Provisional chapter

From Neuronal Differentiation of iPSCs to 3D Neural Organoids: Modeling of Neurodegenerative Diseases

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Abstract

In the last decade, the finding that somatic cells can be reprogrammed into induced pluripotent stem cells (iPSCs) leads to a great improvement of research involving the use of differentiated stem cells as model of diseases. In the field of neurodegeneration, iPSC technology allowed to culture in vitro all the types of patient-specific neurons, not only helping the discovery of diseases' etiopathology but also testing new drugs with a personalized medicine approach. Moreover, iPSCs can be combined with the 3D bioprinting technology, allowing physiological cell-to-cell interactions, given by a combination of several biomaterials, scaffolds, and cells. This technology combines bioplotter and biomaterials which can encapsulate several types of cells, e.g., iPSCs or differentiated neurons, to develop an innovative cellular model. iPSCs and 3D cell cultures' technologies represent the first step to obtain a more reliable model, like an organoid to facilitate neurodegenerative diseases' investigation.

Keywords: cell culture, iPSCs, 3D bioprinting, disease modeling, personalized medicine

1. Introduction

Stem cells represent an unlimited cell source because of their property of self-renewal, and they can also differentiate into almost all adult cell types thanks to their pluripotency characteristic [1]. One of the main problems of stem cell research was the invasively harvesting techniques, such as through bone marrow, adipose tissue extraction by liposuction, or blood apheresis [2]. In 2006, the discovery that adult somatic cells can be reprogrammed into the so-called induced pluripotent stem cells (iPSCs) has allowed to generate stem cell lines with minimally

invasive techniques, like skin biopsy or, more recently, blood withdrawal [3]. These recent findings has led to an outstanding increase in disease mechanisms and drug screening studies involving stem cells, in particular for neurodegenerative diseases because of the impossibility to obtain neural cells from patients. The ability to reprogram patient-specific cells also opens new opportunities for the personalized medicine approach of drug discovery. Moreover, the development of 3D bioprinting provided a useful tool to generate innovative cell cultures, permitting to have a 3D model in which cells can be disposed with a controlled manner and where they can grow in a tissue-like structures [4]. Obviously, 3D bioprinting opened new possibilities in the field of tissue engineering, but it can be helpful also for disease modeling. In fact, the generation of a 3D scaffold that can resemble the human tissues will permit to study neurodegenerative diseases in the so-called brain in dish. Finally, the combination of 3D bioprinting technique with iPSC technology will permit to develop one of the most realistic and reliable in vitro cell cultures, permitting to study organoids with patients' differentiated cells, leading to a personalized medicine approach in drug testing.

2. Stem cells

Stem cell research is considered one of the most promising areas in cell biology and regenerative medicine due to stem cells' unique properties of self-renewing and differentiation into all types of cells. These cells represent nowadays the main tool in the regenerative medicine field because they permit to generate cells needed for transplantation in several degenerative diseases [1], such as rheumatoid arthritis [5], diabetes mellitus [6], heart failure [7], liver diseases [8], and neurological disorders [9–11]. Moreover, stem cells represent an important tool for modeling human diseases, in particular for diseases that affect cells that cannot be easily collected and cultivated. One of the biggest issues in the study of neurodegenerative diseases is the lack of good cellular models that recapitulate the mechanisms underlying their pathophysiology, and in the last decade, stem cells played a major role in the study of these diseases.

2.1. Embryonic stem cells

The first evidence that human stem cells, called human embryonic stem cells (hESC), could be derived from a 5-day blastocyst was reported in 1998 by Thomson and colleagues [12]. ES cells have the ability to proliferate indefinitely and are considered pluripotent cells because they can differentiate into all three germ layers (ectoderm, mesoderm, and endoderm) and, thus, they can generate all the differentiated cells of the adult [13, 14]. Despite that they helped stem cell research, they also opened many controversies because ES cells are obtained from blastocyst, killing the fertilized embryo that has the potential to generate a human being [15]. The big ethical issue on the use of hES cells encouraged researchers to understand the pathways underlying the staminality of this kind of cells.

