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Review

3D bioprinting applications in neural tissue engineering for spinal cord injury repair

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ABSTRACT

Spinal cord injury (SCI) is a disease of the central nervous system (CNS) that has not yet been treated successfully. In the United States, almost 450,000 people suffer from SCI. Despite the development of many clinical treatments, therapeutics are still at an early stage for a successful bridging of damaged nerve spaces and complete recovery of nerve functions. Biomimetic 3D scaffolds have been an effective option in repairing the damaged nervous system. 3D scaffolds allow improved host tissue engraftment and new tissue development by supplying physical support to ease cell function. Recently, 3D bioprinting techniques that may easily regulate the dimension and shape of the 3D tissue scaffold and are capable of producing scaffolds with cells have attracted attention. Production of biologically more complex microstructures can be achieved by using 3D bioprinting technology. Particularly *in vitro* modeling of CNS tissues for *in vivo* transplantation is critical in the treatment of SCI. Considering the potential impact of 3D bioprinting technology on neural studies, this review focus on 3D bioprinting methods, bio-inks, and cells widely used in neural tissue engineering and the latest technological applications of bioprinting of nerve tissues for the repair of SCI are discussed.

1. Introduction

Vital and healthy functions of the body are controlled by a quite complicated system called the Central Nervous System (CNS) [1,2]. Degenerations in the CNS structure caused by physical damages or neurological diseases often cause loss of neuronal cell bodies, axons and glia support [3]. The CNS has a low capacity to replace neurons lost during damage or diseases [4]. One of the CNS diseases, spinal cord injury (SCI), breaks nerve contacts among the brain and other parts of the body. It causes sensory loss and paralysis under the level of damage. 11,000 new incidences emerge every year only in the US, with approximately 450,000 patients living with SCI [5,6]. Traffic accidents, acts of violence, falls, and sports injuries constitute most of the injuries [7]. SCI affects patients' quality of life, forces the families, and causes socio-economic impacts on healthcare systems worldwide [8]. Currently, one of the treatments for SCI is the application of methylprednisolone in overdoses to reduce secondary damage processes [5,9]. However, this therapy is disputable as it causes many critical side effects, containing sepsis, wound infection, gastric bleeding and pneumonia. Furthermore, it has just minor progress in neurological improvement [6,10]. Other therapy options applied for SCI contain the surgical operation for stabilizing, decompressing the spinal cord and multisystem medical administration, hypothermia, and rehabilitative medical care [5,6]. Despite the development of many clinical therapies, therapeutics are still in the early stages for the successful bridging of damaged nerve gaps and complete recovery of nerve functions [11,12].

Recent advances in nanotechnology and tissue engineering offer effective strategies to repair CNS diseases. Neural tissue engineering is focused on the improvement of an appropriate environment that connects the biomimetic scaffold with cells to repair and restore neural tissue function. Physical support for successful nerve regeneration is provided by three-dimensional (3D) scaffold and scaffold-like materials [13,14]. This results in better host tissue engraftation, followed by new tissue development to facilitate cell function [13,15]. An ideal scaffold for neural tissue engineering should meet the following criteria: (i) biocompatibility is an important feature for scaffold to provide cell adhesion, proliferation and differentiation in the lack of immune and cytotoxic reactions; (ii) biodegradability is essential for degradation of the scaffold at a close-matched rate of new tissue formation and finally scaffold removed from the system; (iii) neural transmission is based on the potential of action generated in the synapse. An electrically conductive scaffold can support neurite growth and neural regeneration;

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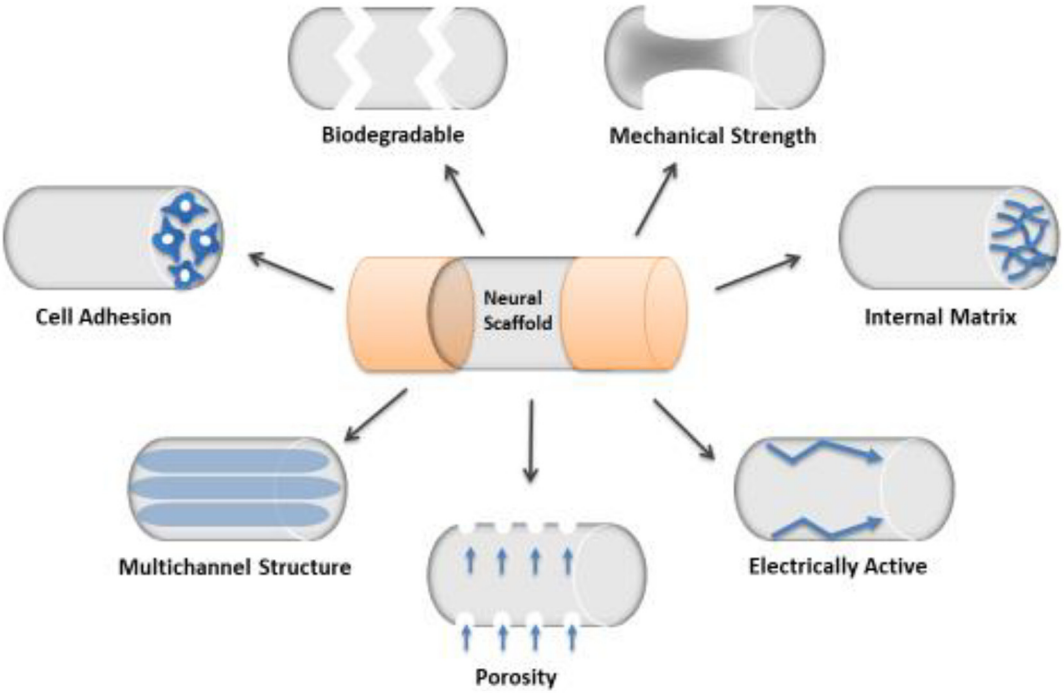


Fig. 1. Schematic illustration of design criteria for neural scaffold.

