava, and corn can also be used [9-75-82].

A hand held bioprinter could be used for *in-vivo* bioprinting of *in vitro* tissues or onto plants growing under a sterile and controlled environment[9-75-82]. For example, a small graft (as a single layer using a 2D printer, or a mass of cells or tissues using a 3D printer) could be printed onto the part of a plant that was damaged by an abiotic stress (e.g., cold- or heat-induced injury) or by a biotic stress (e.g., a fungus or pest) allowing for recovery of dead tissue or covering and strengthening scarred tissue [9-75-82]. As result, chimeric plants that yield multiple fruits can be created[9-75-82 ,198-200].

In plant bioprinting, a scaffold would be used as a mold to shape cells so that they will be aligned prior to differentiation using induction by PGRs [9-75-82]. The proposed printing process can take place in two ways: 2D printing or 3D printing [9-75-82, 198-200]. For 2D printing or monolayer printing, cells are simply directed to spread over a 2D area of nutrient gel scaffold before they are left to grow when placed under optimized conditions [9-75-82]. On the other hand for multilayer or 3D printing, a gel is added gradually to adjust the surface before placing another layer of cells or to enclose the surface [9-75-82]. In some cases, a gel may also contain a specific concentration of PGRs to match the desirable level that would result in a product (tissue (e.g., parenchyma, sclerenchyma, etc.) or organ (leaf, stem, petiole, stigma, tuber, bulb, etc.) [9-75-82]. Thus, more than one gel cartridge may be possible, or necessary. 3D or multilayer printing is expected to be followed by directed organogenesis and differentiation in response to PGRs or other optimal conditions , either optimized *a priori* using assays, or following the published literature [9-75-82, 198-200]. The difference between traditional plant tissue culture (PTC) and the use of a bioprinter will lie in the automation and the precision associated with it[9-75-82, 198-200].

When cells are printed, a bioprinter will align each cell to form a layer of equal size forming the desired scaffold [9-75-82]. When a 2D printer is used, the printing result will only be a single thin layer of cells of variable sizes depending on the size and capacity of the bioprinter[9-75-82]. On the other hand, a 3D printer will add more layers of cells joined by a gelling agent to a desirable height that is limited to a printer's maximum height capacity [9-75-82]. A larger printer would thus be able to print a larger scaffold (3D) or wider base (2D) [9-75-82]. A printed cell or layer of cells (2D horizontal scaffold; or cellular mass (3D scaffold; may be placed on a basal medium carrying a gradient of PGRs or any other nutrient [9-75-82]. The concept of a gradient is not usual in conventional PTC, and is made by using tilted agar medium to make two different concentration gradients that would theoretically affect the growth of the cell layer overlaying it by diffusion[9-75-82, 198-200]. In a 3D gradient, the precision of a computer that guides the printing process is important and a step that is impossible to achieve at present in conventional PTC. This gradient medium serves as an attempt to try and direct the growth of cells that have been printed. The basal medium can either be printed, or is not printed, i.e., it is set *a priori*, e.g., in petri dishes[9-75-82, 198-200].

Bioprinting plant material has foreseeable benefits for ornamental and agricultural purposes, and for biomaterial production [9-75-82, 198-200]. The main concept in bioprinting using plant cells is to arrange cells into a suitable scaffold of a specified area (2D) or volume (3D) that will allow them to develop, in response to an ideal basal medium and additives, including PGRs, directly into a specific organ (i.e., direct organogenesis) [9-75-82]. By being able to bioprint a living structure of living cells with desirable shape, the most obvious manipulation that could be envisioned in the improvement of aesthetic in ornamental plants such as bonsai or *in vitro* flowers [9-75-82, 198-200]. It is also conceivable that plant tissue can be printed as a base for the production of plant-based biomaterial, e.g., lab-grown wood planks or wood blocks for construction decreasing deforestation and creating bioprinted blocks of wood of rare and valuable wood such as sandalwood [9-75-82]. Such wood blocks could be printed by tabletop-sized or larger printers [9-75-82].

A fourth possibility is the use of plant bioprinting to produce designer plant-based food that combines aesthetics, nutraceuticals, and productional aspects, e.g., lab-grown vegetable products, a procedure equivalent to lab-grown meat [9-75-82]. This 3D method would create a desirable and edible plant-based product that can be specific (e.g., only a leaf, root or fruit) or a whole printed plant [9-75-82]. The procedure would also apply to transgenic material [75-82]. Such a bioprinter would allow individuals to manufacture their own fruits or vegetables at home although, relative to

presently used forms of producing fresh produce in mass, the costs of producing a single item would likely be prohibitive [9-75-82]. However, as for most technologies, costs tend to become lower over time[75-82]. Initially, while the prototype is small, the concept would take the form of a table-top printer, using plant suspension cells in liquid medium within cartridges [75-82, 198-200].

As the system develops into a robotized format, the printer interface would allow the user to define the desired cell or tissue to be printed, with the desired shape, nutrients, or colours [75-82]. Such a concept would benefit tissue engineering science and plant tissue culture[75-82]. In addition, there could be untold benefits of plant bioprinting for the production chain and mass production of rare or valuable plant material [75-82]. The production in space of plant products rich in nutrients and with reduced volume through plant bioprinting would be suitable where space may be limited, where delivery of renewable resources may be difficult, or impossible, and thus where plant-based resources would be needed to be printed as needed [75-82, 198-200].

12. Plant Tissue Culture: Applications

Plant tissue culture is an important biotechnological technique solving the problems of modern agricultural providing solutions to major food security issues [83-194, 201-202, 204]. Plant tissue culture can be used for a wide range of purposes with various applications in research and industry [83-194]. There are many applications of plant tissue culture which is used in the production of somatic embryos, plant regeneration via organogenesis, germplasm conservation, synthetic seeds, protoplast culture for the production of somatic hybrids, anther culture for the production of haploids, synthesis of secondary metabolites of pharmaceutical interest, and plant cells used for the bioenergy [83-194]. Plant tissue culture techniques are the most frequently used biotechnology tools ranging from basic to applied investigation purposes in plant sciences [83--194, 204]. Chemical and hormonal control of regeneration, basic and applied aspects of organogenesis, somatic embryogenesis, micropropagation and production of virus-free plants, haploid plants, production of secondary metabolites, and large-scale cell cultures in bioreactors are few landmarks and notable discoveries in plant tissue culture research [83-194].

Plant tissue culture is an in vitro aseptic culture of cells, tissues, organs or whole plant under controlled defined nutritional medium conditions often to produce the clones of plants [83-194]. The resulting clones are true to type of selected genotypes and used for the large scale plant multiplication [83-194, 204]. The beauty of plant tissue culture lies in the culture of small piece of plant material (explant) on a defined nutrient medium to produce large number of plantlets or clones within a limited time in a continuous process, irrespective of season, and weather on year round basis [83--194, 204]. Tissue culture technique offers several advantages over plant propagation under natural conditions[83-194]. It is a rapid procedure as thousands of seedlings can be produced from small fragments (explants) of plants in a short period of time in contrast to conventionally propagated flora [83-194]. Plant in vitro propagation using tissue culture techniques have been exploited for the commercialization of ornamental plants (orchids), vegetable and fruit plants (papaya, mango, and grape), medicinal, woody, and conifers with economically important products [83-194]. Large-scale plant tissue culture has been shown to be an appealing alternative approach to traditional plantation methods since it provides a regulated supply of defined nutrients (including carbohydrate) independent of plant availability [83-194, 204].

Various plant tissue culture systems have been extensively studied to improve and enhance the production and quality of plant metabolites produced by the medicinal plants[83-194]. In vitro plant micropropagation is a powerful technique in order to acquire plant extracts with various commercial applications than using whole plants [83-194]. Tissue culture can be used to propagate perennial woody species, orchids, endangered and threatened plant species irrespective of weather or season [83-194]. This also helps to accelerate the production process of new crop varieties with superior traits as tissue culture experiments required less time and space compared to in-vivo plant growth [83-194]. It also helps in the development of pathogen-free micro-plants saved from various diseases and the new plants produced by tissue culture under aseptic conditions are also disease free plants [83-194]. In vitro micro-propagation is a valuable technology since many secondary plant metabolites cannot be manufactured chemically [83-194, 204].

Tissue culture technique depends mainly on the concept of totipotentiality of plant cells, which refers to the ability of single cell to express the full genome by cell division [83-194]. The totipotency of somatic plant cells is a specific and scientifically exciting phenomenon, which is based on the developmental program of plants [83-194]. It can be best demonstrated in an *in vitro* system where somatic plant cells can regain their totipotency and are capable of forming embryos through the developmental pathway of somatic embryogenesis [83-194, 204]. Under *in vitro* conditions, one or a few somatic cells of the plant or explants have to be competent to receive a signal (endogenous or exogenous). This then triggers the reprogramming of plant cells into the pathway of embryogenic development (commitment) leading to somatic embryo formation [83-194]. The controlled conditions provide the culture of explants on a defined nutrient

medium with the source of carbohydrate in an environment conducive for their growth and multiplication [83-194]. These conditions include proper supply of nutrients, source of carbohydrate, pH of the medium, adequate temperature and proper gaseous and liquid environment [83-194, 204].

Plant tissue culture is a technique in which fragments of tissues from a plant (explants) are developed in vitro in an artificial medium under aseptic conditions [83-194, 201-202]. It involves culturing explants (such as shoot tip, root tip, callus, seed, embryo, pollen grain, ovule or even a single cell) isolated from mother plant on a sterile defined nutrient medium which leads to cell multiplication and plant regeneration [83-194]. Several methods are available for plant tissue culture[83-194]. In organogenesis, the commonly used method, organ formation can occur directly from meristems, or indirectly from dedifferentiated cells (callus) [83-194, 204]. The resultant cultures can then be utilized to mass produce plants (micro propagation) or to develop specific organs (e.g., roots in hairy root culture) [83-194].

Plant tissue culture plays a significant role in basic research in the areas of plant pathology, plant physiology, plant metabolites and conservation [83-194]. Due to advancement in contemporary techniques, several protocols have been developed for the production of a wide variety of plants secondary metabolites on a commercial scale [83-194]. Apart from their use as a tool of research, plant tissue culture techniques have in recent years, become the major commercial importance in the area of plant propagation system, disease elimination, plant improvement, secondary metabolite production and genetic transformation [83-194].