2.2. Induced pluripotent stem cells

The research done with ES cells and the finding of their highly expressed transcription factors, permitted in 2006 to induce mouse's fibroblasts to become pluripotent, by retrovirus-mediated transduction with four transcription factors, i.e., Oct-3/4, Sox2, KLF4, and c-Myc [16]. The following studies allowed to improve the technique, permitting to generate

induced pluripotent stem cells (iPSCs) from adult human cells and to reprogram cells from several tissues [15]. Moreover, it is now possible to generate iPS cells by different transduction methods (**Figure 1**), using different viral and nonviral constructs, as well as integrative and non-integrative system approaches [17]. The best methods to reprogram cells are the non-integrative methods, and the four main groups are available: non-integrative viral delivery, episomal delivery, RNA delivery, and protein delivery [18]. The establishment of human iPS cells has led to have an unlimited source of stem cells overcoming the ethical limit of hES cells. Moreover, iPSCs can be reprogrammed from any somatic cell line of the patients providing a way to study diseases' mechanisms potentially for each patient, opening to the so-called personalized medicine (**Figure 1**). Actually, many iPSCs' lines have been generated from patients with neurodegenerative disease, like Alzheimer's disease (AD) [19], Parkinson's disease (PD) [20], amyotrophic lateral sclerosis (ALS) [21], and Huntington's disease (HD) [22].

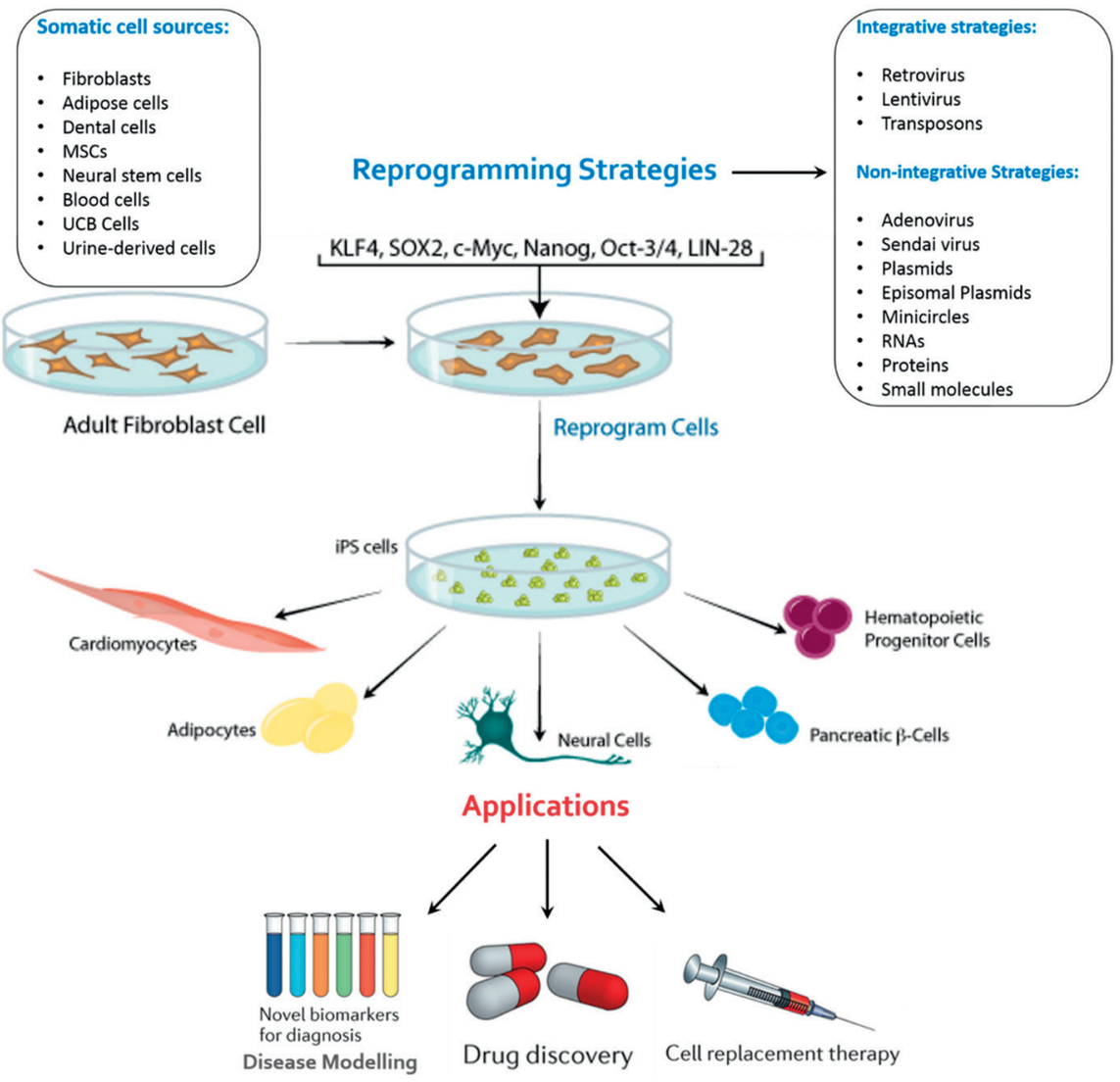

Figure 1. An overview of iPSC technology. Somatic cells can be taken from several sources, like the skin, blood, and urine. There are many reprogramming strategies, and the best ones are the non-integrative strategies. iPSCs can be differentiated into diverse cell lines that can be used for disease modeling, for drug discovery, and for cell replacement therapy (the image was taken from Sharma [23]).