(iv) the scaffold must have appropriate mechanical properties so that it does not increase stress in the lesion region or collapse throughout regular motion; and (v) the scaffold with porous interconnection imitates the extracellular matrix (ECM) of natural tissue, vascularizations, allowing the cells to disperse well and exchange of waste and nutrients. (Fig. 1) [14,15]. Various methods such as gas foaming, melt moulding, electrospinning and phase separation have been used in the production of 3D scaffolds made of synthetic and natural polymers [16,17]. However, the scaffold shape or inner channel configuration and pore size in the scaffold cannot be exactly adjusted by these scaffold manufacturing methods. Besides, these techniques cannot allow fabrication of the scaffolds with cells due to harsh processing conditions.

Recently, 3D bioprinting methods have been noted, which can easily arrange the dimension and shape of 3D scaffold and fabricate scaffolds with cells [18]. 3D printing was described first by Charles W. Hull in 1986 [19]. 3D bioprinting also called additive manufacturing (AM) is the use of rapid prototyping (RP) systems for the printing of cells, growth factors, and different biomaterials in layers form. With this technology, biological structures that highly mimic natural tissue/organ properties can be fabricated [20]. 3D bioprinting utilizes 3D modeling program (*e.g.* computer-aided design (CAD) or computer tomography (CT) scan images) to make 3D solid or gelation objects [21]. Generally, 3D bioprinting system comprises an X-, Y-, Z-axis drive device, 3D modeling program, computers, and bioprinting ingredients/inks. Firstly, a model is formed by utilization CAD or CT images. Then a 3D scaffold structure is generated by bioprinting equipment attached to a computer [22]. In 3D bioprinting, bio-inks are an essential component. Bio-inks consist of biomaterials that can be used cell encapsulation and biomolecule incorporation. Before printing, the basic features of a bio-ink such as cross-linking, viscosity and gelation must be regarded. These features have an effect on printing quality, morphology, cell viability and proliferation [23]. According to the working principles, three basic 3D bioprinting technologies are defined: extrusion, inkjet and laser-assisted (Fig. 2) [24]. Within these technologies, extrusion bioprinting can generate 3D structures in large scale-up by utilization hydrogels with cell-laden. Inkjet bioprinting generally adapted from the commercial two dimensional (2D) printing system and prints liquid droplets with cell-laden for rapid and minor-scale products [25]. Laser-assisted bioprinting can create high-resolution structures using lasers as energy [26]. However, there are considerable advantages and limitations of these technologies in the production of tissue structures such as the neural scaffold mentioned above.

In this review article, various 3D bioprinting techniques such as extrusion, inkjet, laser, plotting used in neural tissue engineering will be discussed in detail. Furthermore, bio-inks and cells available for bioprinting will be described. Finally, bioprinting applications of neural tissues for the repair of SCI will be reported.

2. 3D bioprinting methods for neural tissue applications

Among the 3D bioprinting methods inkjet, microextrusion, bioplotting, stereolithography, and fused deposition modeling have attracted more attention in the neural tissue engineering studies.

2.1. Inkjet bioprinting

Inkjet bioprinting is a cost-effective technique. Bio-inks can be dispersed well by using inkjet bioprinting which has controllable manner. It permits simultaneous and contactless deposition of cells in certain directions in micrometer resolution [27]. Since bio-ink does not have high viscosity in this technique, generally results in structures with low mechanical properties. Besides, the small nozzle dimension and flow rate limit the volume accumulated per drop (< 10 pL). This means that high cell concentrations (> 5 million cells/ mL) must be seeded to maximize the probability that each bio-ink drop will contain a cell [28]. There are two types of inkjet printers: thermal and piezoelectric. In thermal inkjet printers, the temperature provides to heat the printer head and this forms air pressure pulses. These pulses enable droplets to push from the nozzle [27]. This method has high printing speed and a resolution between 20 and 100 μm. It has the potential to print droplets in picoliter volume for obtaining better resolution [29]. In piezoelectric inkjet printing, droplets are formed by applying a piezoelectric actuator. Applied voltage excites the piezoelectric crystal, which is located in the printer head. Deformation occurs both rapid and reversely due to this voltage. The speed and dimension of the droplets ejected can be controlled by changing factors such as time, pulse and amplitude