Plant tissue culture coupled with molecular biological approaches leads towards sustainable agricultural development providing solutions to major food security issues [83-194]. Plant tissue culture plays an important role in plant biotechnology due to its potential for massive production of improved crop varieties and high yield of important secondary metabolites [83-194]. Several efforts have been made to ameliorate the effectiveness and production of plant tissue culture using biotic and abiotic factors[83-194]. Several endangered, rare, threatened, and commercially important plant species have been tissue cultured and successfully micropropagated for large scale production [83-194]. In addition to this plant tissue culture is an efficient technology for the production of somaclonal and gameto clonal variants [83-194]. The micropropagation technology has a vast potential to produce plants of disease resistant and plants of superior quality, well adapted high yielding genotypes with better stress tolerance capacities [83-194, 201-202]. Micropropagation via in vitro culture technique has several advantages over the traditional methods of propagation through seed, cuttings, grafting and air-layering etc. Plant micropropagation, also known as plant tissue culture, is a technique that isolates, sterilizes, and incubates cells, tissues, or organs of chosen plants in a growth-promoting aseptic environment to create a large number of plantlets [83-194]. The isolated cloning technique revealed that, given the right conditions, somatic cells may develop into a complete plant [83-194]. The sterile or endangered flora can also be conserved by plant micropropagation methods [83-194]. Hence, plant tissue culture is an extremely efficient and cost-effective technique for biosynthetic studies and bio-production, biotransformation, or bioconversion of plant derived compounds [83-194, 204]. However, there are certain limitations of in-vitro plant regeneration system including difficulties with continuous operation, product removal, and aseptic conditions[83-194]. For sustainable industrial applications of in vitro regenerated plants on a large scale, these constraints need to be addressed in future studies [83-194, 204].

In vitro micropropagation process is a rapid process that can lead to the production of plants of virus free. In plant cell culture, plant tissues or organs are grown under in vitro fully defined nutrient medium under aseptic conditions and controlled environment [83-194]. Plant tissue culture medium contains all the nutrients required for the normal growth and development of plants. It is mainly composed of macronutrients, micronutrients, vitamins, other organic compounds and plant growth regulators, carbon source and some gelling agents of solid medium [83-194]. There are many mediums used for the in vitro culture and one common medium is the MS medium [83-194]. The Murashigeand Skoog (MS) basal medium supplemented with the required amounts of plant hormones which include auxins, cytokinins, abscisic acid, gibberellins, smoke saturated water, ethylene, and growth regulators with similar metabolic effects [83-194]. Plant micropropagation with different explants like seeds, embryos, calli, anthers, protoplasts, and meristemetic tissues of root/shoot tips is used for large-scale production of industrial products [83-194, 204].

The pH of the nutrint medium (5.8) is also important that affects both the growth and activity of plant growth regulators [83-194]. Plant growth regulators (PGRs) plays an important role as signal molecules and regulators of growth and development in plants. An endogenous auxin pulse is one of the first signals leading to the induction of somatic embryogenesis [83-194, 204]. Auxin (IAA, IBA, NAA and 2,4-D) and cytokinins (BA, TDZ, 24-Epibrassinolide, Melatonin, Smoke saturated water, BAP, Kinetin) are the main PGRs in plants involved in the regulation of cell division and differentiation [83-194]. The higher concentrations of auxins generally favours root formation, where as higher concentration of cytokinin favours shoot formation [83-194]. Endogenous PGR levels, however, can be considered as major factors in determining the specificity of cellular responses to these rather general stress stimuli. In addition to

the absolute requirement of exogenous auxins for sustained growth in *in vitro* cultures, plant cells may produce substantial amounts of the native auxin, indole-3- acetic acid (IAA) [83-194]. A balance of both auxin and cytokinin some times leads to the formation of unorganized mass of cells known as callus [83-194, 204].

Plant tissue culture has many commercial applications and can be applied for the production of disease resistant plants either via organogenesis or somatic embryogenesis. In case of organogenesis, the explants of selected plant produces plants via callus formation [83-194]. Organogenesis is the production of plant organs from a specific tissue in order to develop complete plants [83-194, 204]. It is characterized by being polar, which means that just one aerial organ or root is released and a new complete plant is generated[83-194]. Simultaneously, organogenesis can be direct in which the organogenic shoot is produced directly from the explants, or indirect, in which the organogenic process happens from previously created callus in the original explants [83-194]. Callus is an undifferentiated mass of tissue that forms explants after a few weeks on growth medium with appropriate hormones[83-194, 201, 202]. Callus development is the result of a well-known process known as de-differentiation or re-differentiation[83-194]. To stimulate callus induction and development, several growth hormones are employed [83-194, 204]. Callus induction and development were increased by 2,4-D, NAA, and kinetin. Organogenesis allows for the effective regeneration of new plants from callus [83-194, 204].

Somatic embryogenesis is the process of producing embryos from somatic plant cells (any non-sexual cell) in order to produce a whole plant[83-194]. In contrast to organogenesis, this is a polar process in which the aerial structures and roots of plants develop from the somatic embryo [83-194, 201, 202, 204]. It can either be direct or indirect, depending on whether the process begins with the original explants or with previously produced callus. On the other hand, in case of somatic embryogenesis, the explants cultured under in vitro conditions produces callus tissue [83-194]. The cells of callus tissue are programmed towards somatic embryogenesis and produce somatic embryos[83-194]. There are few specific examples where explants produced somatic embryos directly without callus formation[83-194]. The somatic embryos on germination medium germinate to produce shoot and roots. This can be applied in the conservation of endangered/rare threatened plant species [83-194, 204]. Clonal propagation of high-value forest trees, medicinal plants, endangered /threatened plant species through somatic embryogenesis has the potential to rapidly capture the benefits of breeding or genetic engineering programs and to improve the uniformity and quality of the nursery stock [83-194]. The greatest value of plant cell/ tissue culture rests not so much on their application to mass clonal propagation (micro propagation), but also in their involvement in plant improvement and bio-processing [83-194, 202-202, 204].

Embryo culture has fertilized or unfertilized zygotic (seed) embryos dissected from maturing seeds or fruits and cultivated in vitro until seedling formation [83-194]. Embryo culture is not the same as somatic embryogenesis. In case of conifers, the use of zygotic embryos as explants has several disadvantages including heterogeneity as a result of cross pollination, which may result in the new generation having characteristics inferior to those of the parent (seed-bearing) tree [83-194, 204].

The disadvantages associated with zygotic explants may be overcome if mass propagation of elite, mature trees can be achieved from vegetative tissue explants, such as secondary needles or apical shoots, because the regenerated plantlets will be uniform and possess elite characteristics from clearly defined parents [83-194]. The induction of somatic embryogenesis using shoot apical thin layers has been successful in few conifers (*Pinus roxburghii, Pinus kesiya, Pinus patula,* Scots pine and *Pinus pinea)* [83-194, 204].

In some cases, inter-specific and inter-generic hybrids can be obtained using embryo rescue technique which is not possible through conventional methods [83-194]. Plant tissue culture coupled with biotechnological approaches is applicable to the development of genetically modified plants as well as embryo rescue procedures [83-194, 204].

Tissue culture has been extensively utilized in breeding programs and the hybrids of such crosses are often sterile due to embryo abortion but can be 'rescued' by means of culturing or transplanting the embryos [83-194]. The medium utilized for cell culture can be optimized for the production of desirable products [83-194]. As a result, plant tissue culture is a viable technology for producing desirable bioactive chemicals from plants [83-194]. Plant tissue culture is also used to help save endangered species, as many therapeutic plants are on the verge of extinction due to overuse[83-194]. The development of plant tissue culture techniques will expand the long-term use of therapeutic plants in the future [83-194, 204].

Shoot tip culture is developed from excised shoot tips/buds larger than the shoot apices (used for meristems cultures), and had several leaf primordia [83-194, 204]. These shoot apices are often cultivated such that each one generates several shoots [83-194]. Lateral bud node culture is carried out on a short piece of stem tissue where stem portions

carrying single or many nodes may be cultivated [83-194]. Each bud is cultivated to produce a single shoot. In isolated root culture, a branching root system can be generated by growing roots that are not attached to shoots [83-194, 204].

The second application of plant tissue culture is the cryopreservation [83-194, 204]. Cryopreservation is the successful storage of biological materials at ultra low termperatures -196^0 C [83-194]. The risk of loosing the plant material due to human or accidental error or undesired genetic changes is always present[83-194]. To reduce the costs and risk, cryopreservation is a valuable method for long term preservation of plant material [83-194]. Cryopreservation of embryogenic tissue has generally been considered as a means of avoiding the loss of embryo maturation potential during long-term *in vitro* culture of evading possible somaclonal variation caused by the long-term maintenance of actively growing embryogenic cultures, and of storing a large number of genotypes[83-194, 204]. Similarly, cryopreservation provides a means of storing genetically altered material while field tests are conducted [83-194]. It may also limit the amount of contamination and somaclonal variation resulting from routine subculturing of callus tissue. Furthermore, several plant species generate resistant seeds that cannot be retained for extended periods of time[83-194]. Hence in this situation, tissue culture can be utilized for plant conservation in vegetative state, generally under slow growth conditions or for cryopreservation [83-194].

A third powerful tool in plant biotechnology is genetic transformation in order to transfer relevant genes from bacteria, fungi, animals or plants into plants of interest [83-194, 204]. The most recent feature of plant cell and tissue culture is genetic transformation, which allows the transfer of genes with desirable traits into host plants and the recovery of transgenic plants. This approach offers a high potential for the development of agricultural plants with novel traits. The genetically modified plants exhibit agronomically significant features such as greater yield, improved nutritional quality, improved pest and disease resistance [83-194].

Plant tissue culture plays a pivotal role in vector mediated or vector-independent gene-delivery into plant genome for the production of transgenic plants with improved traits [83-194]. One important aim of genetic manipulation of plants is the increase of resistance against various fungal pathogens. The recent development of molecular tools for genomic analysis of plant species makes it possible to transfer or identify genes controlling agronomic traits [83-194]. However, both the mechanisms for DNA transfer to a plant cell and targeting of the DNA to a complex tissue or organ competent for regeneration is another major issue to be considered for effective and successful transformation [83-194]. Now-a-days there are many genes available for use in plant transformation experiments. However, most of these genes have been used as reporter genes for establishing a model transformation system, and very few have been used for novel phenotypes or for tolerance to various stresses [83-194, 204]. Selectable marker genes have been pivotal to the development of plant transformation technologies because the marker genes allow scientists to identify or isolate the cells that are expressing the cloned DNA, and to select for the transformed progeny [83-194]. Different transcription factors which regulate nutrient assimilation pathways have been over expressed in staple crops that may improve crop yield. There are three methods of genetic transformation, 1) Biolistic method and 2) Agrobacterium method, 3) Protoplast method [83-194, 204].

Biolistic or particle bombardment is used as a direct gene transfer method without using a vector for plant transformation, and relies entirely on physical or chemical principles to deliver foreign DNA into the plant cells. In this method, there is no dependence on bacteria, so the limitations inherent in organisms such as *A*. *tumefaciens* do no apply [83-194]. Plant transformation method by *A*. *tumefaciens*, soil pathogenic bacterium, has become the most used method for the introduction of foreign genes into plant cells and the subsequent regeneration of transgenic plants under in vitro conditions. Various transgenic plants including Arabidopsis, wheat and tobacco are developed through genetic engineering and plant tissue culture conferring resistance against different environmental stresses [83-194, 204].

