The Role of Biomaterials in Implantation for Central Nervous System Injury

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Abstract

Permanent deficits that occur in memory, sensation, and cognition can result from central nervous system (CNS) trauma that causes dysfunction and/or unregulated CNS regeneration. Some therapeutic approaches are preferentially applied to the human body. Therefore, cell transplantation, one of the therapeutic strategies, may be used to benefit people. However, poor cell viability and low efficacy are the limitations to cell transplantation strategies. Biomaterials have been widely used in several fields (e.g., triggering cell differentiation, guiding cell migration, improving wound healing, and increasing tissue regeneration) by modulating their characteristics in chemistry, topography, and softness/stiffness for highly flexible application. We reviewed implanted biomaterials to investigate the roles and influences of physical/chemical properties on cell behaviors and applications. With their unique molecular features, biomaterials are delivered in several methods and mixed with transplanted cells, which assists in increasing postimplanted biological substance efficiency on cell survival, host responses, and functional recovery of animal models. Moreover, tracking the routes of these transplanted cells using biomaterials as labeling agents is crucial for addressing their location, distribution, activity, and viability. Here, we provide comprehensive comments and up-to-date research of the application of biomaterials.

Central nervous system (CNS) diseases include spinal cord injuries (SCIs) and traumatic brain injuries (TBIs). In general, loss of motor, sensory, and autonomic functions appear with SCIs, whereas symptoms of physical, sensory, cognitive, and swallowing deficits, as well as behavioral issues, are the consequences of TBIs. In the process of trauma, damage from a mechanical force is the first harm to the body. Then, inflammation emerges via 2 cell types, microglia and microphages, in the CNS, and this state inhibits myelination. Finally, astrocytes appear in a reactive state to form glial scar tissue that differs from native tissue due to a lack of nutrient supplement function[1,2]. CNS trauma may cause permanent deficits mainly due to an inability of CNS regeneration but also because of glial scar tissue formation. Several methods, such as endogenous cell therapy and exogenous cell therapy, are performed to treat CNS injuries. Cell transplantation is a more achievable therapeutic strategy for CNS injuries because cells are easily obtained compared to organs. However, several barriers to exogenous cell therapy exist, including a low viability of transplanted cells, dispersed cells distributed in the body, and uncontrolled cell differentiation, and these limit the therapeutic efficacy of cells[3–5]. Biomaterials that have flexibility in mimicking natural environments could overcome obstacles of cell transplantation and thereby improve cell

transplantation issues for the therapy of CNS injuries. We review (1) the role of the physical/chemical property of biomaterials on cell behavior, (2) the influence of the

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physical/chemical property of biomaterials on implantation, and (3) the distribution of transplanted cells using a cell tracker employing biomaterials to provide a more comprehensive review of biomaterial application in CNS regeneration medicine.

Role of the Physical/Chemical Property of Biomaterials on Cell Behaviors

A cell's fate can be manipulated by signaling through specific environmental physical/chemical factors, such as the chemistry, stiffness, or topography of a matrix. In this section, we describe the role of electric charges, stiffness, and topography of biomaterial on cellular behavior such as cell adhesion, cell proliferation, and cell differentiation.

Effects of Electric Charges on Cell Behaviors

The effects of electric charges on neural cell cultivation and differentiation have been investigated on carbon nanotubes (CNTs) exhibiting semiconductivity characteristics, which have potential in applying to neural electrodes. Those studies showed that formation of a functional synapse was observed, with evidence of spontaneous synaptic currents and spontaneous action potential frequencies when mature hippocampal neurons were cultured on CNTs[6]. CNT is a candidate material for cell cultivation. A CNT chemistry effect of electric charge (eg., positively, negatively, neutral charge) would affect cell behavior (eg., cell proliferation or differentiation). Hippocampal neuron cells were grown on a positively charged CNT grafted with ethylenediamine (EN), which revealed more outgrowth and branching activities than those of cells grown on negatively charged carboxylic functional groups or neutrally charged poly(m-aminobenzene sulfonic acid) (PABS)[7]. Moreover, a positive charge effect also has been applied in neuronal cell differentiation, such that neuronal stem cells (NSCs) differentiated into a neural lineage without induction factors under cultivation with CNTs. Single-walled CNTs (SWCNTs) and polyethyleneimine (PEI), forming multilayer thin films through a layer-by-layer (LBL) method, showed comparable results in biocompatibility, neurite outgrowth, and neural marker expressions to those of the widely used biopolymer, poly-L-ornithine (PLO)[8]. But a negative charge, such as poly(acrylic acid) (PAA) or poly(methacrylic acid) (PMAA), grafted on CNT also can increase higher neurite outgrowth and neuron differentiation of human embryonic stem cells (hESCs) than that with a conventional PLO substrate[9,10]. These results may be the reason why neural differentiation is preferable for the hESCs and NSCs, and thus neural differentiation is observed after replacement of an inhibition differentiation medium to a general cultural medium. Transdifferentiation was employed in a negative charge using a carboxylated multiwalled CNT (MWCNT) to promote neural differentiation of human bone marrow mesenchymal stem cells (hBMMSCs). One study provided two major roles of carboxylated MWCNTs that promoted hBMMSC neural differentiation by upregulating neural growth factors and the carboxylated MWCNTs that trapped these neural growth factors to create a suitable environment for long-term neural differentiation[11].

Native hydrogels exhibit a property of low cell attachment. Therefore, an electric charge effect becomes a possibility for designing cell-repellent hydrogels. One method is to use a positive charge of material for improving cell cultivation due to the presence of a negative charge of a cellular plasma membrane (head group of phosphatidylserine and phosphatidylinositol) and a negative charge of carbohydrate portions of glycolipids and glycoproteins[12]. Poly(propylene fumarate-co-ethylene glycol) (p(PF-co-EG)) hydrogels have been incorporated with a positively charged arginine polymer to increase the cell density by evidence of an increment of vitronectin on the hydrogels[13]. Moreover, cell-repellent 2-hydroxyethyl methacrylate (HEMA) hydrogels showed the greatest cell attachment and spreading when the cells were cultured on positively charged 2-methacryloxy ethyltrimethyl ammonium chloride (MAETAC)–grafted HEMA hydrogels. The negatively charged sodium 2-sulfoethyl methacrylate (SEMA)–grafted HEMA hydrogels were ranked second, whereas neutrally charged HEMA hydrogels were the worst in terms of cell attachment and cultivation[14].

In addition to synthetic polymer, the chemistry effects on cell behaviors of natural polymers were also investigated. Extracellular matrix, fibronectin (Fn), or hyaluronic acid (HA) was modified in negatively charged alginate, and their influence was compared to the neuronal differentiation. The data showed that mouse embryonic stem cells (mESCs) encapsulated in a group of alginate or alginate-HA exhibited increased differentiation of neurons according to evidence of synaptic and different neuronal subtype markers[15]. Except for differentiation of stem cells, maintaining stemness of a stem cell is an important issue for in vitro cultivation and proliferation. Maintaining stemness of mESCs using a collagen-based, poly(lactic-co-glycolic acid) (PLGA)–based, and positive chitosan-based 3-dimensional scaffold was investigated. Results indicated that all 3 scaffolds could maintain stemness compared with the traditional 2-dimensional dish with feeder cells. When comparing the cell proliferation of 3-dimensional scaffolds, chitosan-based 3D scaffolds had higher cell numbers than those of collagen-based and PLGA-based 3D scaffolds[16].