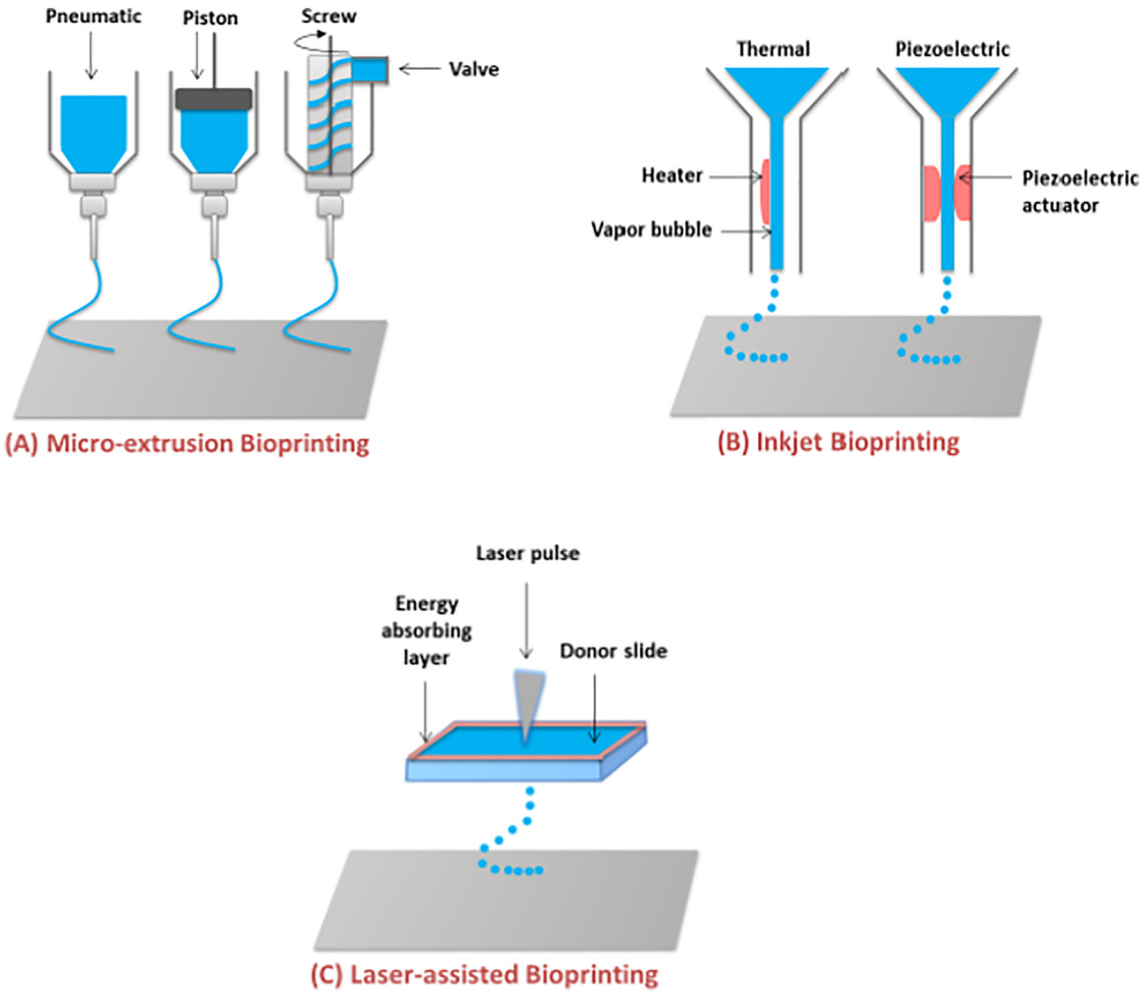


Fig. 2. Schematic illustration of three significant 3D bioprinting technologies according to working principles. (A) Micro-extrusion bioprinting (pneumatic, piston, screw); (B) inkjet bioprinting (thermal, piezoelectric); (C) laser-assisted bioprinting.

[30]. Cell viability is higher since bio-ink is not exposed to heat and pressure in this method [31]. Inkjet printing has high potential in tissue engineering applications and regenerative medicine [28].

2.2. Fused deposition modeling (FDM)

The FDM technique utilizes filament, which is made up from thermoplastic polymer to construct a 3D structure. With the temperature application, the filament is heated in the nozzle to get the printable form (semi-liquid state) and extruded onto the platform [32]. This method is not appropriate for cell printing directly since high temperature is used. Therefore, the cells are cultured onto the construct after the printing process [33]. The use of a thermoplastic polymer filament is an easy way to fuse the filaments during printing and after the printing process, it provides to solidify at room temperature. Layer thickness, width values, and direction of the filaments in addition to the air gap within the identical layer or between layers affect the mechanical behaviour of the printed structures [32]. Inter-layer defects are the main cause of mechanical weakness [34]. Since any solvents are not necessary for the FDM method, it provides to the convenience in the way of material fabricating and handling. This method allows continuous production without having to change the feedstock [35]. The FDM is a simple process and it has low cost and high-speed properties as the main advantage. However, it has disadvantages such as poor mechanical features, layer-by-layer appearance, poor surface property [36] and the limited amount of thermoplastic polymer [32]. There have been many studies by using the FDM technique for both musculoskeletal (cells seeded on the structures) and neural tissue applications (encapsulated cells with structure) [28].