In the last two decades, the technique based on *Agrobacterium rhizogenes* inoculation has gained popularity as a means of creating secondary metabolites generated in plant roots. Organized root cultures can contribute significantly to the generation of secondary metabolites. Hairy root disease is caused by *Agrobacterium rhizogenes* in plants [83-194]. The neoplastic (malignant) roots, generated at rapid growth rate, by *A. rhizogenes* infection are genetically stable and are developed in hormone-free conditions. Hairy roots high stability and productivity allow them to be used as a powerful instrument for the recovery of important secondary metabolites. Hairy roots have high stability and productivity allow them to be used as a powerful instrument for the recovery of important secondary metabolites [83-194, 204].

Protoplasts are plant cells with cell walls removed by enzymatic or mechanical methods [83-194]. Protoplasts are obtained by immersing plant cells in a hypertonic solution, which causes the plasma membrane to shrink off the cell wall due to water efflux Cell wall may be removed using either enzymatic digestion with pectinase and cellulose, or by mechanical techniques Plant protoplasts are the important ideal tools for genetic manipulations such as gene transfer, mutation breeding and somatic hybridization [83-194]. Protoplast culture when coupled with an efficient protocol for

gene delivery and plantlet regeneration serves as an excellent system for the recovery of transgenic plants. Various crops with superior traits have been developed using this technology with enhanced nutritional value and biotic/ abiotic stress resistance that leads to increased crop yield. Conventionally, somatic hybridization, which produces interspecific and intergeneric hybrids, was commonly used as an essential method for plant breeding. The procedure entails the fusion of two somatic protoplasts followed by the selection of desirable hybrid cells and then regeneration of hybrid plants [83-194]. Protoplast fusion is an effective method of transferring genes with desirable traits from one species to another with a great impact on crop development (106). Somatic hybrids created from rice and ditch reed by electrofusion exhibited better results against salt stress (106).

The fourth application of plant tissue culture is the synthesis of synthetic seeds under in vitro conditions [83-194]. Synthetic seed production is a well documented applied technology that capitalizes on the capacity for rapid plant multiplication via somatic embryogenesis. Synthetic seed is also defined as a somatic embryo, or any other vegetative propagule, with an artificial, biodegradable coating entrapped in a nutrient medium supplying carbon sources, mineral nutrients, vitamins and growth regulators[83-194, 204]. The entrapping matrix or coating should not have any adverse effect on the embryo or propagule and should allow germination under both in vitro or ex vitro conditions. The term therefore, reflects the artificial nature of the seed coat as well as somatic origin of the encapsulated propagule [83-194].

The fifth application of plant tissue culture is the anther culture. Anther culture technique is the most viable and efficient method of producing homozygous doubled haploid plants within a short period [83-194]. Agricultural activity demands improved crop varieties with desirable traits such as quality, crop yield, and resistance to environmental stresses. However, the practical application of this technology in plant improvement is still limited by various factors that influence culture efficiency. Therefore, the improved anther culture method can produce doubled haploid plants for which can be useful in different breeding programs that will enable the speedy development of commercially important plant varieties for resource-poor farmers. Although conventional breeding approaches have substantially enhanced cereal productivity, and their progress rate is slow. Thus, there is a need to respond quickly and make rapid changes to drastically reduce breeding cycles to sustain the crop under unpredictable environmental conditions[83-194]. The application of anther culture along with advanced biotechnological approaches could hasten the process to develop superior breeding materials or varieties by decreasing the number of breeding cycles dramatically. Anther culture technique is a biotechnology tool that has found importance and a niche in expediting many crop breeding processes [83-194]. Therefore, in the future, the prospects for anther culture and the integration of genomics tools provide novel opportunities for improving selection efficiency, maximizing genetic grain, and improving varietal development, leading to the early release of rice varieties with desirable traits [83-194, 204].

Use of haploids has emerged as a key strategy for crop improvement. Haploids having a single set of chromosomes in the sporophytic phase have become a valuable source to screen for desired traits or to introduce a mutation in their genetic content. Furthermore, doubled haploids (DHs) can be obtained by spontaneous or induced chromosome doubling. Double haploids are homozygous at all loci, and they can be propagated through seed. Doubled haploids (DHs) achieve complete homozygosity in a single generation[83-194]. On the contrary, the conventional breeding method requires six to seven generations of self-crossing. In vitro production of haploids for crop improvement has been successfully achieved in many crops such as rice, wheat, barley, maize, tomato, potato, brassicas, grapes, sunflower and so on. In addition to crop improvement, doubled haploids (DHs) are an excellent source for gene mapping, cytogenetic research, and evolutionary studies. Various agronomic crops i.e., cereals, fruits, vegetables, ornamental plants and forest trees are currently being used for in vitro propagation [83-194, 204].

The sixth application of plant tissue culture is the production of nanoparticles Nowadays, the addition of nanoparticles as elicitors has, for instance, gained worldwide interest because of its success in microbial decontamination and enhancement of secondary metabolites[83-194]. Nanoparticles are entities in the nanometric dimension range. They possess unique physicochemical properties. Among all the nanoparticles, silver-nanoparticles (AgNPs) are well-known for their antimicrobial and hormetic effects, which in appropriate doses, led to the improvement of plant biomass as well as secondary metabolite accumulation [83-194, 204]. Therefore, the evaluation of the integration of nanotechnology with plant tissue culture is a new advancement of plant biotechnology. The highlight is especially conveyed on secondary metabolite enhancement, effects on plant growth and biomass accumulation as well as their possible mechanism of action. In addition, the use of nanomaterials as potential therapeutic agents is gaining interest worldwide. Elicitation of silver-nanoparticles, as well as nanomaterials, function as therapeutic agents for animal well-being is expected to play a major role in the process[83-194, 204].

The seventh application of plant tissue culture is the mass in vitro propagation of medicinal plants for the isolation of secondary metabolites of pharmaceutical interest [83-194, 204]. In vitro cell culture has the inherent advantage of producing therapeutic proteins such as monoclonal antibodies, antigenic proteins that act as immunogens, human

serum albumin, interferon, immuno-contraceptive proteins, antihypertensive drug angiotensins, and human haemoglobin in certain situations[83-194]. The manufacturing of pharmaceuticals using culture systems of plants can provide remarkable benefits including cost reduction, quick production, and scalability[83-194]. Biotechnological approaches associated with plant tissue culture have increased the scope of medicinal plants along with traditional agriculture used for the industrial production of bioactive metabolites[83-194, 204].

Tissue culture technology advancements showed that transcription factors are effective new molecular tools for plant metabolic engineering to boost the synthesis of important chemicals[83-194]. The in vitro plant propagation has not only made a significant contribution in the knowledge of basic research, but it also offers potential applications as it guarantees a sustainable industry that relies on commercial production of plant-derived compounds[83-194]. Alkaloids are the structurally diverse group of secondary metabolites which possess significant biological activities [83-194, 204].

Plants make them as a defence mechanism in response to biotic and abiotic stressors. Plant tissue culture is a viable technology for producing desirable bioactive chemicals from plants. Plant tissue culture is also used to help and save the endangered species, as many therapeutic plants are on the verge of extinction due to overuse [83-194, 204].

The culture of plant tissues is an effective instrument for the isolation and processing of active compounds, including secondary substances and engineered molecules, from economically important plants[83-194]. Plant tissue culture technique is also used for the micro propagation of medicinal plants in pharmaceutical industry for the isolation of bioactive phytocompounds which has numerous industrial applications. It provides potential benefits for different industries which include food, pharmaceutical and cosmetics [83-194]. Plants are the rich source of phytochemicals with medicinal properties rendering them useful for the industrial production of pharmaceuticals and nutraceuticals. Furthermore, there are numerous plant compounds with application in the cosmetics industry. Cosmetic extracts derived from plant cell cultures suit the markets increasingly stringent safety requirements[83-194]. Plant cell culture cosmetic production is not dependent on appropriate seasonal conditions. Hence, it requires less time and energy. In addition to being free of pathogens, pollutants, and pesticide residues, plant cells generated under aseptic laboratory conditions rarely include any malignant compound or potential allergen, which would otherwise destroy the majority of the plant extracts obtained. Plant tissue cultures are a perfect source of safe and pure components for cosmetic goods since they can be cultivated under controlled conditions with minimum possibility of pathogen or environmental contamination. Utilizing various extraction techniques and solvents while taking advantage of the chemical makeup of plant cell components, plant tissue culture technology allows the isolation of many active ingredients from a single cell culture. Plant cell cultures are now being used for the production of cosmeceuticals, products having cosmetic as well as therapeutic (medical or drug-like) effects that exert beneficial effects on skin health. Plant cell culture extracts with several particular actions for skin care, make-up, and hair care as supplement components are gaining popularity in the cosmetics sector[83-194, 204].

In addition to having moisturizing, anti-ageing, anti-wrinkle effects; plant-derived compounds also possess pharmacological properties such as antiviral, antimicrobial, antifungal, anticancer, antioxidant, anti-inflammatory, and anti-allergy characteristics [83-194]. The in vitro propagation of industrially significant flora is gaining attention because of its several advantages over conventional plant propagation methods. One of the major advantages of this technique is the quick availability of food throughout the year, irrespective of the growing season, thus opening new opportunities to the producers and farmers. The extensive research on plant cell culture has caused a surge in the use of this technique in the pharmaceutical industry. Plants are abundant sources of pharmaceutically significant compounds. However, there is a need to manufacture these compounds within stringent laboratory conditions. Various secondary metabolites having medicinal values can be obtained from plant cell culture. An extensive investigation is being done to investigate the plant sources producing active ingredients, such as antioxidants, ingredients with antimicrobial, anti-viral, anti-cancerous, antifungal, anti-inflammatory, and anti-allergy properties along with moisturizing, anti-ageing, anti-wrinkle and UV protective properties, which are crucial to cosmetics industry [83-194, 204].

Most of the phytochemicals such as polyphenols, phenolics acids, triterpenes, flavonoids, stilbenes, steroids, carotenoids, steroidal saponins, sterols, fatty acids, polysaccharides, sugars, and peptides are extracted with relevant solvents and utilized as an active constituents in cosmetic preparations [83-194, 204].