2.2.1. iPSCs in Alzheimer's disease

Alzheimer's disease (AD) is the most common form of dementia and is characterized by the progressive loss of memory and cognitive functions. The disease leads to a severe form of dementia that causes the death of the patient [24]. The two main hallmarks of the disease are the accumulation of amyloid beta (Aβ) plaques in the extracellular compartment and the aggregation of the tau protein in the intracellular compartment. Only 1–5% of AD cases have a genetic cause, while in the other cases, the real pathogenesis is still unknown [25].

Many groups used and performed several studies on in vitro models with neural and nonneuronal cells derived from iPSCs. For example, higher susceptibility to Aβ1–Aβ42 oligomers was found in neuronal precursors derived from iPSC (iPSCs-NSCs) of a patient with a mutation in the PSEN1 gene (PSEN1-A246E mutation) compared to sporadic AD patient and healthy control [26]. The authors concluded that neurons derived from AD iPSCs could be effective in drug screening, to develop new treatments that protect cells from the toxicity of the Aβ peptides in the AD brain [26]. A similar result was obtained with iPSC-derived neurons of sporadic AD patients and of a patient carrying the pathogenic APP-E693Δ mutation. The study shows that these cell lines produce intracellular Aβ oligomers, resulting in a good cellular model of AD [27]. iPSCs can be used to find new potential biomarkers of the disease, as suggested by Shirotani et al. that developed an innovative method on neurons differentiated from iPSCs [28]. Moreover, induced fAD mutations by genome editing of neurons derived from healthy controls could resemble the pathophysiology of the disease. A decrease in endocytosis and soma-to-axon transcytosis of LDL was found in human neurons with expression of PSEN1ΔE9 induced with genome editing technology. To confirm the potential role of iPSCs in drug discovery, the authors reported that defects were rescued by β-secretase inhibition [29]. Another study reported the generation of an Alzheimer-related protein association network using iPSCs, demonstrating that they can be used as drug screening model and finding a reduction of tau protein after treatment with an inhibitor of γ-secretase [30]. For drug testing, it is important that iPSC-derived neurons are well differentiated, because it was seen that between early and late differentiation stages, cells have different susceptibilities to drugs [31]. Genome editing technology could be used also for mutations' correction, generating an isogenic control. For example, Pires and colleagues reported that A79V-iPSC line in combination of A79V-GC-iPSC line could be used to study pathological cellular phenotypes related to A79V mutation in PSEN [32]. Interestingly, the role of iPSCs in AD research was supported also by analyzing neurons derived from iPSCs of patients with Down syndrome that usually have a high risk of developing AD early. Authors found that such neural cells reproduce AD-like initial cellular hallmark, resulting useful for modeling this variant of AD [33]. Finally, also nonneuronal cells derived from iPSCs could be very useful in disease modeling and drug screening. Many pathological hallmarks were found aberrant in astrocytes derived from iPSCs of fAD and sAD patients suggesting that astrocytic atrophy could be a plausible mechanism for early cognitive impairment and thus opening new therapeutic strategies for AD intervention [34]. Another study reported changes in PSEN1-mutated iPSC-derived astrocytes, revealing the major role of such cells and confirming the importance to implement iPSC technology to support neurodegenerative diseases' study [35].

These researches suggest that iPSC-derived neurons from AD patients can help not only to unravel disease's mechanisms but also to screen new treatment and to find new possible drug targets. Moreover, the authors hypothesize that gene correction is a useful tool to generate isogenic controls or to induce AD mutations in healthy controls. Finally, iPSCs can be differentiated into glial cells, e.g., astrocytes, which in recent years gain an important role in the pathogenesis of several neurodegenerative diseases.

2.2.2. iPSCs in Parkinson's disease

Parkinson's disease (PD) is the second most common neurodegenerative disease after AD, with a prevalence of 1% out of the individuals over age 60 years and 4% of the population with an age over 85 years [36]. The most common mutations, found in about 10% of Parkinson's patients, are present in six genes: SNCA, LRRK2, Parkin, PINK1, DJ-1, and ATP13A2 [37].