The electric charge effect of materials on cells' behavior is summarized in Table 1. Overall, it seems that positively charged materials assisted cell proliferation, whereas negatively charged biomaterials tended to promote the cells to differentiate.

Stiffness Effects on Cell Behaviors

Manipulating the stiffness of material is also a tool for regulating cell adhesion and differentiation. One obstacle of applying a hydrogel in cell cultivation is low cell attachment

Table 1. Electric Charge Effect on Cells' Behavior.

Cell	Material	Result	Reference
Hippocampal neurons	- EN-CNT - Carboxylated-CNT - PABS-CNT	Positively charged EN-CNT revealed most cell outgrowth and branching activities	7
NSCs	- SWCNTs/PEI - PLO	SWCNT/PEI showed comparable results with PLO	8
HASMC	- p(PF-co-EG) hydrogel - Agmatine-p(PF-co-EG) hydrogel	Agmatine-p(PF-co-EG) enhanced cell attachment	13
- MC3T3-E1 - 3T3	- HEMA hydrogels - MAETAC-grafted HEMA hydrogels - SEMA-grafted HEMA hydrogels	Positively charged MAETAC-grafted HEMA hydrogels had best cell adhesion result	14
mESCs	- Collagen based - PLGA based - Chitosan-based scaffolds	Chitosan-based scaffolds had higher cell numbers than those of collagen-based and PLGA-based 3-dimensional scaffolds	16
hESCs	- PAA-CNT - PMAA-CNT - PLO	Both PAA-CNT and PMAA-CNT can increase neurite outgrowth and neuron differentiation of hESCs compared with PLO	9, 10
mESCs	- Alginate - Alginate-Fn - Alginate-HA	Alginate or alginate-HA exhibited increased differentiation of neurons	15

Abbreviations: EN, ethylenediamine; Fn, fibronectin; HA, hyaluronic acid; HASMC, human aortic smooth muscle cell line; HEMA, hydroxyethyl methacrylate; hESCs, human embryonic stem cells; MAETAC, 2-methacryloxy ethyltrimethyl ammonium chloride; mESCs, mouse embryonic stem cells; NSCs, neuronal stem cells; (p(PF-co-EG), poly(propylene fumarate-co-ethylene glycol); (p(PF-co-EG), poly(propylene fumarate-co-ethylene glycol); PAA, poly(acrylic acid); PABS, poly-m-aminobenzene sulfonic acid; PEI, polyethyleneimine; PLGA, poly(lactic-co-glycolic acid); PLO, poly-L-ornithine; PMAA, poly(methacrylic acid); SEMA, sodium 2-sulfoethyl methacrylate; SWCNT, single-walled CNT.

on it caused by low sliding friction on the surface. Therefore, controlling the stiffness or mechanical strength would be an alternative option. The attachment area of NIH3T3 on the surface of a polyacrylamide gel possessed a positive correlation to stiffness when designing materials in the range of 10 to 3000 Pa, but a decreased attachment area of the cells was observed in the range of 3000 to 10,000 Pa[17]. HA, a natural polysaccharide, was also investigated in the relationship between cell adhesion and the stiffness property of materials. A higher stiffness property (storage modules at 17,000 Pa) of HA hydrogels improved cell adhesion of epithelial HeLa cells, preosteoblast cells, and particularly NIH3T3 fibroblasts compared to those of storage modules at 600 and 2500 kPa[18]. In addition to a cross-linker, interpenetrating networks (IPNs) are an alternative method to manipulate stiffness. Arulmoli et al[19]. used IPNs, composed of fibrin-based gels (salmon fibrinogen and salmon thrombin) and thiolated HA, to simulate a brain tissue environment at 202.3 ± 17.33 Pa and reduced human neural stem cell death.

To mimic the environments of native cells, which are cultivated in a 3-dimensional structure in a live body, a series design of a 3-dimensional system of biomaterials was studied. Mechanical strength is also important to control cell behavior, such as survival, metabolite, or growth factor secretion, when cells are encapsulated in a material. Orive et al[20]. compared survival and antibody production of hybridoma cells within 2 mechanical strength–type gels: solid (main force of 11 g per bead for breakdown) and liquefied (main force of 4 g per bead for breakdown) core alginate-agarose beads. They found that the liquefied type can have a higher survival of cells and antibody production compared with the solid type. The team advanced to improve cell survival in solid-type alginate-agarose beads up to 70 days compared to 15 days for the liquefied type when adding another cell line, BHK fibroblasts, and C2C12 myoblasts.

The roles of mechanical strength in stem cell fate determination were studied. Different mechanical strengths mimicking native tissues of brain, muscles, and bone on hydrogels were reported. Naive MSCs are initially small and round but develop increasingly branched, spindle, or polygonal shapes for further differentiation into neuron, myogenic, and osteogenic lineages when cells are grown on matrices of $\sim E$ (0.1-1 kPa), $\sim E$ (8-17 kPa), and $\sim E$ (25-40 kPa), respectively[21]. Importantly, stemness maintenance is an issue for the development of cellular biology. The mechanical strength of hydrogels controlled to 10 kPa was reported to maintain the stemness of hESCs for at least 60 days[22].

Based on the stiffness effect on cells' behavior (Table 2), the stiffness can control cell adhesion, growth, and differentiation. The higher stiffness of hydrogels is suitable for cell culture as they are cultivated in a 2-dimensional environment; in contrast, a softer hydrogel is preferred when cells are grown in a closed 3-dimensional construction. In addition, lower mechanical strength would be preferable for brain tissue cultivation.

Table 2. Effect of Stiffness of Materials on Cells' Behavior.

Cell	Material	Result	Reference
NIH3T3	PAA hydrogel (10–10,000 Pa)	- Cell attachment area has positive correlation with stiffness at 10–3000 Pa - Cell attachment area has negative correlation with - stiffness at 3000–10,000 Pa	17
HeLa cells, preosteoblast cells, NIH3T3	- 600 Pa HA - 2500 Pa HA - 17,000 Pa HA	- All have positive correlation with stiffness	18
hNSPCs, HECFC-ECs	- Fibrin/HA (202.3 ± 17.33 Pa)	- Reducing cell death	71
MSCs	- 0.1–1 kPa PAA hydrogel - 8–17 kPa PAA hydrogel - 25–40 kPa PAA hydrogel	- Neurons are grown on matrices of $\sim$E (0.1–1 kPa) - Myogenic lineages are grown on matrices of $\sim$E (8–17 kPa) - Osteogenic lineages are grown on matrices of $\sim$E (25–40 kPa)	21
hESCs	- 0.7 KPa PAA hydrogel - 3 KPa PAA hydrogel - 10 KPa PAA hydrogel	- 10 kPa maintains the stemness of the cells for at least 60 days	22

Abbreviations: HA, hyaluronic acid; HECFC-ECs, human cord blood–derived endothelial cells; hESCs, human embryonic stem cells; hNSPCs, human neural stem progenitor cells; MSCs, mesenchymal stem cells; PAA, polyacrylamide.