In vitro suspension cultures are created when friable calli are grown on liquid medium in a suitable container and regularly agitated to provide free cell suspension. Conical flasks are utilized because of its enormous surface area, which aids in the retention of liquid medium and the constant exchange of gases. Suspension cultures are classified as batch or continuous cultures [83-194, 204]. At regular intervals, a part of the original cell suspension is collected and sub-cultured on to fresh medium in batch cultures. In continuous cultures, new media is introduced to the same culture on

regular basis, and surplus cell suspensions are discarded. Suspension cultures are commonly utilized in large-scale synthesis of secondary metabolites. Cell suspension culture methods are currently being employed for large-scale plant cell culture from which secondary metabolites are extracted [83-194, 204]. A suspension culture is created by moving the comparatively friable component of the callus into liquid media and maintaining it under appropriate physical conditions of aeration, agitation, light, temperature, and other physical factors. Cell cultures not only produce defined standard phytochemicals in huge quantities, but they also reduce the presence of interfering substances found in field-grown plants [83-194]. The primary benefit of cell cultures is the production of bioactive secondary metabolites in a controlled environment that is independent of climate and soil conditions [83-194, 204].

Chemostat bioreactors are the devices particularly built for large-scale continuous culturing [83-194, 204]. Recent breakthroughs in plant cell culture, molecular biology, enzymology, and fermentation technology indicated that these systems are a viable source of synthesis of important secondary metabolites [83-194, 204]. Plants infected with an engineered virus generate relatively significant amounts of desired chemicals, and these plants can sustain steady levels of protein synthesis without extra intervention. Plants infected with an engineered virus generate relatively significant amounts of desired chemicals, and these plants can sustain steady levels of protein synthesis without extra intervention[83-194]. Plants are used to treat and prevent particular illnesses and diseases in human beings since long time ago. Plants which possess healing metabolites with useful pharmacological effects are referred to as medicinal plants. They are rich in phytochemicals which have the spectacular capability to treat diseases and may be used for the industrial production of pharmaceuticals and nutraceuticals. Applications of plants/flowers extracts in cosmetics are significant which include skin moisturizing, whitening or tanning products, sunscreens, radical-scavenging antioxidants, immune stimulants, and skin thickeners etc [83-194, 204].

Plant micro-propagation has extensively been used to have an insight into plant pathology studies which include factors influencing penetration, infection and multiplication of pathogens, the nature of irregular cell division or growth, and the morphogenetic potential of the diseased cell [83-194, 204]. The employment of plant tissue culture in plant pathology is not only restricted to use plant tissues as a substrate for the pathogens, but it provides the basic understanding of various characteristics of pathological growth, pathogens' attack weapons, and the host response to an infection caused by the invading organism [83-194]. Plant physiology and plant morphogenesis requires the capability to grow plants in vitro that might be best accomplished with plant tissue culture procedures [83-194]. Furthermore, the conservation of plant biodiversity is indispensable for future crops safety due to increasing challenges of biotic and abiotic stresses. In this regard, in vitro techniques permit improvement in various traits associated with plant growth and yield that can later be used for ex-situ conservation [83-194, 204].

The active plant compounds obtained from rare or endangered species can be manufactured by in vitro techniques without adverse environmental effects and in agreement with the bio- sustainability matters that the market demands [83-194]. Plant tissue culture is a powerful tool of agricultural improvement and offers tangible solutions to major crop problems that arise due to constant threat of biotic and abiotic stresses minimizing the crop yield [83-194, 204]. Since plant tissue culture is simple, low-cost and environment friendly, it is imperative to employ this technique for the development of sustainable agriculture in order to meet the food demand of increasing human population. Agricultural diversification to satisfy our future demands necessitates the implementation of innovative agricultural technology. The finest cultural methods, excessive fertilizers, and pest control procedures will not yield the desired results unless the best planting material is used[83-194]. Tissue culture is now widely being used as a viable horticultural propagation. technology, and it has changed the horticultural business [83-194, 204]. This approach is used to achieve mass proliferation and the creation of disease-free stock material. Commercial laboratories are now using tissue culture protocols that minimize somaclonal variations, and working on creating accurate screening and selection approaches for early detection of off-types [83-194, 204]. Although somaclonal variation is deleterious to quick clonal multiplication, some off-types have been discovered that have significant agronomic utility [83-194, 204]. In this regard, plant tissue culture methods hold enormous promise. In the future, plant tissue cultures might not only be a source of novel chemicals with uncharted biological actions, but they might also work as alternative recombinant protein bio-factories, particularly for those whose expression might be problematic or constrained in fermenting microbes. Biotechnological approaches associated with plant tissue culture have increased the scope of medicinal plants along with traditional agriculture used for the industrial production of bioactive metabolites [83-194].

In vitro culture is a method applied for the growth and development of plant cells, tissues, and organs that uses a nutritive culture medium under controlled sterilized conditions [83-194]. This method is considered as one of the most promising and environmentally friendly biotechnological practices for the sustainable supply of biofuels (57-190, 209). There are three main in vitro culture systems including organogenesis (e.g., embryogenesis, direct and indirect shoot regeneration), rhizogenesis, and callogenesis [83-194]. Among these methods, callogenesis can be considered as a robust method for biofuel production. The main advantages of callus culture are- The callus is generally defined as an

irregular bulk of parenchymatous tissue with meristematic cells that are broadly used for the production of different bioactive plant molecules [83-194, 204].

Genetic manipulation of callus for lignin engineering through transient gene transformation is much easier than other methods due to the lack of need for transgenic plant regeneration [83-194]. Somaclonal variations, which are usually seen in callus culture, can result in changes to metabolic pathways and even allows the production of new metabolites [83-194]. Any phenotypic variation during callus culture is referred to as somaclonal variation that can be a result of RNA interference, histone modification, chromatin remodeling, DNA methylation, and spontaneous mutation. Callus culture can be easily scaled up in different bioreactor systems. Callus culture is considered a sustainable and eco-friendly process [83-194]. These callus cultures of many medicinal plants were also used for the production of thin films as biodegradable **biopolymer composite** in food packaging industries [83-194, 204].

The limitations of plant tissue culture methods include difficulties with continuous operation, product removal, and aseptic conditions [83-194, 204]. A few culture systems appear to have the potential to become commercially viable because of these limitations. Plant cell development under in vitro conditions and regeneration into full plants is an asexual process that involves just mitotic division of the cell and, ideally, should not result in variation Clonal multiplication of genetically homogenous plants is the ideal scenario. Uncontrolled and unpredictable spontaneous variation throughout the cultural process is thus an unanticipated and largely undesirable phenomenon. This is attributed to somaclonal variation in production of clones and low secondary metabolite titers. In contrast to these detrimental consequences, its use in crop improvement through the development of new variations is widely recognized. The expense of culture material, electricity, and labour are other issues with in vitro tissue culture cultivation [83-194, 204].

Somaclonal differences that occur during in vitro propagation, commercial phytochemical synthesis, or genetically modified plants can have significant economic consequences are the major impediment to the practical application of plant tissue culture techniques for the production of active metabolites [83-194, 204].

Artificial neural networks (ANNs) are widely used in science and technology, and have been successfully applied in Cannabis sativa plant tissue cultures [201, 202]. Furthermore, Artificial neural networks (ANNs) can also simulate the growth of plants under different in vitro conditions[201, 202]. *Cannabis sativa* micropropagation has largely been an underground effort with few peer reviewed studies. This lack of insight concerning in vitro cannabis techniques has limited the biotechnological utility of Cannabis crop[201, 202]. This is mainly due to the fact that Cannabis sativa found to be recalcitrant under in vitro conditions, restrictions, long legacy of prohibition and stigmatization surrounding this **Indian origin** medicinal plant [201, 202, 204]. Machine Learning (ML) and Deep Learning (DL) are two of the most exciting technological areas of Artificial Intelligence (AI). Data is a power today, and artificial intelligence (AI) can help Cannabis businesses to gather and analyze data in a wide variety of ways. Artificial Intelligence (AI) technology has enhanced Cannabis crop production and improved real-time monitoring, harvesting, processing and marketing[201, 202]. These technologies saves the excess use of water, pesticides, herbicides, maintains the fertility of the soil, and also helps in the efficient use of man power and elevated the productivity and improved the quality of Cannabis products [201, 202]. However, very few and limited in vitro regeneration protocols have been developed in Cannabis and existing protocols highlights only organogenesis [201, 202, 204]. Therefore, there is a golden opportunity for the development of new in vitro regeneration protocols particularly induction of somatic embryogenesis, cryopreservation, protoplast isolation and culture, genetic transformation, production of synthetic seeds, and anther culture for the production of haploids in *Cannabis sativa* [201, 202, 204].

13. 3D Printing: Plant Tissue Culture Lab Ware

3D printing, also called Additive Manufacturing (AM), has the potential to be a technological revolution in the manufacturing industry [1-36-41-82]. It provides an opportunity to produce objects with a high precision rate based on a digital design of the product [1-36-41-82]. Recently, 3D printing has gained immense popularity because of its ability to offer rapid prototypes, make molds and templates to facilitate massive scale production, customer-oriented designs, a high degree of accuracy for complex structures, and efficient and fast customized fabrications for small scale production with economical costs [1-36-41-82]. 3D printing is making significant changes in various sectors, including packaging, Commercial culture vessels are generally manufactured by injection moulding. However, injection moulding requires large upfront investment which makes prototyping new vessels with this process extremely expensive [1-36-41-82]. Additive manufacturing (AM), or 3D-printing, is a technology in which models can be designed using a variety of software and manufactured using techniques such as fused deposition modeling (FDM), stereolithography (SLA), and selective laser sintering (SLS) [1-36-41-82]. Due to mainstream and hobbyist adoption, 3D printers have recently become small, affordable, and user friendly [1-36-41-82]. While these techniques are generally not well suited to large-

scale manufacturing, they allow rapid prototyping and small scale production of specialized/customized parts [1-36-41-82]. This technology allows researchers who are familiar with the problems of their system to develop problem-specific solutions that may not be known to manufacturers or may not be feasible as a commercial product[1-36-41-82]. Recently, this technology has also been employed to make customized lab ware, customized reaction-ware with reaction components printed for various chemistry applications, as well as medical simulation and education [26, 36-41].

Shukla et al., (2017) [41], are of the opinion that due to the complex process of designing and manufacturing new plant tissue culture vessels through conventional means there have been limited efforts to innovate improved designs [41, 42, 195-197]. Further, development and availability of low cost, energy efficient LEDs of various spectra has made it a promising light source for plant growth in controlled environments [41, 42, 195-197]. However, direct replacement of conventional lighting sources with LEDs does not address problems with uniformity, spectral control, or the challenges in conducting statistically valid experiments to assess the effects of light. Shukla et al., (2017) [41], are of the opinion that prototyping using 3D printing and LED based light sources could help overcome these limitations and lead to improved culture systems [41, 42, 195-197]. According to the research work reported by Shukla et al., (2017) [41], a modular culture vessel design in which the influence rate and spectrum of light are independently controlled was designed, prototyped using 3D printing, and evaluated for plant growth[41]. This design is compatible with semi-solid and liquid based culture systems. Observations on morphology, chlorophyll content, and chlorophyll fluorescence based stress parameters from in vitro plants cultured under different light spectra with similar overall fluence rate indicated different responses in *Nicotiana tabacum* and *Artemisia annua* plantlets[41]. The experiment by Shukla et al., (2017) [41]. validated the utility of 3D printing to design and test functional vessels and demonstrated that optimal light spectra for in vitro plant growth is species-specific [41]. According to the research work reported by Shukla et al., (2017) [41], 3D printing was successfully used to prototype novel culture vessels with independently controlled variable fluence rate/spectra LED lighting[41]. This system addresses several limitations associated with current lighting systems, providing more uniform lighting and allowing proper replication/randomization for experimental plant biology while increasing energy efficiency[41]. A complete procedure including the design and prototyping of a culture vessel using 3D printing, commercial scale injection molding of the prototype, and conducting a properly replicated experiment are reported[41]. This open source design has the scope for further improvement and adaptation and demonstrates the power of 3D printing to improve the design of culture systems[41].