Usually, iPSCs are differentiated into dopaminergic (DA) neurons to model PD because the disease is characterized by the loss of DA neurons of the *substantia nigra* in the midbrain. Since monogenic mutations cause an idiopathic-like disease, diverse iPSC lines of patients with Parkin and PINK1 mutations (e.g., 2–4 exon deletions of Parkin and PINK1 Q456X) have been developed. It was seen that these cell lines present abnormalities in mitochondrial and dopamine homoeostasis, microtubular stability, and axonal outgrowth, resulting in an optimal model of the disease [38]. For example, many PD cell phenotypes, i.e., mitochondrial dysfunction, elevated α-synuclein, synaptic dysfunction, DA accumulation, and increased oxidative stress and ROS, were found in iPSC-DA neurons of patients carrying mutations in parkin (V324A) and PINK1 (Q456X) genes [39]. The role in neurons' maturation of elevated α-synuclein caused by SNCA gene triplication was investigated in a cellular model obtained from PD-derived iPSCs. The author has claimed that such triplication leads to the impairment of differentiation and maturation of iPSCs [40]. An electrophysiological characterization of control dopaminergic neurons derived from iPSC was provided by Hartfield and colleagues that confirmed that these cells have the physiological hallmarks of dopaminergic neurons previously reported only on rodent slice. These results suggested that such cells can be considered a useful tool for the physiological study of PD [41]. Moreover, several evidences suggest that PD is not only a brain disease but also a gastrointestinal disorder; thus, Son and colleagues differentiated iPSCs carrying an LRRK2 G2019S mutation in both neural and intestinal phenotypes, providing the first evidence that G2019S mutation causes significant changes in gene expression also in the intestinal cells [42]. Interestingly, the pathologic phenotype was reversed in cortical neurons derived from iPSCs of patients mutated in SNCA using a small molecule found by yeast screening, opening new possibilities in drug screening and testing [43]. Finally, iPSCs have allowed an innovative co-culture of microglial cells and cortical neurons, displaying a unique cytokine profile impossible to obtain without iPSCs [44]. iPSCs were proposed to be used for tissue transplantation, and Kikuchi et al. achieved the transplantation of human iPS cell-derived dopaminergic neurons in a primate model of PD treated with MTPT. The authors reported an increase in spontaneous movement of the monkeys, demonstrating for the first time that such transplantation could be clinically applicable for the treatment of PD patients [45].

The studies previously reported hypothesize that iPSC-derived neurons from PD patients can be very useful in the research of PD pathophysiology and to find new therapeutic targets for innovative drugs. Moreover, the possibility to differentiate iPSCs into nonneuronal cells, such as microglial and intestinal cells, will help to unravel the role of immunity response and the gastrointestinal disorder that affect PD patients.

2.2.3. iPSCs in amyotrophic lateral sclerosis

Amyotrophic lateral sclerosis (ALS) is the most prevalent motor neuron disease and is characterized by the progressive loss of upper and lower motor neurons (MNs), leading to muscle atrophy, paralysis, and finally death usually after 2–5 years from the first diagnosis [21]. Also for ALS the cause is still unknown, but in about 5–10% of cases, several genes are found mutated, among which are SOD1, TARDPB, and FUS [46]. Moreover, in 2013 the GGGGCC-hexanucleotide repeat expansion in C9orf72 locus was found in many familial and sporadic cases of ALS [47].

MNs derived from iPSCs are the most common neural cell type used in ALS involving the use of stem cell differentiation. For example, an increase in oxidative stress and in DNA damage was found in iPSC-derived C9ORF72 MNs, confirming that the reduction of oxidative stress could help to delay patients' death [48]. Moreover, MNs derived from iPSCs with induced mutation in FUS (P525L) were used to investigate the transcriptome and microRNA, finding an alteration of both in pathways with implications for ALS pathogenesis [49]. The role of astrocytes was also investigated in both sporadic and VCP mutant patients, suggesting that in ALS patients, the co-culture between MNs and astrocytes causes alterations in both cell types [50, 51]. Moreover, the genetic correction allowed to study pathways implicated in ALS, like Bhinge and colleagues that found that the activation of AP1 drives neurodegeneration in genetic corrected SOD1 mutant MNs [52]. Small-molecule compounds that regulate IGF-2 expression were found to increase MN resilience, screening the compounds in iPSC-derived MNs [53]. Another example is given by Egawa and colleagues that firstly generated and characterized MNs from iPSCs of patients carrying TDP-43 mutations. They found some pathological hallmark, such as short neurites and abnormal-insoluble TDP-43. Then, they tested trichostatin A, spliceostatin A, garcinol, and anacardic acid and found that the last one, an inhibitor of histone deacetylase, rescued the pathogenic abnormalities like TDP-43 mRNA [54]. All these researches suggest the increasing importance of iPSCs as model for drug screening.