Topography Effects on Cell Behaviors

Topography affects cell adhesion, growth, and in particular differentiation through cellular morphology alternation. Sridharan and colleagues[23] demonstrated that embryonic stem cells (ESCs) in structureless and soft gelatin matrix differentiated into all 3 lineages, but an elongated shape with long filaments and ectodermal lineage differentiation cells were observed when they had a fibril structure with collagen or a collagen-carbon nanotube (CNT) matrix. A micropattern with controlled width/spacing was modeled to examine the differentiation ability of stem cells. The micropatterned microenvironment, using a linear pattern with a width/spacing (W/S) of 40/30 μm, guided neuronal lineage growth, whereas a W/S pattern of 20/40 μm was reported to guide myogenic lineage differentiation[24,25]. The nanopatterned microenvironments also revealed the ability to control stem cell fates. A similar phenomenon was reported in studies of hMSCs that nanopatterning with 350-nm lines resulted in spontaneous neurogenic differentiation of hMSCs, while that of 250-nm-width lines directed hMSCs toward both neurogenic and myogenic differentiation[26,27]. These neurogenic differentiations were associated with the integrin-activated focal adhesion kinase (FAK)[26]. In addition to a line pattern, a square pattern also has been investigated on differentiation. Cells can proliferate and differentiate on the square pattern with 300-μm × 300-μm, 200-μm spacing and 5-μm × 5-μm, 5-μm spacing but not on a pattern with 50-μm × 50-μm, 50-μm spacing. This is because the largest square pattern has enough space for a lot of cells to adhere on a large pattern, and the smallest square pattern can allow cells to extend their filopodia on the one small square pattern, but the pattern with 50-μm × 50-μm, 50-μm spacing limited the cells in one square space. An advanced topographic pattern was built by designing in-line plus a square pattern using CNTs to construct a neural network environment[28].

Poor cell adhesion on a cell-repellent hydrogel could be improved by using topography. Polyethylene glycol–based hydrogels are materials with low cell adhesion. To improve this issue, linear micropatterned Acr-sP(EO-stat-PO) hydrogels with a 10-μm groove were designed, and results showed an improvement in cell adhesion. Moreover, the spread of L292 cells increased on linear micropatterned hydrogels without a coating of the bioadhesive molecule, vitronectin, during the culture period, whereas the cells on nonmicropatterned smooth hydrogels were only adsorbed but did not spread on the hydrogel after 1 day[29]. In addition to cell adhesion and proliferation that can be manipulated through topography, cellular morphology can also be directed by topography for promoting cell differentiation. Surface wrinkles were designed on a poly(2-hydroxyethyl methacrylate) (PHEMA) hydrogel surface to verify their effect on hMSC morphology and differentiation. The hMSCs on lamellar wrinkles of the PHEMA hydrogel possessed higher aspect ratios of cell adhesion area and differentiated into an osteogenic lineage, whereas those on hexagonal wrinkles of the PHEMA hydrogel showed lower aspect ratios of cell adhesion area and differentiated into an adipocyte lineage[30].

A 3-dimensional structure can also control topography through interior porous structures. Mouse induced pluripotent stem cells (miPSCs) cultured on a poly(lactic-co-glycolic acid) (PLGA) porous scaffold, through salt leaching/solvent evaporation, reached a maximum cell number after 10 days postseeding, which was 5 to 6 times higher than those on the nonporous controls[31]. Khayyatan et al[32] used a freezing temperature to control the porosity of collagen scaffolds. The highest infiltration of human induced pluripotent stem cell–derived neural progenitors (hiPSC-NPs) was observed when cultured in a smaller porosity collagen scaffold (<50 μm) prepared at –196°C, suggesting that a smaller porosity has a higher surface area in the interior of the collagen scaffold to create more adhesion space

Table 3. Effect of Topography of Materials on Cells' Behavior.

Cell	Material	Result	Reference
hNSCs	- 300 × 300 × 200 μm (l × w, s) - 50 × 50 × 50 μm (l × w, s) - 5 × 5 × 5 μm (l × w, s)	- 300 × 300 × 200 μm and 5 × 5 × 5 μm (l × w, s) promote the proliferation and differentiation of cells	28
hMSCs	- 350-nm-width lines - 250-nm-width lines	- 350-nm lines resulted in spontaneous neurogenic differentiation - 250-nm-width lines directed toward both neurogenic and myogenic differentiation	26, 27
hMSCs	- 40/30 μm (W/S) - 20/40 μm (W/S)	- 40/30 μm guided neuronal lineage growth - 20/40 μm was reported to guide myogenic lineage differentiation	24, 25
hNSCs	- 300 × 300 × 200 μm (l × w × s) - 50 × 50 × 50 μm (l × w ×s) - 5 × 5 × 5 μm (l × w × s)	- 300 × 300 × 200 μm and 5 × 5 × 5 μm (l × w × space) promote the proliferation and differentiation of cells - 30-μm and 5-μm line patterns allow cells to adhere on the surface - 5-μm line pattern allows one cell to adhere on the surface	28
L292 cells hMSCs	- 10-μm groove on hydrogel - Lamellar wrinkles PHEMA hydrogel - Hexagonal wrinkles PHEMA hydrogel	- Improvement in cell adhesion - Lamellar wrinkles promoting cells into an osteogenic lineage differentiation - Hexagonal wrinkles promoting cells into an adipocyte lineage	29 30
miPSCs	- 80- to 120-μm pore size - 45 to 80-μm pore size - 25 to 45-μm pore size	- 6 times increase of cell density in group of 80-120 μm porosity - 5 times increase of cell density in group of 45-80 and 24-45 μm porosity - Gene expression of SOX2 and Otx2 decreased with porosity - Protein expressions of SOX2 and PAX6 decreased with porosity	31
hiPSC-NPs	- 150-μm pore size - 100-μm pore size - 25-μm pore size	- Length of cell infiltration: group of 25 > 100 > 150-μm pore size	32

Abbreviations: hiPSC-NPs, human induced pluripotent stem cell–derived neural progenitors; hMSCs, human mesenchymal stem cells; hNSCs, human neural stem cells; l × w, s, length × width, space; miPSCs, mouse induced pluripotentstem cells; PHEMA, poly(2-hydroxyethyl methacrylate); W/S, width/spacing.

for cells. However, homogeneous pore size could not be easily achieved by the above-described method. The inverted colloidal crystal (ICC) scaffold using polystyrene beads with a mean diameter of 158 μm could tightly control pore morphology and provide interior pores' connection of the scaffold. Their results showed that ICC-made scaffolds increased the adhesion and viability of miPSCs compared to those of freeform ones[33].

We summarize the effect of materials' topography on cell behavior in Table 3. In general, the major application of topography control is in cell differentiation because the cells can be limited in the space of the topography pattern to achieve cell morphology manipulation. In addition, amounts of cell infiltration also can be controlled through porosity of the 3-dimensional scaffold.

We summarize environments that are suitable to cell cultivation and differentiation through manipulating physical/chemical properties of the biomaterials in Figure 1. With these approaches, matrices can be designed to elicit enhanced cell attachment for a cell-repellent-based substrate, maintenance of stemness for cell banking, or directed cell differentiation into specific lineages for cell therapies.

Influence of Physical/Chemical Property of Biomaterials on Implantation

Cell transplantation appears to be a promising regenerative medicine, but the cells alone seem not fully satisfactory to the outcome of treatment. Biomaterials providing flexible characterics in regulating cell behavior in the above description would be a possible solution to resolve the bottleneck. Designation of an implanted material would render them with biocompatibility preferentially and other tailored characteristics such as biodegradability, a niche-creatable environment, or a nonrestricted size or shape property[3,34]. To fully address biomaterials' effect on implantation, we review the issue of biomaterial implantation based on the effect of its chemical properties, topography, and stiffness. The following contents describe the Chemical/ECM of Biomaterials on Implantation; the citation/reference of the issue has been cited in the sentence.