14. 3D printing: Plant-derived compounds for Nozzle Design

3D printing makes it possible to offer personalized packaging for clients by making different prototypes at high speed according to their demand [43]. In this way, waste parts can be reduced, protecting the environment, and improving efficient production in manufacturing[1-43]. It can even go beyond that by making the packaging a part of the products [1-43-82]. There are different additive manufacturing technologies, and more importantly, there are various materials for 3D printing[1-43]. The prevalent challenge in applying 3D printing in different industries is to apply proper material with essential properties that act as a substrate [1-82]. 3D printing materials are primarily from polymers such as polylactic acid, nylon, acrylonitrile, and butadiene styrene[1-82]. Also, ceramics, metals, and thermoplastics are commonly used [1-82]. However, these materials are not economical and have hazardous impacts on the environment and humans[1-82].

Additive manufacturing technology has been developed in the manufacturing industry; however, limited choice of materials and low printing speeds in large-scale production make 3D printing challenging in the industry [1-82]. Wood and cellulose-based materials have recently drawn a lot of attention for use as 3D printing materials due to their unique properties such as environmental friendliness, cost-effectiveness and abundancy [1-82]. However, because these compounds are derived from various natural sources, their different particle sizes can result in low 3D printing quality[1-82]. The packaging industry can significantly benefit from 3D printing technology for cellulose-based materials by producing high-quality recyclable economical packaging on a large scale according to the clients' demand[1-82]. The proposed nozzle design enables selecting different geometrical cross-sections of the nozzle dies and any number of extrusion points along the nozzle die simultaneously during the 3D printing process [43]. These capabilities lead to advanced performance and improved speed of 3D printing in large scale manufacturing [1-82]. The proposed nozzle design provides a novel technique for 3D printing of plant-derived compounds with remarkable advantages such as providing selective variable extrusion and multiple nozzle dies [43]. Compared to other existing 3D printing techniques, the proposed nozzle abilities make it a promising option with higher speed and better functionality for the packaging industry[1-82]. Without separating into its basic components, wood can be employed in 3D printing by considering the temperature settings and nozzle requirements of the printer [43.]. Bringing wood to 3D printing to

provide opportunities such as producing wood-like products and printing complex wooden objects in combination with wood was investigated [43]. The effect of wood content in 3D printing materials on the properties of 3D printed parts was investigated [1-43].

The utilization of wood and cellulose-based materials in 3D printing has gained increasing attention due to their significant advantages such as more environmental sustainability, cost-effectiveness, abundancy, biodegradability, renewability, and energy efficiency[1-43]. The packaging industry can benefit remarkably from applying cellulose based materials in 3D printing by producing recyclable cost-effective packaging. However, employing cellulose-based materials in 3D printing can cause problems[1-43]. Fiber sizes and properties of plant derivatives can result in nozzle clogging and low-quality printing products. As nozzle is a vital component of a 3D printer, designing a nozzle, which expedites the 3D printing process and promotes the quality of resulting products, seems inevitable.

One of the study by Sharma et al., (2021) [43], reported that the advantages and limitations of using wood, nanocellulose materials, microcrystalline cellulose, ethers/esters, hydrogels, and lignocellulosic materials as single components or combination with other materials in 3D printing have been investigated[43]. More importantly, a novel nozzle has been designed based on FDM technology. The proposed nozzle mechanism has significant prominences over other existing 3D printing methods, including selective variable extrusion and the possibility to have multiple nozzle dies [43]. According to the study conducted by Sharma et al., (2021) [43] reported that the suggested nozzle consists of a base cylinder, a rotating cylinder, nozzle dies, moving flexible selectors, and stepper motors [43]. By changing the length of flexible selectors, various extrusion points can be selected, and the opening space for material extrusion can be controlled [43]. Moreover, nozzle dies with various shapes and sizes can be provided. The mechanism allows the 3D printer to choose any number of extrusion points along the nozzle die for material flow[43]. Furthermore, it provides the possibility of switching multiple nozzle dies with different cross-sections to extrude materials with different shapes according to the geometrical cross- sectional area of the selected nozzle die[43]. According to the study conducted by Sharma et al., (2021) [43] reported this new nozzle design can be applied easily for 3D printing of packaging parts using plant derived compounds [43]. All the packing components can benefit from the proposed nozzle design, especially when prototypes of complex structures or individual customized products are on demand. Besides, it can reduce the printing time and improve the printing quality [43]. These potencies make it competent to be utilized for large-scale production where printing speed and quality are determinative factors [43]. However, the designed nozzle must be fully constructed[43]. Besides, a slicer software interface should be developed, which can use this prototype printing method to slice 3D objects and enable the users to customize slice settings based on the desired nozzle die [43]. The nozzle printing process can be improved by providing more degrees of freedom along the printing axis. All of these would be the future research directions [43].

15. 3D Printing: Ecological Research

According to Behm et al., (2018) [44] **,** ecological research often involves sampling and manipulating non-model organisms that reside in heterogeneous environments[44]. As such, ecologists often adapt techniques and ideas from industry and other scientific fields to design and build equipment, tools, and experimental contraptions custom-made for the ecological systems under study[44]. Three-dimensional (3D) printing provides a way to rapidly produce identical and novel objects that could be used in ecological studies, yet ecologists have been slow to adopt this new technology[44]. Behm et al., (2018) [44], reported that overall, 3D printing can provide time and cost savings to ecologists, and with recent advances in less toxic, biodegradable, and recyclable print materials, ecologists can choose to minimize social and environmental impacts associated with 3D printing [44]. The main hurdles for implementing 3D printing—availability of resources like printers, scanners, and software, as well as reaching proficiency in using 3D image software—may be easier to overcome at institutions with digital imaging centers run by knowledgeable staff [44]. As with any new technology, the benefits of 3D printing are specific to a particular project, and ecologists must consider the investments of developing usable 3D materials for research versus other methods of generating those materials[44]. The ecological research areas in which 3D printing is predicted to be the most impactful and current studies that have already used 3D printed objects [44]. The methodological workflow has been designed for integrating 3D printing into an ecological research program and give a detailed example of a successful implementation of 3D printing workflow for 3D printed models of the brown anole, *Anolis sagrei,* for a field predation study[44]. After testing two print media in the field, this study by Behm et al., (2018**)** [44]**,** showed that the models printed from the less expensive and more sustainable material (blend of 70% plastic and 30% recycled wood fiber) were just as durable and had equal predator attack rates as the more expensive material (100% virgin plastic) [44]. Recent studies have highlighted the benefits of 3D printing in terms of cost and time efficiency [44], yet ecologists wanting to implement 3D printing for the first time must still traverse a steep learning curve[44].

Behm et al., (2018) [44] are of the opinion that 3D printing technology has the promise to reduce the time and cost invested in creating custom materials used in ecological research, while at the same time increasing the ease at which collaborations occur within and outside the scientific community[44]. Although there is a learning curve for developing 3D image files, there are ample online libraries of 3D files, plus tech savvy students and 3D printing center staff can be extremely helpful [44]. Recent advances in print materials may reduce the footprint associated with this new technology [44]. Overall, as with any new technology, ecologists must weigh the costs in terms of time and monetary investments into developing usable 3D materials for research versus other methods of generating those materials[44]. If ecologists are in the position to commit the initial investment in securing printing resources and navigating the technological learning curve, the resulting ability to implement 3D printing into future studies could save time and money on the long term[44].

In the commercial and medical sectors, 3D printing is delivering on its promise to enable a revolution [1-43-82]. However, in the fields of Ecology and Evolution we are only on the brink of embracing the advantages that 3D printing can offer [47]. Walker and Humphries (2019) [47] also showed that the potential that 3D printing offers in terms of improved experimental techniques, greater flexibility, reduced costs and promoting open science, while also discussing its limitations[47].

In the commercial and medical sectors, 3D printing is delivering on its promise to enable a revolution. However, in the fields of Ecology and Evolution there are only on the brink of embracing the advantages that 3D printing can offer [1-47]. Therefore, few examples where the process has enabled researchers to develop new techniques, work with novel species, and to enhance the impact of outreach activities. According to Walker and Humphries (2019) [47] aim is to showcase the potential that 3D printing offers in terms of improved experimental techniques, greater flexibility, reduced costs and promoting open science, while also discussing its limitations[47]. By taking a general overview of studies using the technique from fields across the broad range of Ecology and Evolution, Walker and Humphries (2019) [47] showed that the flexibility of 3D printing technology aimed to inspire the next generation of discoveries. According to Walker and Humphries (2019) [47] following points to be noted on ecological research [47].

- 3D printing enables the rapid production of items for low unit cost by commercial suppliers, or if many experimental pieces are to be printed then this can be done in-house[47]. Printed items can be models of delicate bones, complex biological structures, microfluidic chambers, labware, or even hypothetical ancestral structures[47]. 3D printed models can remove the reliance on the use of museum-preserved specimens[47].
- A major limitation to the adoption of 3D printing is the initial cost of the printer, but this is falling[47]. The cost of CT or laser scanning any structure to be modeled can also be high, but with suitable CAD software, many items can be approximated to a reasonable degree and custom items designed[47].
- The impact of 3D printing on the environment is only just being studied [47]. Many materials are not recyclable, and some plastics may release chemicals into the environment. The amount of chemicals released and the effects these may have is unknown[47].
- Sharing of 3D models online is creating a large repository of objects that can be downloaded and printed, allowing anyone with a 3D printer to produce them[47]. This can facilitate replication of experiments in a way never before possible[47].
- The overarching advantage of 3D printing is the freedom given to researchers, allowing them to print custom objects, quickly and at relatively low cost[47]. The application of 3D printing in Ecology and Evolution has begun but the technique offers many more opportunities for the future[47].