2.3. Stereolithography (SLA)

The stereolithography method consists of an ultraviolet (UV) laser and has the functionality to focus the laser on a hydrogel or resin that has been photo-sensitized by the addition of a photoinitiator [37]. UV laser provides to solidify the liquid, especially, covalent bonds between neighboring polymer chains are created by the energy supplied by the laser [38]. The layers in the 3D structure are obtained by immersing the stage in the tank of liquid and changing its movement from up to down to equal the distance to the height of the layer [38,39]. SLA bioprinters offer a very high print resolution, considering factors such as laser power, time of exposure, size of the laser spot, light wavelength value [39,40]. However, the SLA method is somewhat slow, costly and has restricted types of materials for 3D printing applications. Furthermore, the kinetics of the reaction and the curing treatment are complex. The thickness of each layer depends on the light source energy and time of exposure [41]. Desired mechanical performance can be obtained by applying post-treatment processes such as heating or photo-curing to fabricated parts [42]. The SLA technique can be applied effectively to produce the additional complex nanocomposites [43]. It can print photosensitive polymeric material polymerizing to soft substrate materials, which generally have mechanical markings similar to neural

tissue types, thereby assisting the differentiation of seeded cells into neuronal subtypes [44].

2.4. Micro-extrusion bioprinting

Micro-extrusion-based bioprinting comprises of extruding a continuous bio-ink stream [45]. The continuous accumulation of bio-ink provides excellent structural integrity in this technique [46]. In micro-extrusion printing, the cells are mixed into the bio-ink, and then the biomaterials are dispensed through nozzles or needles [45]. The micro-extrusion head accumulates the material into the substrate as beads using instructions come from CAD-CAM program. First, the beads are put in the x-y direction, and then the extrusion head is moved along the z-axis to fabricate complex 3D constructs [47]. In this method, since high viscosity bio-ink can be fabricated with micro nozzle sizes, there is a broad range of bio-ink usage [48]. The piston, screw or pneumatic pressures are the parts and driven forces of the extrusion bioprinting [49]. The major advantage of this process is that the cell density with high values can be fabricated with a rapid production rate [50]. However, there is a problem which is the shear stress occurs in the cells. The applied pressure can affect the viability in a pressure-based system, and nozzle diameter is another parameter that affects the viability [51]. On the other hand, optimization parameters such as the concentration of the components, pressure and diameter of the nozzle are the critical values to solve these problems. The micro-extrusion technique has been successfully applied to develop constructs for tissue engineering [52]. For example, aortic valves [53], tumor models [54] and vascular tissues [55] were printed with this technique. Also, cell viability in biological structures developed by micro-extrusion is reported to be higher than 90% [56].

2.5. 3D bioplotting

In the 3D bioplotting technique, dropwise extrusion of a viscous liquid medium into a gel-like material using a pressure syringe [57]. The loaded material is extruded from the micro-sized needle and distributed as micro-strand [58]. Using a crosslinking agent or UV radiation, the hydrogel material is solidified over time. The key point of this technique is the 3D dispersion of a viscous printing material into a liquid medium having a similar density. 3D bioplotting can use a broad range of different materials such as polymer melts, thermoset resins, polymer solutions, high fillers, cement and bioactive proteins [57]. However, these materials can be too stiff or have low elasticity inappropriate for neural tissue engineering. 3D bioplotter can print co-cultured scaffolds and tissue structures [28]. Scaffolds produced by this technique generally demonstrate a smooth surface due to the extrusion and hardening procedure. Studies have demonstrated that scaffold surface topology greatly affects cell adhesion and proliferation [59]. Accordingly, it is necessary to create surface roughness by scaffold surface modification to increase cell adhesion on bioplotted structures.

3. Biomaterials and hydrogels for neural tissue engineering

SCI contusions result in an irregularly shaped cavity surrounded by preserved white matter. The ideal way of inserting a biomaterial for repair is to place it into the cavity, which would reduce extra damage to the nerve tissue. Thus, a large number of research groups have highlighted injectable, *in situ* gelling substrates for spinal cord repair [60,61]. 3D bioprinting is a novel process to construct complex structures on the biomimetic scale [62]. The scaffold material has a significant effect on printability and cell growth. In addition to traditionally used biomaterials, 3D bioprinting technology has shown advances to the development of the ideal printable biomaterials which provide to mimic the natural structure of the ECM. Biomaterial-based scaffolds enable microenvironment for cells. Also, these materials provide to axonal regrowth in lesion sites and reinstate the neural circuitry to regain its functionality [63]. This scaffold and cell combination regenerate growth potential by developing scaffold integration [64]. A hydrogel in the form of the biomaterials is water-swollen crosslinked polymer networks that can mimic mechanical and to some extent, the architecture of soft spinal cord tissue [65]. Injectable hydrogels are that has an important role in delivering cells and growth factors into an injury with less invasive operative interferences by constructing a 3D matrix imitating native extracellular matrix to adjust cell fate [63]. Some features of the hydrogels that maybe include: porosity, degradation, functionality, biocompatibility, *in situ* gelation and elastic modulus to optimize their applications in spinal cord repair [66–68]. Hydrogels are under consideration as a bio-ink due to their ability to form 3D hydrophilic polymer networks [69]. Therefore, bioprinting methods mainly utilize the hydrogels to print in a bio-ink form [70]. Hydrogels used as bio-ink can be formed with synthetic and natural polymers. Natural polymers such as collagen, hyaluronic acid, chitosan, gelatin, agarose, alginate, fibrin, and synthetic polymers such as Poly D, L-lactic acid (PLA), Poly-ϵ-caprolactone (PCL), Poly-lactic-co-glycolic acid (PLGA), Poly-glycolic acid (PGA), Polyethylene glycol (PEG) are widely used in neural tissue engineering for the repair of SCI. These biomaterials are given in Table 1 in detail [71–98].