CNS Disease Treatment by Biomaterials Alone

To promote angiogenesis in the brain, basic fibroblast growth factor (bFGF) and epidermal growth factor (EGF) were adsorbed into a porous block scaffold hybrid of gelatin and 3-(glycidoxypropyl) trimethoxysilane (GPSM), and the gelatin/GPSM/bFGF/EGF scaffold was used to implant into a cavity in the cerebral cortex. The gelatin/GPSM/bFGF/EGF scaffold resolved the problem of low cell survival and remained for 60 days in the brains of animals. The scaffold formed an integrated connection with the host brain, and newborn cells represented by vascular endothelial (NAGO-positive), astroglial (GFAP-positive), and microglial (Iba1-positive) cells were increased to 2-fold in the presence of
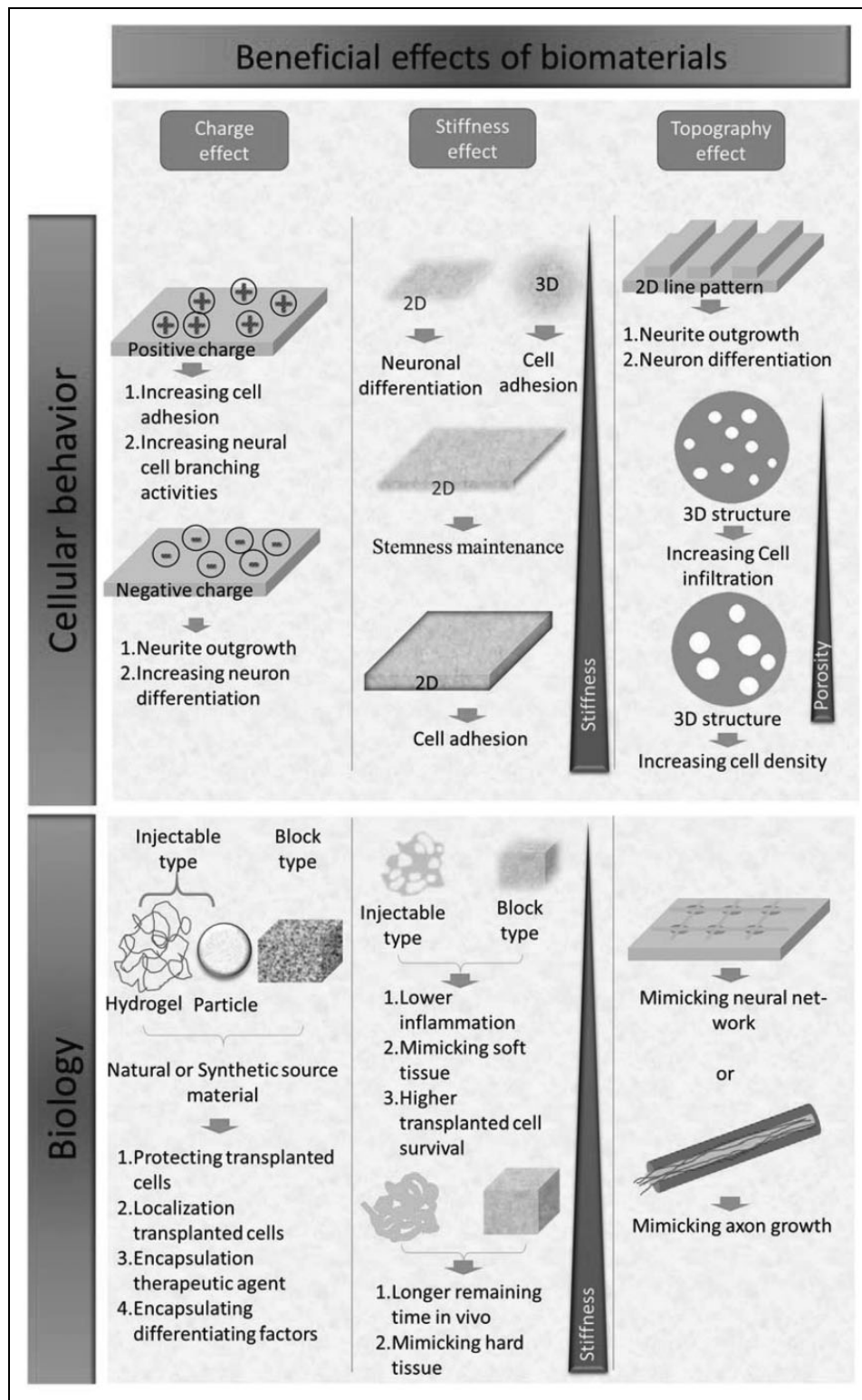
Figure 1. Beneficial effects of biomaterials on cellular behavior and biology through the physical/chemical properties of biomaterials.

bFGF/EGF in the implanted scaffold[35]. However, the additives did not promote neuronal cell formation and migration and an unbiodegradable block used in vivo. Hydrogels are a cross-linked network within a porous structure with a dynamic hydration status, which is similar to the extracellular matrix (ECM) environment serving as a complicated microenvironment for supporting cell and tissue structures, regulating cellular behaviors, and promoting cell-to-cell

communication. A neurite-promoting peptide sequence, IKVAV, was grafted to a biocompatible material, HA, to form an injectable material and then implanted into the lesion area of the cortex for 6 weeks. The results showed that invasion of host cells, especially nerve fibrils, was observed[36]. Collagen and other injectable biocompatible materials grafted with glycosaminoglycan were implanted into the cerebral cortex for neural regeneration in TBI. The results showed that more migratory cells (DCX-positive cells) and neural progenitor cells (NPCs) (NeuN-positive cells) were increased about 7-fold at 21 days. Importantly, fewer inflammatory cells (marker of ED1) in the implanted matrix and lesion boundary zone were observed compared to the nonimplanted group[37].

How to control cell differentiation in vivo is also an issue in regeneration medicine. The other type of injectable biomaterial, not hydrogel based, was used for trophic factor delivery through the biodegradable property of materials to achieve controlled release. Biodegradable poly(ester-amide) microspheres, composed of adipic acid, L-phenyl-alanine, and 1,4-butanediol, were designed to load with differentiation factors, including Wnt3A, BMP4, and cyclopamine. After the microspheres had been incubated with hiPSC-derived neuroepithelial-like stem cells, cortical differentiation of the cells was observed in vitro. Moreover, the biodegradable poly(ester-amide) microspheres did not evoke a significant inflammatory response after transplantation into an intact rodent brain[38].

Based on the delivery route, block biomaterials and injectable biomaterials are 2 major types with regard to implantation. The advantage of the block type is that it can last longer after implantation, whereas the injectable type can deliver the therapeutic agent more conveniently and with less of a surgical area requirement.

CNS Disease Therapy by Combinations of Cells and Biomaterials.
Although biomaterials can act as scaffolds or trophic factor carriers for treating diseases of the CNS, less differentiated cells and a low amount of newborn endogenous cells ($\sim$500 cells/ mm2) still need to be overcome in regeneration medicine[35,39,40]. Therefore, exogenous cells integrated into biomaterials become an alternative strategy in regeneration medicine.