16. 3DPrinting: Plant-based pharmaceuticals--- without plants

Earth is facing a concerning trend—the rapid depletion of potential plant-derived drugs [45]. Globally, tens of thousands of flowering plant species play vital roles in medicinal applications, but many of the pharmaceuticals dominating the United States market heavily rely on imported raw plant materials that require very particular climate conditions for optimal growth [45]. The threat to many plant species is intensified by factors such as climate change, invasive pests and diseases, and farming practices struggling to meet the large demand for end products [45]. To address these problems, a team of 10 at University of Rochester, New York, USA [45], undergraduate students pioneered new technologies to more efficiently replicate useful chemicals found in plants, including those endangered by Earth's changing climate[45]. Calling themselves "Team RoSynth," the students created an affordable 3D-printing system for optimizing production of in-demand, plant-derived drugs and pharmaceuticals [45]. As a test case, the team biochemically synthesized rosmarinic acid (RA) [45]. RA is typically extracted from plants such as rosemary, sage, and fern [45]. It is used as a flavoring and in cosmetics and has also been shown to have antioxidant and anti-inflammatory properties [45]. Team RoSynth designed their 3D bioprinter to print hydrogels—jelly-like substances made of water

and polymers that can hold and release biological molecules[45]. The Rochester team's system is unique because it prints genetically engineered bacteria and genetically engineered yeast in adjacent hydrogels, which are then submerged in a liquid nutrient broth[45]. The complex work of making the final product chemical is divided among the two different types of microbes, making the process go more easily and quickly[45].

17. 3D Printing: Viable Plant Cells Successfully Bioprinted

3D bio-printing of plant cells has emerged as a promising technology for plant cell immobilization and related applications [200]. Despite the numerous progress in mammalian cell printing, the bioprinting of plant cells is still in its infancy and needs further investigation[200]. One of the systematic study by Zhou et al., (2025) [200] reported on optimizing the 3D bioprinting of plant cells, using carrots as an example, towards enhanced resolution and cell viability [200]. This study by Zhou et al., (2025) [200] investigated the effects of cell cluster forms and nozzle size on the rheological, extrusion, and printability properties of plant cell bioinks, as well as on the resultant cell viability and growth [200]. This study by Zhou et al., (2025) [200] found that when the printing nozzle is larger than 85% of the cell clusters embedded in the bioink, smooth extrusion and good printability can be achieved together with considerable cell viability and long-term growth [200]. Specifically, this study optimized a bioink composited with suspension-cultured carrot cells, which exhibited better uniformity, smoother extrusion, and higher cell viability over 1 month culture compared to those with the regular callus or fragmented callus [200]. This work provides a practical guideline for optimizing plant cell bioprinting from the bioink development to the printing outcome assessment[200]. It highlights the importance of selecting a matched nozzle and cell cluster and might provide insights for a better understanding and exploitation of plant cell bioprinting [198-200].

A recent study has established a method to print viable plant cells using a 3D printer [35, 46]. Researchers at North Carolina State University (NC, USA) [35, 46] have developed a method to 3D print various types of plant cells using bioinks for a reproducible way to study cell-to-cell communication in three dimensions [35, 46]. This method could be used to gain insight into how plant cells communicate amongst themselves and with their environment, which is key to understanding plant cell function[35]. This could eventually lead to improved crop varieties and optimized growing conditions. A plant root has a lot of different cell types with specialized functions [35]. There are also different sets of genes being expressed; some are cell-specific [35, 46].

Capturing cell-to-cell signals in a three-dimensional (3D) environment is key to studying cellular functions [35, 46]. A major challenge in current culturing methods is the lack of accurately capturing multicellular 3D environments [35, 46]. In this study, the team of researchers established a framework for 3D bioprinting plant cells to study cell viability, cell division, and cell identity[35, 46]. This team has established long-term cell viability for bioprinted *Arabidopsis* and soybean cells[35, 46]. To analyze the generated large image datasets, the team developed a high-throughput image analysis pipeline[35, 46]. Furthermore, they showed the cell cycle reentry of bioprinted cells for which the timing coincides with the induction of core cell cycle genes and regeneration-related genes, ultimately leading to microcallus formation[35]. Last, the identity of bioprinted *Arabidopsis* root cells expressing endodermal markers was maintained for longer periods[35, 46]. The framework established here paves the way for a general use of 3D bioprinting for studying cellular reprogramming and cell cycle re-entry toward tissue regeneration[35, 46].

A new study from North Carolina State University, USA showed a reproducible way of studying cellular communication among varied types of plant cells by "bioprinting" these cells via a 3D printer [35, 46]. The researchers bioprinted cells from the model plant *Arabidopsis thaliana* and from soybeans to study not just whether plant cells would live after being bioprinted – and for how long – but also to examine how they acquire and change their identity and function[35, 46]. The process of 3D bioprinting plant cells is mechanically similar to printing ink or plastics, with a few necessary tweaks [35, 46]. "Instead of 3D printing ink or plastic, they used 'bioink,' or living plant cells[35, 46]. "The mechanics are the same in both processes with a few notable differences for plant cells: an ultraviolet filter used to keep the environment sterile and multiple print heads – rather than just one – to print different bioinks simultaneously[35, 46]. Live plant cells without cell walls, or protoplasts, were bioprinted along with nutrients, growth hormones and a thickening agent called agarose – a seaweed-based compound[35, 46]. Agarose helps to provide cells strength and scaffolding, similar to mortar that supports bricks in the wall of a building [35, 46]. The research showed that more than half of the 3D bioprinted cells were viable and divided over time to form microcalli, or small colonies of cells[35, 46]. The researchers also bioprinted individual cells to test whether they could regenerate, or divide and multiply [35, 46]. The findings of this research work showed that *Arabidopsis* root and shoot cells needed different combinations of nutrients and scaffolding for optimal viability [35, 46]. Meanwhile, more than 40% of individual soybean embryonic cells remained viable two weeks after bioprinting and also divided over time to form microcalli [35, 46]. Finally, the researchers studied the cellular identity of the bioprinted cells [35, 46]. *Arabidopsis* root cells and embryonic soybean cells are known for high proliferation rates and a lack of fixed identities[35, 46]. In other words, like animal or human stem cells, these cells can

become different cell types [35, 46]. They found that bioprinted cells can take on the identity of stem cells; they divide and grow and express specific genes[35, 46]. This experiment were able to examine the genes expressed by individual cells after 3D bioprinting to understand any changes in cell identity [35, 46]. The researchers planned to continue their work studying cellular communication after 3D bioprinting, including at the single-cell level[35]. This research work has been published in *Science Advances* [35, 46].

18. 3D Printing: Teaching Botany

Science education is most effective when it provides authentic experiences that reflect professional practices and approaches that address issues relevant to students' lives and communities. Such educational experiences are becoming increasingly interdisciplinary and can be enhanced using digital fabrication [55, 72]. Digital fabrication is the process of designing objects for the purpose of fabricating with machinery such as 3D-printers, laser cutters, and Computer Numerical Control (CNC) machines[55, 72]. Historically, these types of tools have been exceptionally costly and difficult to access; however, recent advancements in technological design have been accompanied by decreasing prices[55, 72]. Digital fabrication in the form of 3D-printing has emerged as an innovative pedagogical approach to enhance Science, Technology, Engineering, and Mathematics (STEM) learning across a range of settings and for a variety of purposes[55, 72]. Evidence from the learning sciences suggests that individuals learn when they engage in the process of making through digital fabrication[55, 72].

3D printing and its applications are constantly growing, mainly in terms of technological improvements for its most diverse uses, both in industry and for personal use, and the possibility for educational purposes through teaching models [70, 71]. 3D printing technology is an instrument that can make it possible to create educational models in a personalized and adapted way according to the needs of students, which can be worked on for scientific experimentation, illustrate concepts, facilitate the visualization of biological structures as parts of plants and others [70, 71]. The ways of teaching and learning have advanced over the years [70, 71] The use of 3D technologies is constantly growing. This technology can be applied in teaching through didactic models[70, 71]. At the same time as these technological and scientific advances occur, many may leave aside traditional knowledge and its respective importance, especially in the school environment[70, 71]. One of the study by

Miranda et al., (2023) [70] showed the 3D printing of teaching models of the main parts of Amazonian medicinal plants to be used as learning tools in teaching botany [70, 71]. Through bibliographical and field research, three well-known plants widely used medicinally in the Amazon region were chosen: Andiroba, Buriti, and Tucumã. Using 3D modeling programs, seeds and fruits, the materials most used in traditional medicine for these plants, were printed [70, 71]. The results focused on printing models depicting the anatomical structure of the species so that their medicinal importance could be highlighted through the structural visualization of their active principles so that their applications could be understood [70, 71]. Therefore, this study showed that didactic models can awaken interest in learning by enabling a visual and tactile experience of botanical structures that may not be part of students' lives, making it possible to rescue traditional [70, 71, 72].

19. 3D Printing: Green Bioprinting of Plant Cells

3D printing has demonstrated tremendous possibilities offered for tissue engineering and regenerative medicine, where complex structures of living cells and biocompatible materials were shaped into functional tissues and organs [1-35, 46-82]. Unlike animal cells, the use of green plant cells (green algae and land plants cells) for 3D bioprinting is still at its very early stage[1-35, 46-82]. The main feature that could be explored by green plant 3D bioprinting is the totipotency of the land plant cells[1-35, 46-82]. This asset enables any given part of the plant to regenerate a complete individual, identical or almost identical to the original one[1-35, 46-82]. Moreover, due to their high growth rate, green algae appear to be a handy tool to characterize the process of printing plant cells, called "Green bioprinting"[1-35, 46-82]. Successful transfer of 3D bioprinting of green plants could lead to a breakthrough in the understanding of the so-called in planta behavior, but also in innovation within plant-based industry for food industry or bioproduction of active biomolecules of plant-based (bio)materials[1-35, 46-82]. The concept of entrapping green plant cells in a hydrogel is called immobilization[1-35, 46-82]. The entrapping green plant cells within bioink prior its deposition for later cultivation could be considered as an advanced cell immobilization technique [9-35, 46-82].

Biological printing "Bioprinting" enables the precise deposition of cells in a viscous biomaterial in a specific spatial arrangement using a computer-aided printer [9-35, 46-82]. The evolution of this technology from mainstream 3D printing has demanded increasingly complex printing methods to enable the biological roles of the ink, such as cell adhesion, and proliferation to be served in addition to the mechanical properties required of conventional 3D printing

ink [9-35, 46-82]. Due to their embedded cellular material, bioinks are printed at much lower temperatures and demand mild, non-cytotoxic cross linking methods to preserve cellular viability, and function [9-35, 46-82].