3.1. Cell types

Cell selection is an important factor in tissue and organ printing for the proper function of the produced structure. Cells selected for printing must imitate the physiological situation of the cells *in vivo* also sustain their functions under optimized conditions [99]. Different viable cell types have been used to promote neural regeneration. These can contain Schwann cells, neural stem cells (NSCs), olfactory ensheathing cells (OECs) and mesenchymal stem cells (MSCs). Viable cells may be embedded into the scaffold or placed in the printing medium during the fabrication operation of the scaffold [62].

Activated Schwann cells have a crucial role in directing axon elongation by forming new myelin after peripheral nerve injury. In this context, it may be a useful approach to assemble Schwann cells on tissue engineering scaffolds. Gu et al. [100] developed scaffolds modified with Schwann cell-derived ECM to connect a 10 mm sciatic nerve space. In this study, they observed that the number and size of regenerative neural fibers were higher in the Schwann cell-derived ECM than in the cell-free scaffold [100]. NSCs are capable of producing neurons and glia cells. Also, engrafted NSCs may differentiate into astrocytes that facilitate glial scar formation at the injury site [101]. It was supported by some works that MSCs increased differentiation of NSCs to oligodendroglia [102]. When transplanted into the injured spinal cord, OECs can penetrate astrocytic scars and a result ease axonal growth along a wound barrier [103,104]. Furthermore, OECs may myelinate growing axons, thus improve axonal transmission [105]. Latest works have investigated the effect of embryonic stem cells (ESCs) in the therapy of SCI in animal models. Keirstead et al. [106] showed that ESCs have differentiation potential into mature oligodendrocytes and human ESCs can support healing following SCI in rats. Baharvand et al. [107] observed an enhancement in the locomotor function of rats after transplantation of collagen scaffolds containing human ESCs in SCI rats. Another type of cell used in neural tissue applications is pluripotent stem cells [62]. These versatile cells have large differentiation potential and their viability has been examined in different clinical studies [62,108]. Since undifferentiated pluripotent stem cells can cause the formation of teratoma, stringent differentiation procedures have to be performed to generate certain cellular phenotypes before *in vivo* studies [62].

4. 3D bioprinting applications of neural tissue

Several studies have been tried with nerve scaffolds for CNS regeneration. Table 2 summarizes 3D bioprinting applications for neural

Table 1
Biomaterials for treatment of injured spinal cord.

Materials	Appropriate properties of the materials for the nervous system	Ref.
Collagen	-Low immune response after transplantation. -Enables binding sites to reinforce cell adhesion, migration, proliferation, and differentiation. -Shows enhanced astrocyte migration, oriented axonal growth and decreased scar invasion in the injury site, in a transection SCI model in rats.	[71–74]
Hyaluronic acid	-Highly compatible and supportive material to design scaffolds for SCI. -Exhibits alleviated inflammatory cell infiltration and decreased glial scar formation *in vivo* studies.	[75–77]
Chitosan	-Supports the re-fabrication of spinal tissue, and modifies the inflammatory response in SCI. -Promotes axonal growth and cell adhesion. -Increases differentiation of neural stem cell (NSC) *in vivo*.	[75,78,79]
Gelatin and gelatin-based biomaterials	-Have the ability for filling the cavity, inhibiting inflammation, and diminishing necrosis and apoptosis for SCI repair.	[75]
Agarose	-Uses to regulate the cell adsorption and neurite outgrowth. -Promotes differentiation of neural progenitor cell (NPC) to oligodendrocytes.	[75,80–82]
Alginate	-Can fill the injured spinal cord. -Promotes axonal outgrowth.	[75,83,84]
Fibrin	-Has no toxic effects or contrary reactions to the recipient. -Conducts reconstructed blood vessels and axonal regeneration in the SCI model in rats.	[75,85]
Self-assembling peptides	-Good biocompatibility. -Promote NSC growth and migration.	[75]
Acellular scaffolds	-Act as a skeleton structure for cell attachment, proliferation, migration, and differentiation. -Can provide functional recovery through the facilitation of axonal regeneration.	[75,86]
Poly D, L-lactic acid (PLA)	-Provides the direction to improve axonal expansion *in vitro*. -Decreases cavity volume in SCI models. -Can be used as a drug carrier.	[87–89]
Poly-ϵ-caprolactone (PCL)	-High mechanical property. -Slow degradation rate. -Induces cell differentiation and neuronal extension.	[75,90]
Poly-lactic-co-glycolic acid (PLGA)	-Enhances the therapeutic profits in animal models by binding the cells. -Encourages axonal outgrowth and NCS viability more than PCL and PLA.	[75,91,92]
Poly-glycolic acid (PGA)	-Has a nerve-like structure with 3D scaffolds. -Mostly limited to bind a short nerve cavity because of unstable structure.	[93–95]
Polyethylene glycol (PEG)	-Can repair the injured neuronal membrane. -Diminishes the mitochondrial-derived oxidative stress to save the damaged spinal cord. -Supports NPC differentiation, adhesion and proliferation. -Demonstrates good therapeutic impact in rat SCI models.	[75,96–98]

tissue engineering.