These works suggest that MNs derived from iPSCs of mutated and sporadic ALS patients can be a helpful tool to study both disease mechanisms and drug screening. Several investigations can be done in iPSC-derived MN cellular models, e.g., oxidative stress, DNA damage, and transcriptome. The co-culture between astrocytes and MNs can give information about how they interact with each other and whether this interaction could have a pathophysiologic role in ALS.

2.2.4. iPSCs in Huntington's disease

Huntington's disease (HD) is characterized by loss of neurons mainly in the caudate nucleus, the putamen, and the cerebral cortex with affection in a later stage of other areas, e.g., the hippocampus and hypothalamus [55]. Despite other neurodegenerative diseases, the cause of HD is well known; in fact it is an autosomal dominant genetic disorder caused by an

expansion mutation of the trinucleotide (CAG) repeat in the HTT (IT15) gene, encoding a 350-kDa protein called Huntingtin (HTT) [56]. Even though the genetic cause is clear, the mechanisms through which mutant HTT results in the degeneration of some types of neurons are still unclear. Thus, studies on HD models are needed in order to discover treatments.

As the neurodegenerative diseases previously reported, also for HD, neurons differentiated from iPSCs of patients helped to understand the role of mutant HTT gene and the mechanisms that lead to the pathology. For example, early molecular changes in intracellular signaling, expression of oxidative stress proteins, and p53 pathway both in iPSCs and in neurons differentiated from them were reported [57]. Another study reported changes in neuronal development and adult neurogenesis, exploiting the iPSC capacity to model also embryonal development [58]. The generation of iPSCs from a patient that presents an expansion in the HTT gene without any symptom is very intriguing. The generation of iPSCs in an early stage of HD will allow to study the pathological process and the abnormal changes that lead to the pathology [59]. The possibility to differentiate iPSCs into neurons opened the possibility to discover new therapeutic targets, e.g., pre-mRNA trans-splicing modules [60]. Finally, the role of glial cells was investigated in several studies, among these who studied it were Hsiao and colleagues that reported that HD astrocytes provide less pericyte coverage by promoting angiogenesis and reducing the number of pericytes [61]. Finally, in a mouse cell model of HD, many but not all pathological hallmarks of HD were found. This result suggests that nonhuman iPSCs must be used carefully when translated into human pathology [62].

The researches previously reported highlight the importance to have a realistic model of the disease to study mechanisms that lead to neurodegeneration and iPSC-derived neurons that represent as a useful tool. They can be used also to perform a study of drug discovery and drug screening, to better understand the effect of chemicals in neurons. Moreover, the possibility to differentiate iPSCs in nonneuronal cells, such as astrocytes, helps to discover the role of glial cells in HD pathogenesis.

3. 3D bioprinting

The term bioprinting was used for the first time in 2009 by Mironov with the release of the first issue of the journal *Biofabrication*, a magazine that took its name from the eponymous term biofabrication. While the term biofabrication is intended to indicate natural processes such as biomineralization, the term bioprinting is defined by Guillemot in 2010 as [63, 64].

The use of computer-aided transfer processes for patterning and assembling living and nonliving materials with a prescribed 2D or 3D organization in order to produce bio-engineered structures serving in regenerative medicine, pharmacokinetic and basic cell biology studies.

3D bioprinting is an emerging technology, used for the manufacture and the generation of artificial tissues and organs [65], adding new approaches to tissue engineering (TE) and regenerative medicine, such as the manufacture of scaffold to support cells, as well as in situ deposition of cell suspensions [63]. Bioprinting technology has allowed to overcome several limits, such as the control of in vitro 3D biological structures and cellular distribution [66].

Bioprinting, through the use of hardware and software, has been used in particular for the design of three-dimensional structures, allowing the creation of "organoids" for biological and pharmacological studies, and to repair and replace human tissues.

3.1. Bioprinting and bioplotter techniques

Bioprinting can be distinguished on the basis of the bioink printing technique, allowing to change the printing processes according to the needs that the different cell types require: ink-jet, laser, and extrusion (**Figure 2**) [65]. In addition to the specific printer characteristic, each bioplotter must have common functionalities. The most important is the presence of a robotic displacement system that can move along the three Cartesian axes, x, y, and, for the 3D characteristics, z. Usually, the bioink is extruded from a dispenser, but it is possible to have more dispensers, permitting to have different bioinks in the same scaffold. One of the most recent techniques allows a coaxial extrusion, with a bioink that is surrounded by a second bioink. The sterility of the printout is usually guaranteed by the presence of sterile chamber with laminar flow system. If the bioplotter is quite small, this problem could be overcome by simply placing the bioprinter under a classic cellular hood. Finally, the presence of a dedicated software for the supply of bioink and for the high-resolution control of the design of the construct to be printed is essential [65].