3D bioprinting uses three major types of printing technology: inkjet, laser-assisted and extrusion[9-35, 46-82]. Inkjet bioprinting uses low viscosity solutions, such as cell suspensions that are deposited at high shear rates as droplets of 50μm (micrometer) diameter [9-35, 46-82]. In contrast, laser assisted bioprinting focuses a laser toward a laser-absorbing biomaterial layer which in turn produces a local pressure to enable ink deposition[9-35, 46-82]. Extrusion based bioprinting, or bioplotting, is the most common subtype, comprising the deposition of cells, and bioink using a nozzle driven by pneumatic, piston or screw forces[9-35, 46-82]. Although slower than laser and inkjet printing approaches, extrusion bioprinting enables structural deposition, and solidification as each layer is deposited and is associated with good cell viability [9-35, 46-82]. Each type of printing technology requires bioinks of differing viscosities for successful deposition: droplet and inkjet printers require viscosities of 1–300 mPa^{-s} whereas extrusion based printers require a minimum of 30–6×107 mPa^{-s}[9-35, 46-82]. As such, bioinks with tunable viscosity are most likely to offer the versatility required to undergo printing using different technologies [9-35, 46-82].

Green bioprinting has been yet tested for several biotechnology applications with successful results [9-35, 46-82]. The technology already demonstrated its capability to provide significant size constructs (> 3cm3), populated with cells from various origins, i.e., algae and superior 490 plants [9-35, 46-82]. The few published studies screened the main topics, green bioprinting must tackle, from biocompatibility to proliferation supporting biomaterial, through bioink rheology and cell survival, to printing fidelity and technology screening[9-35, 46-82]. Finally, these studies demonstrated that even with a similar basis than for mammalian cell bioprinting, green bioprinting needs new approaches and tools specifically developed for plant cells [9-35, 46-82]. As an example, looking at the exponential development of bioink observed in the biomedical field and mammalian cell bioprinting, it is expected that dedicated plant cell bioinks will be proposed in the next decade [9-35, 46-82]. For instance, today very few studies were targeting plant cell growth within the hydrogel post-printing, thus advanced proliferative bioink will have to be explored to reach scalability of such processes. One of the study already listed the parameters which will impact the cell growth and development, enhancing the capabilities of such *ex planta* tissue production [9-35, 46-82]. Most of them being very similar to the ones impacting mammalian bioprinted tissue maturation (i.e. gas exchange, cell/matrix interaction, matrix mechanical properties, cell-to-cell signaling, interaction between gelling agent and media constituents etc.) [9-35, 46-82]. Aggregation of 3D bioprinting technology and knowledge on modulating the cells micro-environment, might ultimately allow for controlling the fate of the cells[9-35, 46-82]. This will in a near future allow the achievement of plant organogenesis, inducing the production of specialized plant tissue, like wood, one of the most valuable renewable natural resources[9-35, 46-82]. Food production is also a major field of application, enabling the production without ecological constraints related to seasons, soil availability and water use[9-35, 46-82]. This is one of the main claims of contributors, to support and emphasize the breakthrough of green bioprinting [9-35, 46-82].

Another very recent work presenting green bioprinting and which could be considered as precursor for future applications of 3D bioprinting in plant science is the work of Beckwith et al. on the *Zinnia elegans* plant model [9-35, 46-82]. In this study, plant cell differentiation was induced by plant hormones modulation within the 3D construct [9-35, 46-82]. By tightly regulating ratios of auxin and cytokinin hormones in the cultivation medium of their bioprinted structures, after 12 days cultivation, the authors managed to obtain different ratios of lignification of the constructs [9-35, 46-82]. This was aligned with the detection of other markers of plant cell differentiation, namely cell viability, enlargement, and elongation[9-35, 46-82]. This study represents a key step for the *in vitro* formation of wood and opens the way to the study/control of the structural properties of such cell-filled architectures [9-35, 46-82]. Finally, in a non-bioprinted but still 3D printing approach, Wightman's team demonstrated new plant cell behaviors and morphologies induced by the interaction between 3D micro and nanostructures, and plant cells [9-35, 46-82]. Here, 3D scaffolds composed of polyethylene terephthalate (PET) microfibers and polylactide (PLA) nanofibers were produced through a shear spinning method and seeded with *Arabidopsis thaliana* and *Zinnia elegans* cells [9-35, 46-82, 198-200]. While being isolated as spherical or ellipsoidal cells, they were adapting to the fibers shape and wrap themselves around them, a new behavior and morphology never observed *in planta*[9-35, 46-82].

Green bioprinting, just like mammalian cell bioprinting, is targeting 1 to 10 dm^3 prints 200 size which will be populated with tremendous amounts of cells reaching up to 1.8 × 103 $cells.dm^{-3}$ [9-35, 46-82]. It is important to permit *in situ* proliferation during sterile culture, in order to minimize inoculation density within the bioink [9-35, 46-82, 198-200]. It is also of great importance to develop a dedicated hydrogel blend to grow and cultivate the plant cell-laden scaffold [9-35, 46-82]. Similarly, to mammalian cells bioprinting, the materials with which the cells are mixed must be biocompatible and exhibit rheological properties allowing for cell protection and high shape-fidelity of the printed constructs[9-35, 46-82]. Among the technologies available for bioprinting, the micro-extrusion technologies are to date the most explored ones for green bioprinting[9-35, 46-82, 198-200].

For now, green bioprinting was applied to the two categories of "green plants" being photosynthetically active organisms, land plants or micro-algae [9-35, 46-82, 198-200]. As presented up to 80% of the published work targeted land plant cells with only seldom work targeting micro-algae[9-35, 46-82]. Within these published works, bioink seeding necessitates an important amount of cell biomass which implies to deploy dedicated *in vitro* cell plant culture strategies[9-35, 46-82, 198-200]. Seeding cell densities is still a parameter that is not strongly controlled for most of the studies[9-35, 46-82]. Indeed, as an example, some studies worked on concentrated biomass mass 10 to 40g of basil cell plant concentrated biomass per g of bioink [9-35, 46-82]. Furthermore, 50% wt per bioink volume of rice plant cells while others numerate the cell biomass to seed at precise cell densities (2 x 224 10^6 cell/g of bioink for *C. reinhardtii* or *C. sorokinian*a [9-35, 46-82]. For plant cell bioprinting, single or clustered cells in suspension have been used[9-35, 46-82, 198-200]. A recent study also evaluated a bioprinting strategy with 100 cells' agglomerates, reaching diameters up to 150 µm [9-35, 46-82]. It is important to note that working with large cell aggregates necessitates the use of large bioprinting nozzles [9-35, 46-82I. In one of the study, micro-extrusion of live cells was only achieved with nozzles of 610µm inner diameter, while smaller nozzle sizes were clogged during the 230 extrusion process [9-35, 46-82]. As for the upfront bioproduction of plant cells biomass, growth protocols were inherited from plant tissue culture (PTC) field [9-35, 46-82]. Two types of cell expansion protocols should be distinguished, (i) the growth of unorganized plant cell aggregates or (ii) the growth of single plant cells suspension[9-35, 46-82, 198-200]. In addition to biological and rheological properties, in order to be valid in vivo tissue replacements, bioinks must have adequate mechanical strength to support de novo tissue formation [9-35, 46-82]. Common mechanical assessments for bioinks include compressive, storage and elastic moduli, residual, and maximum compressive stresses[9-35, 46-82, 198-200].

The culture methodologies of bioprinted plant cells could be assimilated to the culture of immobilized cells [9-35, 46-82, 198-200]. Thus, similar problematics like lightning homogeneity, or oxygenation and mass transfer of molecules within the cellularized matrix will apply [9-35, 46-82]. In fact for now, none of the environmental parameters induced by 3D bioprinting (shear stress during micro-extrusion, mechano-transduction/encapsulation) were described as influencing the callus formation or maintenance [9-35, 46-82]. Thus, the only specificities of the plant cell culture in bioprinted architectures holds in the quality of the hydrogel used for bioprinting [9-35, 46-82]. Indeed, culture matrices/bioink must be sufficiently transparent to allow for light diffusion, and hydrogel microporosity should allow for both nutrients, O_2 and CO_2 access to the cells [9-35, 46-82, 198-200]. An interesting example is the study of Balasubramanian et al., (2021) [44] in which microalgal cell density and chlorophyl content of the bioprints were compared for two lightning conditions : dark or light/dark (12:12 h) cycles with a light intensity of 23 µmol m^{-2} s^{-1} using Grow light LEDs strip (Red:Blue (4:1) [9-35, 46-82]. As for mammalian cell culture, the composition of nutritive media is cell specific and no dedicated formulations designed for 3D cell plant cultures[9-35, 46-82]. The only resides in the use of the 3D printed support as a nutritive substrate as for the study of Balasubramanian et al. (2021) [44] where *C. reinhardtii* was grown on bioprinted bacterial cellulose [44]. A primary challenge facing the translational potential of bioprinting for medical use is the pursuit of appropriate, biocompatible materials [9-35, 46-82, 198-200].

The most commonly used medium for 3D plant cell culture is the Murashige and Skoog (MS) medium, which can be specifically adapted for each plant species [9-35, 46-82]. Indeed, carbohydrate is brought thanks to the supplementation of sucrose and adjusted for each cell needs (30g/l for carrot and basil cells: or 10g/L) for *Zinnia elegans* [9-35, 46-82]. Another main adaptation consists in fine tuning the amino acids or hydrolysates contents fulfilling the plant nitrogen needs or adding specific vitamins, like Gamborg's vitamins pool applied by Park et al. (2020) for carrots plant cell growth [9-35, 46-82].

As for 3D bioprinting of plant cells, one of the main challenges to reach green bioprinting consists in formulating a biocompatible, printable bioink allowing to reach high shape-fidelity while offering a suitable environment for cells viability, proliferation, and differentiation [9-35, 46-82, 198-200]. The choice of the bioink formulation will depend on the cell specificity, the necessary rheological properties and the bioprinted object consolidation conditions ensuring cell survival during the whole process (cross-linking, gelation, post-processing techniques) [9-35, 46-82]. Many well-known and widely used biomaterials are derived from plants or microorganism and were therefore evaluated[9-35, 46-82]. Gellan gum, agarose, methylcellulose, nanocellulose, bacterial cellulose, carrageenan, pectin, silk, starch and alginate have been successfully applied to plant cell encapsulation and green bioprinting [9-35, 46-82, 198-200]. Within this list of available biomaterials, alginate, a polysaccharide derived from brown algae, is used in 70% of the studies [9-35, 46-82]. This biopolymer, commonly used in mammalian cells' bioprinting, was also proven to be suitable for plant encapsulation and green bioprinting[9-35, 46-82]. Indeed, it was demonstrated to support both viability and activity of encapsulated plant cells[9-35, 46-82]. *Morinda citrifolia* (Noni) cells were for example entrapped in alginate micro beads and demonstrated a change in their metabolism while being cultured in 3D [9-35, 46-82]. Also, *Nicotiana tabacum* (Tobacco) cells were encapsulated in an alginate matrix with the aim of increasing the secreted Scopolin bioproduction yield, when compared to cell suspensions[9-35, 46-82, 198-200].