Wong et al. [109] developed scaffolds in different architectures using a 3D printer and investigated their effects on spinal cord regeneration. Porous PCL scaffolds were fabricated in macro-designs of the cylinder, hollow tube, five-channel, open-path with core, and open-path with no core. These scaffolds implanted to transection rat SCI model for 1 and 3 months. *In vivo* studies have shown that the open-path architectures resulted in less scar and provided elongation of nerve fibers across the length of the defect in the scaffold. On the other hand, cylinder, tube and channel designs had a double defect length and have not provided a favorable environment for spinal cord regeneration. Lee et al. [110] produced collagen hydrogel scaffold with murine neural stem cells (NSCs, C17.2) and vascular endothelial growth factor (VEGF)-containing fibrin gel using inkjet bioprinting technique. As a result of printing, C17.2 cells demonstrated > 92% cell viability. When C17.2 cells were printed in VEGF-fibrin gel, they demonstrated significant morphological alterations, multiplication and migration compared to cells in the control group. This technique may be efficient to evaluate cellular behaviour and nerve tissue regeneration applications. In another work, Owens et al. [111] produced cellular nerve graft by 3D bioprinting. Firstly, mouse bone marrow stem cells (BMSCs) and Schwann cells were formed as cylindrical units as bio-ink components. Then, they were printed in layers to create a nerve graft. As a result of the work, *in vivo* tests have shown that cellular nerve graft restores both motor and sensory functions to certain levels. This work approved that the bioprinted graft was superior to an autologous graft for neural damages. Hsieh et al. [112] developed thermo-responsive polyurethane (PU) hydrogel scaffold for traumatic brain injury in zebrafish. Firstly, they produced PU hydrogel and then embedded NSCs into the hydrogel. Then, they printed the PU hydrogel scaffold by fused deposition manufacturing (FDM) technique. As a result of the study, improved cell proliferation and differentiation were observed. Scaffold showed great success in the neural injury of zebrafish. Cell-loaded PU has the potential to repair the function of damaged CNS. Tse et al. [113] printed neuronal NG 108–15 cells and Schwann cells by utilization a piezoelectric model inkjet printer and produced neural tissue. After printing, 86% and 90% neuronal and glial cell viability was observed. Also, printed cells showed similar proliferation rates than unprinted cells. This study demonstrated that inkjet bioprinting supplies perfect cell viability and has the potential to form a neural connection for printed structures. Furthermore, Gu et al. [114] performed micro-extrusion bioprinting of frontal cortical NSCs supplemented with alginate, carboxymethyl chitosan and agarose hydrogel to obtain neural mini-tissue structure. With this study, it was observed that the cells could survive in the structure and become functional neurons *in situ*. These differentiated neurons may create synaptic connections and show an increased calcium response due to bicuculline. Wei et al. [115] used stereolithography bioprinting to promote nerve regeneration. In the study, a new nano-bioink consisting of gelatin methacrylamide, bioactive graphene nanoplatelets and NSCs was developed, and its applicability was evaluated. The results showed that the cells were distributed homogeneously throughout all scaffolds and neurites spread from soma after 14 days of culture. Huang et al. [116] produced a biodegradable polyurethane hydrogel containing graphene or graphene oxide. NSC loaded hydrogel was printed using a 3D bioprinter. Cell viability, differentiation, gene expression and oxygen metabolism were observed in the scaffold with a low rate of graphene. This study supports the use of 3D tissue structures containing cells in neural tissue engineering applications. In another study, Zhu et al. [117] produced GelMA and PEGDA scaffold *via* stereolithography-based bioprinting. Next, NSCs

Table 2
3D bioprinting applications of neural tissue by different printing methods and various cell types and bio-inks.

Bio-ink	Cell type	Printing method	*In vitro/in vivo*	Outcome	Ref.
PCL	–	3D printing	*In vivo* (transection rat SCI model)	The open-path scaffold designs preserved their length of defect up to 3 months and increased successfully spinal cord regeneration by comparison to the cylinder, tube and channel designs.	[109]
Collagen and fibrin, fibrin loaded with VEGF	NSCs	Inkjet bioprinting	*In vitro*	92% high cell viability observed after printing. Cells demonstrated morphological changes, migration and proliferation in VEGF-fibrin gel.	[110]
Collagen	BMSCs and Schwann cells	3D bioprinting	*In vivo*	Cellular nerve graft repaired both motor and sensory function.	[111]
Polyurethane	NSCs	Fused deposition manufacturing	*In vitro* *In vivo* (zebrafish TBI model)	Great cell growth and differentiation were observed on the scaffold. PU showed improved motor function and survival rate in adult zebrafish with induced TBI.	[112]
Cell suspension	Schwann cells and neuronal NG 108-15 cells	Piezoelectric inkjet bioprinting	*In vitro*	86% and 90% neuronal and glial cell viability was observed.	[113]
Alginate, carboxymethyl chitosan, and agarose	Cortical NSCs	Micro-extrusion bioprinting	*In vitro*	Cell viability and differentiation were observed. Differentiated neurons showed an increased calcium response due to bicuculline.	[114]
GelMa, graphene nanoplatelets	NSCs	Stereolithography	*In vitro*	Cells distribution throughout all scaffolds homogeneously was observed and neurites spread from soma after 14 days of culture.	[115]
Polyurethane and graphene or graphene oxide	NSCs	3D bioprinting	*In vitro*	Cell viability, differentiation, the expression of gene and oxygen metabolism were observed in the scaffold with a low rate of graphene.	[116]
GelMa and PEGDA	NSCs	Stereolithography	*In vitro*	Light stimulation increased NSC neuronal differentiation and inhibited the generation of glial cells.	[117]
Carbon nanotube nanocellulose	Neuroblasto ma cells	3D bioprinting	*In vitro*	The nanosized scaffold had good electrical conductivity. Cell growth and adhesion were observed.	[118]
PEDOT:PSS	Dorsal root ganglia (DRG) cells	Stereolithography	*In vitro*	Conductive hydrogel improved regulation and stimulation of cell behaviour.	[119]
Alginate, HA and fibrin	Schwann cells	3D bioplotting	*In vitro*	High viability of SCs (> 90%) was observed.	[120]
SPCL and gellan gum	Oligodendrocyte-like cells	3D bioplotting	*In vivo* (hemisection rat SCI model)	Scaffolds were well fused within injury and did not trigger chronic inflammatory processes.	[121]
Collagen–heparin sulfate	NSCs	3D bioprinting	*In vivo* (rat SCI model)	Improved locomotor function was observed.	[122]
Gelatin/fibrin and GelMa	NPCs OPCs and	Micro-extrusion bioprinting	*In vitro*	Bioprinted NPCs differentiated and extended axons throughout microscale scaffold channels.	[123]
PEGDA-GelMa	NPCs	Micro-scale continuous projection printing (μCPP)	*In vivo* (rat SCI model)	Injured host axons were regenerated in the scaffolds and formed synapse onto NPCs implanted into the scaffold.	[124]