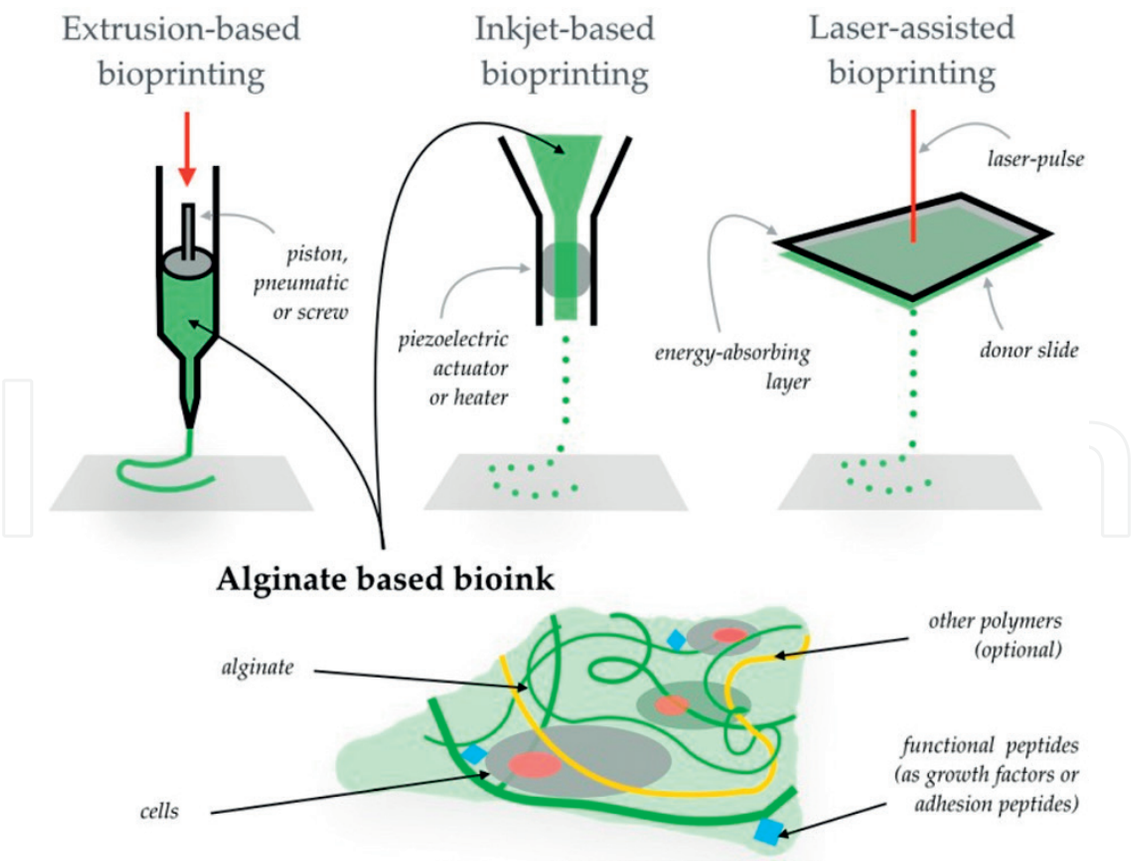

Figure 2. On the top the most used printing processes are extrusion-based (performed by piston, pneumatic method, or screw), inkjet-based operated by a piezoelectric actuator, and laser-assisted (composed of an energy-absorbing slide and a donor slide that collect the discharged bioink droplets). On the bottom the complexity of the 3D bioprinted construct, composed of a natural biocompatible material (e.G., alginate), cells, functional peptides, and other biocompatible materials. (the image was taken from Axpe and Oyen [67]).

First, bioplotters that appeared on the market were intended for a purely industrial use, since they had a high price. The costs limited their development and related researches. With the advent of technology and knowledge of bioprinting, we have witnessed the birth of multiple models of bioplotters, each with characteristics that reflect the needs of the individual creator [12, 13], e.g., increasing the number of nozzles for simultaneous extrusion of several materials [68] and changing the type of technology that controls the nozzle.

3.1.1. Inkjet bioplotter

Inkjet bioplotter was the first technique used in the 1980s in offices and then for domestic use. It was readapted around the year 2000 to be used as a biological printer, replacing the normal ink with a bioink, containing cells and biocompatible materials [69]. Droplets of the biomaterial are extruded from very small orifices, deposited on a substrate, maintaining good cell dispersion, viability, and functionality, even with different cellular types [70]. The stream can be continuous, command-driven (drop on demand), and electrodynamic. Both piezoelectric and thermal inkjet printers have been readapted for biological printing, offering many advantages in terms of simplicity, versatility, and material control, both in terms of quantity and speed in the printing process [71].