Block Type Cells/Biomaterials. Natural materials are to be employed to bridge a spinal cord injury. Three materials—collagen, chitosan, and fibrin—were constructed as a block type and mixed with cells to compare their influence on transplanted cellular survival and the in vivo material degradation rate[34]. The authors used microfiber with a double-coaxial microfluidic device to embed neural stem/progenitor cells (NS/PCs) and basal materials and then used collagen (positive charge at pH 7[41]), chitosan (positive charge at pH 7[42]), or fibrin (negative net charge at pH 7[43]) to assemble the construction. The results showed that microfibers with collagen successfully bridged host tissues and promoted the

differentiation of 3 neural lineages. In addition, it was also found that the chitosan-coated microfibers also bridged transected spinal cord by a scar, but the highest cell proliferation and a more complete scar formation were observed as cells were engrafted in the collagen-coated microfibers during in vivo transplantation. The authors suggest that chitosan-coated microfibers exhibit too dense a structure in the interior compared with the others to inhibit host cell invasion. The lowest cell survival and scar formation were observed in the group of fibrin-coated microfibers, which might have been caused by the composition of fibrin, which is quickly digested in the microenvironment[34].

Injectable-Type Cells/Biomaterials. The effects of ECM on cell survival and transplantation in injectable-type hydrogels were also investigated. He and colleagues[44] demonstrated that cells can be protected when they are encapsulated in ECM materials before implantation. Studies showed that detached human embryonic stem cell–derived endothelial cells (hESC-ECs) kept in Matrigel, a complex extracellular environment secreted from Engelbreth-Holm-Swarm (EHS) mouse sarcoma cells, at 4°C could regain expression of cell adhesion and ECM molecules compared to those suspended in phosphate-buffered saline (PBS) as evidence of gene expression patterns. Moreover, engrafted Matrigel-encapsulated hESC-ECs showed more long-term survival of hESC-ECs than those of the cell-alone group postimplantation[44]. A series design based on ECM for neural regeneration has been reported. ECM types and their effects on neural stem cell (NSC) transplantation were investigated, and the results showed that a laminin-based collagen type I scaffold can enhance the transplanted cells' survival compared with a fibronectin-based collagen type I scaffold. Furthermore, using a laminin-based collagen type I scaffold alone did not improve the damage to cognitive behavior[45], suggesting that a scaffold effect in transplantation plays a role in protecting transplanted cells rather than repairing the damage site. Guan and colleagues[46] further used collagen type I alone to mix human mesenchymal stem cells (hMSCs) for treatment of TBI. The highest biodistribution of transplanted hMSCs was observed when the cells were mixed with collagen scaffold, which was enhanced by 79% in the target site at 12 hours compared with that of the cell-alone group. The modified neurological severity score (mNSS) and Morris water maze evaluations all indicated significant improvement at day 28 after cell transplantation. Moreover, the researchers found that implanted hMSCs differentiated into neuronal cells (4.6%), oligodendrocytes (1.8%), and astrocytes (0.8%) in the presence of collagen more than those of the cell-alone treatment group[46]. Although transplanted hMSCs can transdifferentiate into neural-type cells after transplantation, major reasons for TBI improvement should be caused by other factors because of the few differentiated cells in the damage site. Qu et al[47]. had evaluated the expression levels of angiogenesis factors, neurogenesis factors, and tissue plasminogen activator (tPA) of the cells in the

presence or absence of collagen type I scaffold. Both the cells alone and cells with collagen type I presented higher angiogenesis factors (NOTCH4, VEGFA, and TGFB) than neurogenesis factors (MDK, BCL2, and BIRC5). However, the cells cultured with the collagen type I scaffold showed more increased expression levels of factors in angiogenesis, neurogenesis, and tPA than that of the cell-alone group[47]. It revealed that the role of implanted scaffolds in the cell engraftment procedure is to provide an adhesion environment for transplanted cells and maintain their ECM molecular of transplanted cells and thus increase survival of engrafted cells, which possess neurotrophic factor release and assist the generation of newborn cells. This hypothesis was further explored by Ballios et al[48]. They demonstrated that HA-based injectable hydrogel increased survival of NSCs, the mechanism of which is through HA receptor CD44 on the cell. Based on the results, Führmann et al[5]. advanced to render material having the capability of controlling exogenous cell differentiation and maintenance of exogenous cell survival through Arg-Gly-Asp (RGD) peptide and PDGF-A when applied to spinal cord injury. Higher transplanted cell survival and migration were found in the hydrogel group compared to the media-only group. However, a teratoma with 3 germ layer cells was observed in the cell-only group and injectable hydrogel-containing cell group. In addition, an attenuated teratoma appeared in the hydrogel-containing cell group, suggesting that a grafted PDGF-A in the hydrogel enhanced more contact opportunity between human-induced pluripotent stem cell–derived oligodendrocyte progenitor cells (hiPSCs-OPCs) and PDGF-A.

The role of biomaterials can support not only cell replacement in regenerative medicine but also the cells that secrete the therapeutic agents to treat CNS disease[49]. It has been demonstrated that CNS disease can be improved after the treatment of trophic factors or therapeutics, such as dopamine required for Parkinson's disease or anti–amyloid-β (Aβ) antibodies to target Alzheimer's disease (AD). For this purpose, a long-term secreting device design would be a key issue. In general, injectable hydrophilic-type biomaterials are employed to achieve long-term therapeutic agent secretion through a cell-encapsulating platform. The most used hydrophilic types in the encapsulation system are alginate, agarose, chitosan, collagen, poly(ethyleneglycol), or polyvinylalcohol[50]. In addition, immune reactions of the host would involve encapsulated cell survival. Agarose/poly(styrene sulfonic acid) (agarose/PSSa) encapsulated with tyrosine hydroxylase-positive PC12 cells was investigated. Results showed a similar host reaction in the injection tracks and the place around the encapsulation cell gel, as evidenced by GFAP expression, meaning a lower host response was observed when using encapsulating system. Also, the agarose/poly(styrene sulfonic acid) (agarose/PSSa) encapsulated system extended the survival of encapsulated cells at least for 5 weeks[51]. To prolong the period of trophic factor secretion, researchers developed a device that integrated a membrane and a cell encapsulation system together. The integrated device can prolong cell survival at a high density for at least 1 year by managing the parameter of hydrogel stiffness and permeable membrane porosity[50]. The integrated device has been applied as passive immunization for Alzheimer's disease to misfolded toxic proteins by continuous antibody delivery. Results showed that Aβ 40 level, Aβ 42 level, and amyloid plaque burden were decreased in the brain, and downregulated levels of phospho-tau pathology in the hippocampus were prevented[52].

Most of injectable hydrogel is hydrophilic, which is easy to mix with cells. On the other hand, it requires a different procedure for delivering the cells when using a hydrophobic-based material such as PLGA. PLGA-based microparticles have been designed as a cell carrier to deliver NSCs into the stroke lesion cavity in the brain[53]. Researchers have used an oil-in-water (O/W) emulsion technique to prepare 100- to 200-μm microparticles and then coated fibronectin on the microparticles for cell attachment. The implantation results showed that primitive tissue formation was observed within 7 days after implantation into the lesion cavity. To provide a more adequate environment for de novo tissue formation, vascular endothelial growth factor (VEGF) was encapsulated into a PLGA-based cell carrier microparticle. It was observed that endothelial cells of the host integrated into this primitive tissue through released VEGF, attracting the formation of a neovasculature, and moreover, part of endothelial cells interspersed into grafted human neural stem cells (hNSCs)[54].

Table 4 summarizes the effect of various sources of materials postimplantation. Choosing proper raw materials or using additive factors encapsulated in biomaterials would improve the outcome of cell therapy treatment, as well as achieve structure support and increase delivered cell survival in CNS therapy.