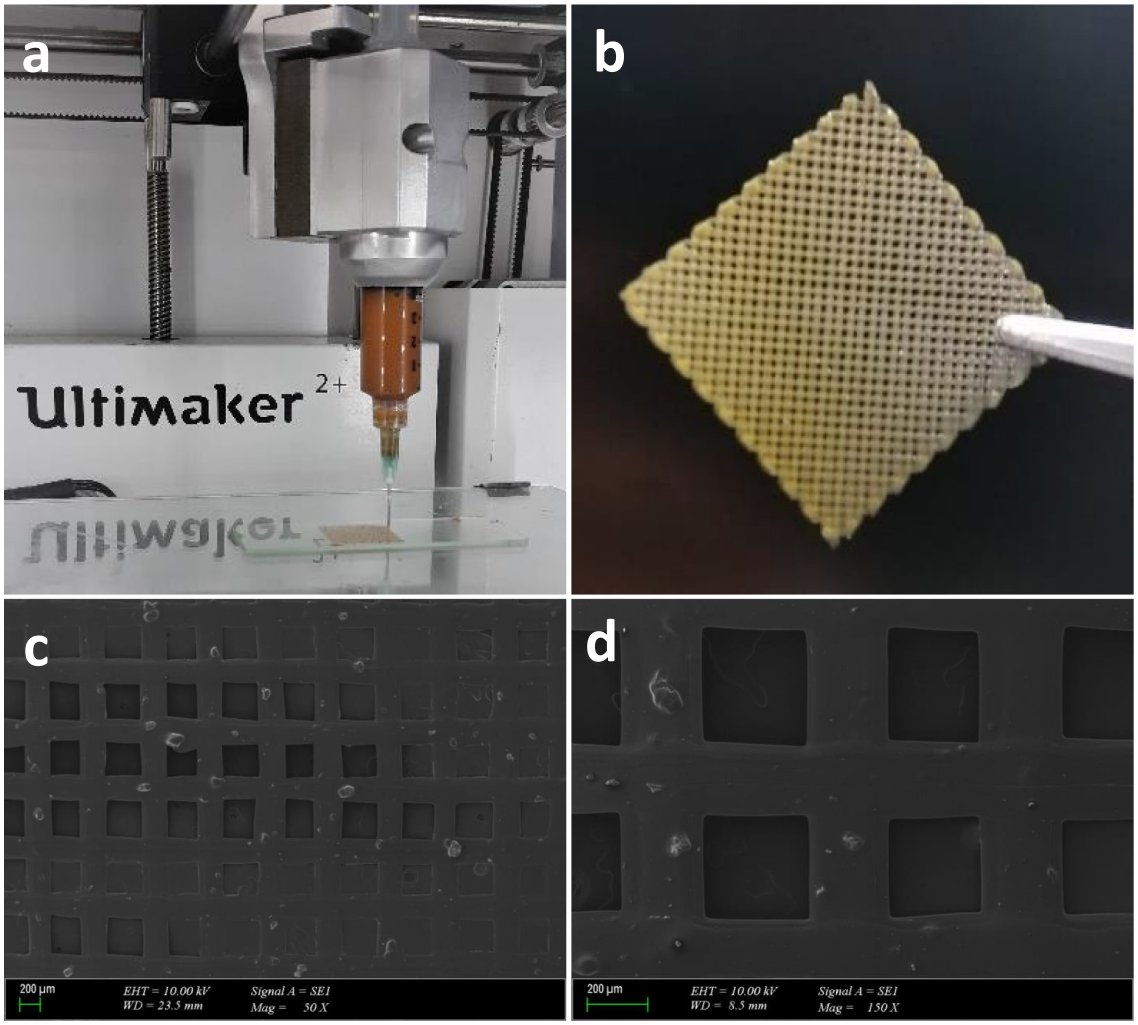


Fig. 3. Production of BFO doped PLA scaffold by the modified 3D printer (a), BFO doped PLA scaffold (infill 70%) (b), SEM images of BFO doped PLA scaffold (infill 70%) (c,d).