3.1.2. Laser technology

Laser-based direct writing was introduced in 1999 and is one of the most used laser-based bioprinting techniques [72]. The technique involves a layer with biological material (donor layer) and a layer that collect cells (acceptor layer) that are pushed by the laser through the first layer. The pulsation of the laser creates bubbles which in turn generate a shockwave, forcing the cells to pass from the donor layer to the acceptor layer. This technique allows to have a good resolution but has some disadvantages, such as irreversible damage to the cells because of the heat and light generated by the laser [65].

3.1.3. Extrusion-based bioprinter

The advent of TE has allowed extrusion technology to be thoroughly studied and applied to the field of bioprinting, for the generation of living tissues. Extrusion technique includes a combination of different delivery systems combined with an automatic robotic system for extrusion and 3D printing [73]. Deposition of the material takes place through extrusion to form a cylindrical filament made of a biocompatible gelatinous material, in which the cells are encapsulated, maintaining the desired three-dimensional structure [74]. This type of extrusion can be pneumatic or mechanical. Mechanical extrusion involves the use of a piston, which guides the deposit of the material allowing a good flow control through the nozzle, or a screw, which allows the extrusion of more viscous substances, but which can cause leakage of pressure, causing damage to cells. This technique also has some limits, particularly during the development of the biomaterial which must have the right viscosity to be extruded and the ability to be easily homogenized with cell suspension and to maintain three-dimensional structure [65].

3.2. Hydrogel and bioink

One of the fundamental elements that characterize the bioprinting process is the development of biomaterial, which must have specific characteristics: biocompatibility, printability, and

ability to maintain a three-dimensional structure once printed and maintained in culture [65]. The main feature of the hydrogel, biocompatible material used as a three-dimensional support for cell growth, is the ability to be extremely hydrophilic, making it an excellent candidate in terms of biocompatibility for its use in bioprinting. It was initially used in TE because it was able to simulate the extracellular matrix, guarantying cell growth and communication [75]. Biomaterials are divided, According to their derivation, biomaterials are divided in natural or synthetic compunds. There are naturally derived polymers such as sodium alginate, gelatin, collagen, chitosan, fibrin, and GelMA [76–78] and synthetic polymers such as Pluronic®, polyethylene glycol (PEG), and polyurethanes [79, 80]. Over time, it has been seen how the natural compounds are more performing when placed in contact with the cells than the synthetic ones. Several cell types associated with different biomaterials to compose the bioink have already been used in several research areas, where cellular viability and motility have been demonstrated, as well as a spatial organization similar to in vivo tissue [81]. To create a new biomaterial, we must consider different physical, mechanical, and biological characteristics that are close to the tissue we want to recreate. Thus, researchers tend to create a combination of biomaterials for each cell type, and with well-defined printability specifications, so as to make the process as standardized and reproducible as possible, despite being a very open field and full of new developments. New-generation bioinks are now able to maintain each of these characteristics, thus improving the success in terms of bioprinting. All this is possible if particular attention is paid to the following chemical, physical, and biological properties: rheological studies (viscosity, thinning, viscoelasticity), biofunctional analysis, biodegradation, and polymerization (cytocompatibility, cell adhesion, migration, proliferation, and differentiation) [82]. One of the most important features that has different biomaterials is the ability to cross-link once the bioink has been printed, reticulating the bioink in which cells are encapsulated, without affecting the viability, the differentiation, and the capacity of migration [83]. The ability to polymerize depends on the material used; for example, collagen needs chemical cross-link, through covalent bonds that bind free amines or carboxylic groups of collagen that is able to reticulate, also through a biological process, and through the interaction with transglutaminase. Other compounds, such as sodium alginate, use an ionic cross-link: divalent cations such as Ca^{2+} bind to two sodium alginate residues, cross-linking the structure. UV radiation is a very promising cross-link technique given its reaction speed but with many questions regarding the possible damage induced to the cells included in the material. Other materials, such as gelatin and agarose, are heat-sensitive, so they are used during the printing process at the melting temperature and then stiffened with cooling [84–87]. One of the most common types of bioink used in bioprinting techniques is the so-called cell-laden hydrogel, which includes natural hydrogels such as agarose, sodium alginate, chitosan, collagen, gelatin, fibrin, and hyaluronic acid and synthetic hydrogels such as Pluronic® and PEG. Hydrogels can be used with the most common bioplotter that mount different printing techniques, allowing the creation of bioinks that combine the advantages of the natural material with the advantages of synthetic materials [88]. Recent findings have shown also the possibility to transfect cells with target DNA or plasmid, directly during the bioprinting process [80]. A new promising method to develop bioinks is the base on decellularized extracellular matrix. This kind of bioink consists in eliminating cells from a tissue of interest, keeping intact