Stiffness Effect of Biomaterials on Implantation

CNS Disease Treatment by Biomaterials Alone. Mechanical strength affects the inflammation status of the tissue. The mechanical property of the brain in the body is the softest, but that of electrodes which may be implanted into the brain is several orders of magnitude rigid. Moshayedi and colleagues[55] investigated the effect of matrix stiffness on inflammation when materials were implanted into a body. They rendered one with a softer mechanical property like brain ($G' = 100$ Pa) and the other with a stiffer property like muscle ($G' = 30$ Pa) through crosslinking agents in polyacrylamide. The results showed that softer implanted material showed less inflammatory gene expression postimplantation[55]. In addition to inflammation of body response, stiffness of materials would decide their standing time in the body. Higher mechanical strength of materials would deposit in the body longer than soft materials did[56].

CNS Disease Therapy by Combinations of Cells and Biomaterials
Block-Type Cells/Biomaterials. Mechanical strength of implanted materials has been chosen according to the site

Table 4. Effect of Chemistry of Materials Postimplantation.

Purpose	Applied Site	Materials	Type	Cell	Evidence	Ref
Angiogenesis scaffold	Cerebral cortex	Gelatin/GPSM/bFGF/EGF	Block scaffold	—	1. Integrity of the brain shape 2. The scaffold remained for 60 days in the brain 3. Newly produced cells 4. Expressions of GFAP, Iba1, and NAGO	35
CNS regeneration scaffold	Cerebrum	HA-IKVAV	Injectable hydrogel	—	Host cell invasion into scaffold	36
Brain trauma scaffold	Cerebral cortex	Collagen-GAGs	Injectable hydrogel	—	1. 7-fold increase of migratory cells and NPCs at 21 days 2. Fewer inflammatory cell 3. BDNF and GDNF were increased	37
Generation of cortical neurons	Rodent brain	PEAs/Wnt3A/BM P4/cyclopam ine	Injectable particles	—	1. Released for 1 month in vitro 2. Promoted cortical differentiation of cells in vitro 3. Did not evoke a significant inflammatory response in vivo	38
SCI scaffold	Spinal cord	- Microfiber s/collagen - Microfiber s/chitosan - Microfiber s/fibrin	Block scaffold	Mice NS/PCs	- Cell survival: microfibers/collagen > microfibers/chitosan > microfibers/fibrin - Scar formation: microfibers/collagen > microfibers/chitosan > microfibers/fibrin - Neural lineages in microfibers/collagen: astrocytes > oligodendrocytes > neurons	34
TBI scaffold	Cortex	Collagen type I	Injectable hydrogel	hMSCs	- Enhanced by 79% in the target site at 12 hours - mNSS and Morris water maze are improved at 28 days - Higher differentiated neuronal, oligodendrocyte, and astrocyte cells of hMSCs in hydrogel group than those of cell-only treatment	46
TBI scaffold	Cortex	- Laminin/collagen type I - Fibronecti n/collagen type I	Injectable hydrogel	NSCs	- Highest implanted cell survival in laminin-based scaffold - Most improvement of cognitive behavior in laminin-based scaffold	45
SCI scaffold	Spinal cord	HAMC-RGD/PDGF-A	Injectable hydrogel	hiPSCs-OPC	- Higher cell survival in hydrogel (46%) than in media (29%) - More migrated cells in hydrogel than in media by 33% - Reduced cystic cavitation area by 88% - Attenuated teratoma formation in hydrogel vs. those in media	5
Stroke brain repair	Brain	- HA hydrogels - R-Y-I/HA hydrogels - Y-I/BDNF, BMP-4/HA hydrogels	Injectable hydrogel	iPS-NPC	- R-Y-I/HA hydrogels promote iPS-NPCs differentiated into neurons (NF200) in vivo - Y-I/BDNF, BMP-4/HA hydrogels promote cell proliferation (Ki-67) and astrocytic differentiation (GFAP) in vivo	56
Dopamine secretion	Brain	Agarose/PSS a	Injectable particles	PC12	1. The encapsulated cells survived at least for 5 weeks 2. The encapsulated cell necrosis was not apparent 3. Similarity of expression of GFAP around encapsulation cell gel particles and injection tract	51
Anti-Aβ antibody secretion	SC	PEG plus 0.45-μm porous membranes	Device implantation	Chimeri c-C2C12	1. Long-term survival of encapsulated cells over more than 10 months in immunocompetent allogeneic recipients 2. Aβ 40 and Aβ 42 levels decreased 3. Amyloid plaque burden decreased 4. Preventing phospho-tau pathology in the hippocampus	52
Neovascularization	Stroke brain	VEGF/PLG A	Injectable particles	hNSCs	More angiogenesis was found in microparticle-released VEGF than in microparticles without VEGF	54

Abbreviations: Aβ, amyloid-β; BDNF, brain-derived neurotrophic factor; GAGs, glycosaminoglycans; GDNF, glial cell line–derived neurotrophic factor; GPSM, 3-(glycidoxypropyl) trimethoxysilane; HA, hyaluronic acid; HAMC, hyaluronan and methylcellulose; hiPSCs-OPCs, human induced pluripotent stem cells–derived oligodendrocyte progenitor cells; hMSCs, human mesenchymal stem cells; hNSCs, human neural stem cells; iPS-NPC, human induced pluripotent neural precursor; mNSS, modified neurological severity scores; NS/PCs, neural stem/progenitor cells; PEAs, poly(ester amides); PEG, polyethylene glycol; PLGA, poly(D,L-lactic acid-co-glycolic acid); PSSa, poly-(styrene sulfonic acid); SC, subcutaneous; SCI, spinal cord injury; TBI, traumatic brain injury; VEGF, vascular endothelial growth factor.

Table 5. Effect of Stiffness of Materials Postimplantation.

Purpose	Applied site	Materials	Type	Cell	Evidence	Reference
Brain inflammation	Brain	PAA gel: - $G' = 30$ kPa - $G' = 100$ Pa	Block scaffold	—a	Softer material has less inflammatory gene expression postimplantation	55
Brain stroke repair	Brain stroke	HA gels: - $G' = 100$ Pa - $G' = 350$ Pa - $G' = 1000$ Pa	Injectable scaffold	—a	- Volume of gel remaining in the brain: 1000 Pa > 350 Pa >100 Pa HA gels - 350 Pa HA gel with the lowest remaining volume of infarcted tissue	56
SCI scaffold	Spinal cord	50:50 PLGA/PLGA-PLS	Block scaffold	mNSCs	1. Functional recovery: scaffold/cells > scaffold >> cells > nontreatment group 2. Reducing formation of glial scar (GFAP) in scaffold/cells and scaffold group 3. New formation of NF of host origin in scaffold/cells and scaffold group	57
HI scaffold	HI brain injury	PGA scaffold	Block scaffold	mNSCs	1. Parenchymal loss was dramatically reduced and filled by scaffold plus cells complex 2. Blood vessel and NF formation of exogenous cell by scaffold plus cells complex 3. Mononuclear cell infiltration and astroglial formation minimized by scaffold plus cells complex	58
Cell survival	TBI	$G' = 0.8$ Pa (laminin based) $G' = 20$ Pa (fibronectin based)	Injectable scaffold	NSCs	Highest survival of implanted cell in softer material	45