were transplanted onto the scaffold. The study was performed using low rate light treatment to the recovery of injured nerve tissues. Thus, the cells were further stimulated and achieved a greater curative effect. This study demonstrated that bioprinted neural scaffold with low rate light treatment enhances cell growth and expression of the neural marker. Besides, neural stem cells can be further differentiated into neural cells by using the laser. The use of this technique in neural regeneration can be effective. Kuzmenko et al. [118] developed a carbon nanotube nanocellulose scaffold using the 3D printing method. Then, neuroblastoma cells were embedded on the printed scaffold. This scaffold that nano-sized demonstrated high electrical conductivity. Also, increased cell growth and binding were observed in cells cultured on the scaffold. The results showed that composite and electrically active scaffolds have the potential for neural tissue regeneration. Zhang et al. [119] fabricated a conductive hydrogel for the treatment of neurological diseases using stereolithography bioprinting for biological signal recording and inducement of living tissues. The electrical conductivity to the scaffold was provided by a poly(3,4-ethylene dioxythiophene) PEDOT: polystyrene sulfonate (PSS) solution during printing. In this study, a 3D hydrogel scaffold that conductive has been developed for regulation and stimulation of cell behaviour. This study supports applications for regeneration of neural tissue. Ning et al. [120] produced hydrogels, containing alginate, HA and fibrin by the submerged cross-linking method. Then, they printed the hydrogel scaffold including Schwann cells using 3D bioplotting method. The results of this study have demonstrated the applicability of bioprinted scaffolds that has low-viscosity bio-inks and proper for cell migration in the scaffolds to repair neural damage. Similarly, Silva et al. [121] developed a new biphasic tubular scaffold *via* 3D bioplotting method to support regeneration in SCI sites. This scaffold consists of a mixture of starch = poly-ε-caprolactone (SPCL) and gellan gum. As a result of the *in vitro* study, it was observed that combined scaffolds could promote oligodendrocyte-like cell culture. Furthermore, *in vivo* studies conducted in a hemisection rat SCI model demonstrated that the scaffolds are well fused within the wound and did not induce chronic inflammatory processes. This study demonstrated that these 3D structures could be used in future SCI regeneration approaches. Chen et al. [122] performed crosslinking of collagen using a natural polysaccharide heparin sulfate. NSCs seeded on the 3D bioprinted collagen–heparin sulfate scaffolds. This study improved a strategy to promote axonal regeneration and functional recovery for a spinal cord injury. Research on Sprague-Dawley rats reported that improvements in locomotor function according to electrophysiological examinations. Joung et al. [123] developed a scaffold for SCI using an extrusion-based bioprinter. Then, induced pluripotent stem cells (iPSC)-derived neural progenitor cells (NPCs) and oligodendrocyte progenitor cells (OPCs) were implanted into the printed scaffolds. Cell position was checked up *via* a point-dispensing printing method. The results showed that NPCs differentiate and elongate axons along the micro-scaffold channels. Properly bioprinting of OPCs with NPCs supports multicellular neural tissue

engineering applications. The capability to direct the modeling and combination of transplanted cells may be useful in reconstructing axonal links along with the CNS injury sites. This work may be used to create new biomimetic scaffolds that model CNS structure *in vitro* and to develop novel clinical approaches to cure neurological defects, containing SCI. Koffler et al. [124] fabricated 3D biomimetic PEGDA-GelMa scaffolds using a micro-scale continuous projection printing method (μCPP) to form a complicated CNS structure for regenerative medicine applications in the spinal cord. μCPP can print the hydrogel scaffolds adapted to the size of the rodent spinal cord in 1.6 s. Also, it can be scaled to human spinal cord dimensions and lesion geometries. After that, they loaded NPCs onto the 3D-printed scaffolds to support axon regeneration and form new 'neural relays' across sites of complete spinal cord injury *in vivo* in rodents [125,126]. As a result of the study, they found that the damaged host axons were regenerated into the scaffolds. Also, these axons synapse onto NPCs placed into the device and that implanted NPCs, in turn, elongate axons out of the scaffold and into the host spinal cord below the damage to repair the synaptic conduction and develop functional results. This study demonstrates that 3D biomimetic scaffolds are an effective option to increase CNS regeneration [124].

Our laboratory has carried out studies using 3D printing technology for applications of tissue engineering. In a recent study, we successfully produced PLA scaffold with bismuth ferrite (BFO) nanoparticles on a modified 3D printer. This produced biocompatible and electrically active scaffold has excellent potential for use in applications of neural tissue engineering (Fig. 3).

5. Conclusion

SCI is a CNS disease that does not have an adequate treatment yet and affects the individual's life negatively. Disruptions in the CNS structure result in loss of neuronal cell bodies, axons, and associated glia support. Neural tissue engineering is focused on the improvement of an appropriate environment that connects the biomimetic scaffold with cells to repair and restore neural tissue function. 3D bioprinting is an attractive technology for the production of complicated neural grafts where different bioactive factors and cells can be easily combined and used synergistically for developed neural regeneration. On the other hand, the production of complicated constructions with multiple cell types still a challenge for this technology. In this review article, various 3D bioprinting methods such as extrusion, inkjet, laser, plotting utilized in applications of neural tissue have been discussed in detail. Furthermore, bio-inks and cells available for bioprinting have been explained. Finally, applications for models of 3D neural tissue designed by bioprinting methods, especially for the repair of spinal cord injury has been described.

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