In the last ten years, there has been increasing interest in the use of green plant cells (land plant & microalgae) for biotechnology and bioproduction [9-35, 46-82]. Indeed, plant 3D tissue/culture represents a tremendous tool for plant micropropagation and crops production, for the extraction of high-value compounds in the biopharmaceutical field, and last, but not least, for its application in space programs [9-35, 46-82, 198-200]. As for the bioproduction, it is now clearly established that organ plant culture through the process of *in vitro* redifferentiation is generally associated with an improved synthesis of secondary metabolites [9-35, 46-82]. In the spectrum of the available techniques to achieve organized plant cell culture, green bioprinting offers great potentials [9-35, 46-82]. Other attempts to regenerate whole plant organs under *in vitro* conditions (i.e. shoots or roots), from explants or from callus, demonstrated an improved production of secondary metabolites with a produced metabolites pattern similar to the field-grown parent plant [9-35, 46-82, 198-200]. Thus, Green bioprinting, very similarly to bioproduction with other cell immobilization techniques retaining cells entrapped in a microporous biomaterial, is expected to benefit to several bioprocess practices[9-35, 46-82, 198-200]. Exploration of the use of 3D structured photosynthetically active geometries is also a research field almost unexplored [9-35, 46-82, 98-200]. The work carried out by Balasubramanian and his team (2021) [44] has highlighted the possible production of live photosynthetic materials, with applications in smart textiles [9-35, 46-82]. They have particularly worked on the development of suitable methods for the culture of plant cells and their differentiation in 3D bioprinted structures [9-35, 46-82]. However, green bioprinting is in its infancy and needs to be explored and consolidated [9-35, 46-82, 198- 200]. There are still many aspects to be investigated, including the interaction between the cells and the hydrogel, the cell-cell interactions after 3D structuration, and a thorough structural analysis of the behaviour of the cells in these scaffolds [9-35, 46-82]. Overall, 3D bioprinting methods are suitable for all kinds of applications[9-35, 46-82, 198-200].

A new study demonstrates a reproducible method of studying cellular communication among different types of plant cells using a 3D printer [1-82, 198, 199]. Understanding plant functions requires a better understanding of how plant cells communicate and their surroundings. Plant cell bioprinting is mechanically similar to printing ink or plastics, with a few minor differences. Bioprinted with nutrients, growth hormones, and agarose were live plant cells without cell walls or protoplasts [1-82, 198, 199-200]. Like mortar for a brick wall, agarose contributes to cell strength and scaffolding. According to the findings, more than half of the 3D bioprinted cells were viable and divided over time to form microcalli or small cell colonies[1-82, 198, 199]. Arabidopsis root and shoot cells required different combinations of nutrients and scaffolding for optimal viability. Individual cells were also bioprinted to see if they could regenerate, divide, or multiply. Researchers discovered that bioprinted stem cells could take on the identity of stem cells. After 3D bioprinting, they could examine the genes expressed by individual cells [1-82, 198, 199-200].

20. 3D Printing: Plant derived Biomaterials

According to Jovic et al., (2019) [67] the applications of plant derived biomaterials for 3D bioprinting are immense [1-67]. Further applications of these natural materials may extend to wound dressings, implantable medical devices and drug delivery systems, potentiated by coupling to bioprinting technologies for truly customizable, and bioactive medical therapies such as wound dressings and drug delivery systems[1-67]. Irrespective of the current challenges for translation to clinical practice, this renewable, natural, and abundant set of versatile biological materials hold great promise for revolutionizing the future of bioprinting [1-67-82].

Alginate is one of the most commonly used materials in 3D bioprinting [67]. Alginate is renewably sourced from brown algae, commonly Laminaria hyperborean, *Laminaria digitate, Laminaria japonica, Ascophyllum nodosum,* and *Macrocystis pyrifera*[67]. The rheological properties of alginate-based bioinks must be considered in the context of 3D bioprinting. Nanocellulose, a versatile and abundant natural biopolymer, has shown great promise due to its robust mechanical properties, unique surface chemistry, desirable biological characteristics (high biocompatibility, low biodegradability, and non-toxicity), and cost efficiency [1-67]. From a morphological viewpoint, nanocellulose boasts an array of desirable properties for 3D bioprinting, and tissue engineering [1-67-82]. Agarose is derived from red seaweed and has garnered interest in the plant derived 3D bioprinting sphere due to its ability to be prepared as a thermal-reversible gel. Carrageenan is a relatively under researched polygalacton derived from the Rhodophyceae members of red algae seaweeds [9-35, 46-82]. The material is composed of alternating long chains of a-1, 3 Dgalactose, and b-1, 4 3, 6-anhydro-galactose with ester sulfates which emulate the structure of mammalian glycosaminoglycans[1-67]. Pectin is a natural constituent of plant cell walls, possessing high molecular weight, and hydrophilicity, making it an ideal candidate for hydrogel formation and subsequently 3D bioprinting [67-82]. Starch is a highly abundant storage polysaccharide derived from cereals and tuber plants such as potatoes[9-35, 46-82]. It is, however, important to appreciate that although primarily related to the 3D bioprinting of edible products, these printing specifications remain to be translated into application for 3D tissue engineering [67-82]. Furthermore, starch molecules may be compounded with other biopolymers to augment properties for bioprinting applications[1-67]. As such, research into the role of starch-based polymers for 3D bioprinting has been commenced, in particular for bone tissue engineering [67-82].

Fucoidan is an anionic sulfated, water-soluble polysaccharide derived from marine brown algae[67-82]. The material has garnered interest owing to its potential antioxidant, anti-inflammatory and angiogenic[1-67].

According to Li et al., (2023) [73], the types of biomass materials for 3D printing need to be further expanded. Although biomass has been studied and applied in 3D printing technology, the proportion of biomass in 3D printing raw materials is relatively small [73-82]. At the same time, improving the mechanical properties, stability, heat resistance, durability and compatibility of biomass materials with other materials is imminent. The addition of functional properties to biomass 3D printing materials should also be considered [1-73].

According to Li et al., (2023) [73], it is necessary to further enrich the types of 3D printing technology and update the existing technology to be suitable for biomass 3D printing [73]. Improving the printing speed and accuracy of 3D printing technology to meet higher end, finer and more complex applications is also an area that needs to be explored [73]. Finally, it should be put on the agenda to speed up the application field of biomass 3D printing products. The non-toxic and environmentally friendly characteristics of biomass resources can provide convenience for their use in various fields. With the development of biomass chemistry and processing technology, 3D printing technology using biomass as a raw material will be able to customize and mass-produce according to different needs [1-73].

21. Limitations

3D printing has revolutionized various industries by enabling the production of complex designs and shapes. According to Iftekar et al., (2023) [74], the potential of new materials in 3D printing has led to an exponential increase in the technology's applications [74]. However, despite these advancements, the technology still faces significant challenges, including high costs, low printing speeds, limited part sizes, and strength[74]. In some cases, researchers did not provide sufficient explanations and details, and many studies focused only on a limited range of materials, with insufficient information, a lack of in-depth study, and a smaller number of samples, thereby reducing the overall understanding of the consistency of the experiments[74]. Additive manufacturing is expected to continue advancing and improving, but it will take some time to overcome the challenges, particularly those related to the cost and speed of 3D printing[74]. As technology becomes more efficient, faster, and cost-effective, it will become more accessible to a wider range of users worldwide [9-35, 46-82]. Additionally, the industry will focus on sustainability, developing eco-friendly materials, and adopting circular economy models [74]. Overall, the future of additive manufacturing looks promising, and it will be fascinating to witness the emergence of innovations and applications in the years to come [1-74].

22. Conclusion

3D bioprinting is a rapidly evolving field of biomedicine, merging the disciplines of tissue engineering, materials science and 3D printing technologies to yield viable biological structures in customized spatial arrangements. The implications of the technology are immense, lending itself to fuel advancements in fields such as drug delivery, reconstructive transplantation surgery, and implantable medical devices. Bioprinting technology is becoming an increasingly available and affordable technology to support tissue engineering research, with many research groups now opting to customize their own in-house devices owing to the widespread availability of open source software and firmware. A major limitation of bioprinting for biological constructs is the choice of a suitable bioink, one not only biologically conducive of cell growth but in possession of the necessary rheological properties such as viscosity, shear thinning, and crosslinking to optimize post-printing fidelity. Biomass materials are mainly degradable materials made from woody, herbaceous, gramineous and vine plants, as well as their processing residues and waste, through physical, chemical and biological means. Biomass materials have the advantages of being green, widely sourced and cost-effective. They have broad development potential as green 3D printing consumables. They have broad development potential as green 3D printing consumables. In recent years, as the concepts of green and low-carbon have been emphasized, the research of biomass materials in 3D printing has been gradually developed. Biomass resources have been applied in various technologies of 3D printing and the types of biomass materials used in 3D printing are also constantly enriching. The post-treatment of 3D printed specimens of biomass also includes various methods such as drying, solidification, heat treatment and surface modification, which can improve the performance of printed specimens. It is not an exaggeration to say that the advantages of 3D printing technology, such as high material utilization, low energy consumption and personalized customization, combined with the sustainable, non-toxic and low-cost advantages of biomass, will have a significant impact on the sustainable development of society and promote continuous progress in fields such as biomedicine, electronic equipment, food, textiles, architecture and furniture. Overall, while 3D printing technology has advanced significantly in recent years, there are still many challenges that need further development rather than investigation. Thus, considerably more research is needed to explore the potential of this technology, develop new materials, and improve the cost and speed.

Unlike animal cells, the use of green plant cells (green algae and land plants cells) for 3D bioprinting is still at its very early stage. The main feature that could be explored by green plant 3D bioprinting is the totipotency of the land plant cells. Green bioprinting has been yet tested for several biotechnology applications with successful results. 3D bio-printing of plant cells has emerged as a promising technology for plant cell immobilization and related applications. As for 3D bioprinting of plant cells, one of the main challenges to reach green bioprinting consists in formulating a biocompatible, printable bioink allowing to reach high shape-fidelity while offering a suitable environment for cells viability, proliferation, and differentiation. There are many applications of 3D printing technology in plant science for example, plant cell printing, plant phenotyping, ecology, printing of secondary metabolites without plants, used as model system in teaching botany, and viable cell printing. There are many applications of plant tissue culture which is used in the production of somatic embryos, plant regeneration via organogenesis, germplasm conservation, synthetic seeds, protoplast culture for the production of somatic hybrids, anther culture for the production of haploids, synthesis of secondary metabolites of pharmaceutical interest, and plant cells used for the bioenergy. However, green bioprinting is in its infancy and needs to be explored and consolidated. There are still many aspects to be investigated, including the interaction between the cells and the hydrogel, the cell-cell interactions after 3D structuration, and a thorough structural analysis of the behavior of the cells in these scaffolds. In turn, 3D printing technology is now being adopted across many scientific disciplines for rapid prototyping with emerging utility in plant science. Additive manufacturing technology has been developed in the manufacturing industry. However, limited choice of materials and low printing speeds in large-scale production make 3D printing challenging in the plant science.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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