Abbreviations: HA, hyaluronic acid; HI, hypoxic-ischemic; mNSCs, murine neural stem cells; NF, neurofilament; NSCs, neural stem cells; PAA, poly(acrylic acid); PLGA, poly(lactic-co-glycolic acid); PLS, poly-L-lysine; SCI, spinal cord injury.
aNot applied.

of treatment. Researchers designed a block biodegradable scaffold using PLGA to deliver exogenous cells for a midline lateral hemisection in the spinal cord of an adult rat. They simulated spinal cord architecture that contained an inner structure of gray matter having neural stem cells and an outer structure with white matter exhibiting long and axially oriented pores for axonal guidance and radial porosity to allow fluid transport. Open-field locomotion results showed significant improvement in scaffold plus exogenous cells compared with the lesion-control groups. Moreover, GAP43 (axonal marker) and BDA tracking could be observed rostral and caudal to the injury when rats received scaffold plus exogenous cells therapy. Furthermore, both new neurofilament formation of the host and a reducing glial scar (GFAP-positive) formation were observed in the scaffold plus exogenous cells group. Although the scaffold plus exogenous cells group showed promise in functional recovery and reduced epidural and glial scar formation, the scaffold-alone group also received a significantly improved outcome[57]. This might be the reason why mechanical support is mandatory in the treatment of spinal cord injury. In contrast, in the brain, which is a softer tissue in the body, a polyglycolic acid (PGA) scaffold, a hydrophilic, and low mechanical strength materials have been employed to coimplant with NSCs for brain injury. Results confirmed that the PGA plus exogenous NSCs can fill the cavity of a hypoxic-ischemic brain with loss tissue and promote blood vessel formation. The authors also found that biobridges appeared between host tissue and donor cells[58].

Injectable-Type Cells/Biomaterials. Different mechanical strength of materials would affect the cells in which the materials are delivered. Different types of ECM exhibit various mechanical properties. Tate and colleagues compared a laminin-based ($\sim G' = 0.8$ Pa) and fibronectin-based ($\sim G' = 20$ Pa) collagen type I scaffold in encapsulating NSCs to implant into the TBI site. The highest survival of implanted cells was observed in the group that received the laminin-based collagen type I scaffold, which had 2 times and 7 times as much as the fibronectin-based and cell-alone groups, respectively. Moreover, the laminin-based collagen type I scaffold showed better functional recovery in cognitive behavior than cells alone and fibronectin-based collagen type I[45].

From previous studies (summarized in Table 5), it seems that choosing a mechanical strength of implanted materials would depend on the injured site and the implanted cells delivered by biomaterials. Stiffer materials would be required for injury sites that exhibit higher mechanical strength and vice versa.

Topography Effect of Biomaterials on Implantation

Biomaterials not only play a role in supporting cell survival but also are tailorable for tissue morphology in regeneration medicine. Kato-Negishi and colleagues[59] prepared a

Table 6. Effect of Topography of Materials Postimplantation.

Purpose	Applied Site	Materials	Type	Cell	Evidence	Reference
Premade neural network transplantation	Cortical tissue	Premade neural cell network by PDMS	Block Scaffold free premade neural network	Rat cerebral cortices	1. Highly orderly neural structure as in PDMS template with condition of 100, 100, and 200 mm (diameter, depth, and distance) 2. Spontaneous [Ca2]i responses 3. Synaptic connections between transplanted and host cells	59
Restore lost long-distance axonal pathways	Rat brain	CMC/ agarose/ ECM/cells	Block scaffold	Cerebral cortical neurons	1. An axonal cytoarchitecture created 2. Facilitated robust neuronal viability (22 days in vitro) 3. Minimally invasive implantation	39

Abbreviations: CMC, carboxymethyl cellulose; ECM, extracellular matrix.

network structure of neurospheroids in vitro through topographic microchambers for precisely controlling the organization of transplanted cells. They constructed polydimethylsiloxane (PDMS) microchambers with various diameters (50, 100, 150, and 300 μm), depths (50, 100, 150, and 300 μm), and distances (100, 200, 300, and 600 μm). In the condition of 100, 100, and 200 μm (diameter, depth, and distance), more orderly network structures between neurospheroids were observed. The authors further stamped the neurospheroid network (NSN) onto the cerebral cortex by simply peeling off PDMS microchambers from the network neurospheroid after a 1-day in vitro cultivation. Their results demonstrated that the NSN stamped onto brain tissue showed spontaneous [Ca2+]i responses for more than 8 days and synaptic connections between the stamped NSN and host neurons in the cortical tissues. Although material-free cell transplantation can prepare an organizational structure of neural tissue, it will be hard to apply to surgical procedures in the future. Another type of scaffold was designed in the columnar shape, named micro–tissue-engineered neural networks (micro-TENNs), to form an axonal architecture to restore neural circuits for long-distance deficits. The researchers used an agarose-based micro-column structure, the interior of which was filled with ECM suitable for cerebral cortical neuron cell culture and the exterior of which was coated with carboxymethyl cellulose (CMC) for enhanced stiffness. The micro-TENNs minimized the invasively implanted area in rat brains through enhanced stiffness on the exterior of micro-TENNs[39].

We summarize the topography effect of materials postimplantation in Table 6. Topography applied in the implantation has more limitations due to product realization in clinical use in recent years. The potential of topography is to simulate the architectural structure of tissues or organs in the body, and this characteristic may be more useful in the designation of organoid structure in vitro.

The chemistry, stiffness, or topography of biomaterials can be employed in cell transplantation to assist cell therapy, but cellular responses in the human body are not mediated by only one parameter. Moshayedi et al[56]. combined these 3 parameters in a hydrogel and mixed them with human NSCs for stroke brain therapy. They found the lowest remaining volume of infarcted tissue when the mechanical strength of hydrogel was at 350 Pa. In topography design, they created environmental spatial cues by grafting matrix metalloproteinase (MMP)–degradable peptide on the hydrogels for vascular cell invasion into the hydrogels. Moreover, the effects of chemistry, adhesion motifs, and growth factors on neural progenitor differentiation were explored by a statistical design-of-experiment (DOE) approach. The data showed that adhesive peptides played an important role in neuronal differentiation of human neural progenitor cells (hiPSC-NPCs) in vivo rather than those of growth factors. Moreover, the hydrogel containing both adhesion peptide motifs and growth factors (BMP4 or BDNF) promoted the differentiation of hiPSC-NPCs to astrocytes more than that of neuronal differentiation in vivo.

Overall, CNS injury can be treated by biomaterials alone or complex cell/biomaterial methods through maintaining the shape of the injured site; creating some space with a permissive interface for the invasion of glial cells, blood vessels, and axons cells; protecting transplanted cells; or delivering therapeutic agents. The effect of chemical properties of biomaterials would be a role in providing structural support to the injured site and protecting exogenous cell survival. The stiffness properties of biomaterials would affect host inflammation, exogenous cell survival, and therapeutic agent release; moreover, they determine the standing time of materials in the body. The topography effects of biomaterials are major in the environment, mimicking in particular the architectural design. The influence of 3 parameters of biomaterials on biology is summarized in Figure 1. In addition, delivery types of materials play an important role for the design of implantation materials. We summarize the advantages and disadvantages of various delivery types of biomaterials for further design of implantation materials in Table 7. Both block- and injectable-type biomaterials can act as a backstop to support the trauma site. However, the

Table 7. Advantages and Disadvantages of Various Delivery-Type Biomaterials.