the extracellular matrix that is then pulverized and subsequently used as bioink once dissolved in a saline buffer. Finally, the cell suspension bioinks, characterized by a print that does not have a support material, like a scaffold, are also very common. It uses aggregates of cells in culture medium, placed in mono- or multicell spheres [73]. This technique is based on the liquidity of the tissue and its fusion, such as to allow cells to assemble, merge, and create cell-to-cell interconnections [89, 90]. Organovo was the first medical research company that used this technique to create functional human tissues. They developed a liver model using parenchyma cells and an extrusion printer of their own creation [91].

4. 3D bioprinting and neurodegeneration

In the last decade, the possibility of replacing dead cells in degenerative processes affecting the central nervous system opened the way for a more intense and accurate study of stem cells and their possibility of replacing damaged tissue [92]. It was also thought to exploit the ability of stem cells to secrete cytokines and growth factors, offering benefits such as anti-inflammatory effects, protection of neural cells, and endogenous recovery systems. Transplanting these cells into damaged sites presents various problems such as low cell survival and limited engraftment [93]. To minimize these problems, it was decided to use three-dimensional scaffold printing that mimics the complexity both from the biological and functional points of view of the tissue to be replaced [94].

The manufacture of three-dimensional prefabricated scaffolds has already given positive results in the treatment and repair of spinal and nerve damage but with a great limitation in terms of control of the external shape of the scaffold and of its internal architecture [95, 96]. These problems have been overcome with the 3D bioprinting, which leaves the operator complete freedom regarding the shape, the material, and its internal architecture. The recent developments in the field of 3D bioprinting are mostly aimed to the field of regenerative medicine, to respond to the growing demand for tissues and organs for transplants, arriving only later for this technology to be applied to basic scientific research. Until now only few studies have focused on using 3D printing applied to the creation of neural tissue compared to other widely studied tissues such as the skin, bones, heart tissue, and cartilaginous structures [97]. The few studies published so far, in which they use nerve cells in 3D printing processes [98, 99], show a poor characterization of bioinks to be used for nerve cells, due to the delicacy of the tissue to be recreated and of the characteristics necessary for the optimal growth of the nervous tissue [94]. Recently, researchers also think that the nervous tissue printed in 3D may be used for the neural regeneration, a huge possibility in the field of neurodegeneration to replace degenerated neural tissue [78, 80].

The creation of nerve tissue by bioprinting is also used for pharmacological studies, for toxicological screening, and for basic research. It is necessary to underline how this field is still in its infancy and how it is necessary to validate this model for the applications described up to now, to be sure that the model completely recapitulates the pathophysiology that we want to investigate with this tool [94] in particular with regard to neurodegenerative diseases.

5. Conclusions

In the last decade, two groundbreaking discoveries, i.e., somatic cell reprogramming into iPSCs and 3D bioprinting, changed the way to modeling diseases, in particular for those pathologies which are hard to study in simple cell cultures, such as neurodegenerative diseases. The first one is permitted to obtain neural cell cultures in few months starting from adult somatic cells, like fibroblasts and PBMCs, while 3D bioprinting consists in the print of hydrogel and cells, to generate models that imitate tissue characteristics. While iPSCs are differentiated into neurons in many papers for disease modeling, 3D bioprinting is actually used for few tissues, like the cartilage, bone, and heart. Neural 3D cell cultures are still in development, there are no target bioinks, and the studies that combine neuronal cells and 3D bioprinting are more complicated than other tissues because of the fragility of such cells. Despite this hurdle, the possibility to create an in vitro neural tissue would open many fields of research that today are unreachable, first of all the opportunity to study the 3D spatial connection between different neuronal populations and how they communicate with each other. In combination with iPSC technology, we can create a physiological model to understand physiological and pathological mechanisms and to better understand mechanisms underlying neurodegenerative diseases.

Finally, the combination between 3D bioprinting and iPSC technology will open not only new possibilities in many fields, drug screening, replacing expensive in vivo experiments, and overcoming animal models' issues, but also personalized medicine thanks to the use of cells derived from patients. More intriguingly, the generation of a 3D neural tissue composed of patient's cell will allow the so-called neuro-regeneration, opening the possibility to replace a degenerated tissue.

Conflict of interest

The authors declare that there is no conflict of interest.

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