	Advantages	Disadvantages
Injectable hydrogels	- Easy to mix with transplanted cells - Easy to mix with hydrophilic therapeutic agents - Easy to handle in surgical process - Easy to simulate mechanical strength of soft tissue	- Shorter remaining time in body - Limitation in simulating a higher mechanical strength of hard tissue
Injectable particles	- Controlled release of trophic factors or therapeutic agents - Easy to handle in surgical process	- Lower protection in delivered cells in the host
Block-type materials	- Long-term remaining time in body - Easy to simulate a higher mechanical strength of hard tissue	- Requiring bigger surgical area

Table 8. Development of Cell Labeling Tracker Applied in the Central Nervous System.

Application	Labeling Agent	Cell No./ Delivery Route	Instrument	Outcome	Reference
Stroke model	Fluorescent plus magnetite nanocluster	5 × 105 mBM-MSCs/IC	T2-weighted FSE sequence MRI	1. The sizes of labeling agent are 80 ± 10 nm 2. Above 85% cell viability 3. Iron payload for MSC is 18.42 ± 1.7 pg/cell 4. Sensitive enough for monitoring the migration of a small number of cells 5. Labeling does not affect MSC functions	60
Stroke model	Fluorescent plus mesoporous silica-coated SPIONs (fmSiO4@SPIONs)	- 5 × 105 C17.2 cells/IC - 1 × 106 C17.2 cells/IV	T2-weighted SE sequence MRI	1. The size of the labeling agent is 50 nm 2. Above 85% cell viability 3. Iron payload for MSC is 10 pg/cell 4. Sensitive for monitoring the cells injected from the intravenous to ischemic hemisphere 5. Migrated labeled cells have cell function with highly expressed nestin	61
Stroke model	MRI/SPECT/fluorescent tri-modal probe (125I-fSiO4@SPIOs)	- 1 × 106 MSCs/IC - 1 × 106 MSCs/IV	T2-weighted FSE sequence combined with SPECT	Quantifiable and real-time visualization of implanted cells: 1. 35% of intracerebrally injected MSCs migrate to the lesion area at 14 days 2. 90% of intravenously injected MSCs trapped in the lung at 14 days	62
Stroke model	19F-MRI contrast agent with fluorescence dye	3.75×106 hNSCs/IC	T2-weighted MSME MRI combined with diffusion SMES sequence MRI	1. Above 75% cell viability after labeling 2. Injected biomaterials and normal cells are distinguishable	63

Abbreviations: FSE, fast spin echo; hNSCs, human neural stem cells; IC, intracerebrally; IV, intravenously; mBM-MSCs, mouse bone morrow mesenchymal stem cells; MRI, magnetic resonance imaging; MSCs, mesenchymal stem cells; MSME, multislice multiecho sequence; SE, spin echo; SMES, stimulated multiecho trace; SPECT, single-photon emission computed tomography.

standing period in the body, surgical area, and delivery method of biomaterials are different. In general, block-type biomaterials are employed in SCI due to advantages of higher mechanical strength and long-term performance of block-type biomaterials. In contrast, it is better to use injectable-type biomaterials for TBI due to advantages of less surgical area required in the treatment and trophic factor delivery for promoting regeneration.

Cell Tracking Application of Biomaterials in the CNS

The application of biomaterials is used not only for assisting in cell therapy but also in cell labeling for tracing the distribution of implanted cells. A dual-functional probe has been developed with optical and magnetic properties for doubly confirming the location of implanted cells[60]. The researchers used a polystyrene magnetite nanocluster (PMNC) as the inside core, and the outside of PMNC was coated with 2 layers of silica sandwiched with a layer of rhodamine to form a fluorescent-magnetite nanocluster (FMNC). After synthesis of FMNC, mesenchymal stem cells (MSCs) were incubated with FMNC to examine the efficacy of cell tracking in the ischemic mouse brain. Iron payload for MSCs can achieve 18.42 ± 1.7 pg/cell and does not affect MSC functions. Moreover, small quantities of FMNC-labeled cells can be detected in magnetic resonance imaging (MRI) for more than 1 month[60]. One team further designed a cell-labeling agent with higher MRI sensitivity and efficiency with material composed of mesoporous-type silica and a small pore size of labeling agents, which avoided a too high uploading of the labeling agent in the cells to diminish the labeled cells' function. Their results showed that a homing effect of labeled cells to the ischemic hemisphere was observed when the stroke animal received an intravenous injection of 1×10^6 cells, and those homing cells were functional with highly expressed nestin[61]. The cell tracking using MRI can observe its distribution with high-resolution images in the brain, but it is difficult to use the tracking agent in a whole-body scan. Tang et al[62]. developed a tracking agent combining probes of MRI, single-photon emission computed tomography (SPECT), and fluorescence to study the quantification of implanted cell distribution after intravenous or intracerebral injection into stroke rats. The percentage of implanted cells migrating to the lesion area was 35% after intracerebral injection, whereas 90% of implanted cells were trapped in the lung by intravenous injection. Nevertheless, less implanted cells were observed in the lesion area, and the delivery route showed significant improvement in neurobehavioral outcomes after 14 days of MSC treatment[62]. The transplanted cells could be observed by using a labeling agent, but implanted supportive materials could not easily distinguish them from the damaged cavity area with real-time visualization. To achieve visualization of the stroke pathology, tissue regeneration, and transplanted cells at the same time in an animal model, Bible and coworkers[63] used a highly sensitive 19F-MRI contrast agent in a T2- and diffusion-weighted MRI session with multinuclear MRI. The cells were labeled with 19F-MRI contrast agent, mixed with extracellular matrix derived from decellularized matrix, and then implanted into a middle cerebral artery occlusion (MCAo) animal model. They found that the distribution of 19F-labeled implanted cells, implanted extracellular matrix, and stroke pathology can be clearly distinguished by T2- and diffusion-weighted MRI.

We summarize the development of a cell tracker in Table 8 to provide more comprehensive information for supporting the designation of biomaterials contained with exogenous cells when they are implanted into the body.

Conclusions and Future Perspective

Tissue engineering requires 3 elements—cells, signals, and a scaffold—to construct an organ, the goal of which is to regenerate or replace a damaged part. For many years, several efforts have been carried out by exogenous cell transplantation, growth factor treatments, scaffold implantation, or a complex of cell/biomaterial implantation in tissue engineering/regenerative medicine[64]. Moreover, tracking implanted therapeutic materials is involved in the trend of regenerative medicine. However, the goal of tissue or organ replacement is still unmet in this field. In one study, the organoids, which were produced in vitro and exhibited a 3-dimensional microanatomy structure, were found from a patient's teratoma and studied to understand cancer formation[65]. In recent years, scientists have attempted to use stem cells or primary cells for constructing a therapeutic organoid, such as human intestinal organoids as the therapeutic organoid for inflammatory bowel disease[66]. Due to the complicated brain structure and microenvironment, most studies of brain organoids have focused on drug screening and explored the mechanisms of brain disease[67–69]. Few studies have applied a brain organoid to treat brain diseases[70], indicating the complicated nature of the forebrain, midbrain, and hindbrain brain structures, functions, and cell types. Therefore, application to brain therapy through organoids may employ biomaterials to precisely manipulate the cell behavior, which has tissue specificity. In addition, in vitro cultivation system improvement (e.g., coupling to a perfusion system for dynamic control of nutrients and gas exchange to increase cell viability of interior organoids) may be an alternative method to speed up the developments of organ replacement[67-69].

Declaration of Conflicting Interests

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