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Role of Biomaterials in Neural Stem Cell Fate

By Athira K. S. & Paulose C. S.
Indian Institute of Science, India

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Abstract- In this article we review the types of natural and synthetic materials that are being used in brain tissue engineering applications for traumatic brain injury and stroke. We analyse modifications of scaffolds including immobilizing drugs, growth factors and extracellular matrix molecules to improve central nervous system regeneration and functional recovery. This review attempts to outline the varieties of biomaterial parameters that are applied as biophysical and biochemical signals to direct NSC fate and behaviour. The understanding on the interaction of NSCs decision and biomaterial parameters is helping to advance NSCs-based clinical approaches for nerve tissue regeneration and repair.

We conclude with a discussion of some of the challenges that remain to be solved towards repairing and regenerating the brain. This review seeks to describe the current types of scaffolds and evaluate their use in combination with stem cells for TE applications. Finally, conclusions about the current state of biomaterial scaffolds containing stem cells for TE applications are drawn and suggestions for the future direction of the field are given. An overview of the available biomaterials for use in combination with directed stem cell differentiation as means of replacing diseased or damaged tissues are given. In this review, current and emergent approaches based on stem cells in the field of TE are presented for specific applications of human tissues and organs. The combination of stem cells and TE opens new perspectives in tissue regeneration for stem cell therapy because of the potential to control stem cell behaviour with the physical and chemical characteristics of the engineered scaffold environment. Niche includes a biomaterial with appropriate biochemical and mechanical factors for the cells and tissues studied. In this review, we examine the mechanisms that contribute to the death of transplanted cells. We review both the in vitro data, where biomaterial scaffolds are designed to enhance cell survival, and the in vivo data, where scaffolds are shown to improve cell survival following transplantation into the damaged brain and spinal cord.

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I. Introduction

Brain neurological disorders, such as stroke/cerebral ischemia, traumatic brain injury (TBI) and neurodegenerative diseases, are lack of effective treatments in the past years due to the extensive loss of cerebral parenchyma [1]. Recently, trans-plantation of stem cells has been becoming an important approach for injured brain tissue regeneration. Neural stem cells (NSCs), which have been isolated from various regions in the developing and adult nervous system, are capable to differentiate into all kinds of neural cell types including neurons, astrocytes, and oligodendrocytes, offering promising prospects for the treatment of brain diseases [2]. However, stem-cell therapy for central nervous system (CNS) diseases is of challenge because the blood–brain barrier limits the diffusion of neurotrophic molecules into the brain by traditional oral or intravenous routes. Moreover, the lesion brain cannot afford a suitable microenvironment for NSCs regeneration because inflammation, glial scar formation, release of inhibitory molecules, and absence of growth-guiding astrocytes [3]. Therefore, it is necessary to develop advanced biomaterials generating bioactive artificial microenvironments, which closely mimic the natural niche to support NSCs growth without losing ''stemness'' or undesired differentiations.

In the body, stem-cell populations physically reside within instructive local tissue niches that maintain and regulate stem cells fate [4]. Artificial microenvironment of NSCs can be designed with new material syntheses and processing techniques to feature an intense signal to maintain NSCs stem cell fate, or a myriad of signals that address the biologically relevant sequence of events leading to stem cell lineage commitment. Biomaterials could also be used as delivery vehicles for NSCs transplantation to deliver trophic factors, support residual neurons around the injury site, maintain of replacement cells, provide contact guidance for directed axonal outgrowth, and minimize hostile inflammatory reactions. In this article we review progress to date employing tissue engineering to promote cell replacement using neural precursors (NPs) to restore neurological function after traumatic brain injuries and stroke. The therapeutic value in transplanting NPs is extremely high due to the inability of neurons to undergo mitosis and the incapacity of the brain to repair large injuries on its own.

The study of NSC-biomaterial interactions would advance our understanding on the mechanisms of NSCs-fate specification and self-renewal which could in turn pave way for the rational design of new scaffolds that encourage successful incorporation, survival, and integration of NSCs into diseased or injured regions of the CNS [5]. Specifically, changes to one or more parameters at the initial time point of cell culture could ultimately influence long-term functional differentiation and gene expression [6]. This paper reviewed the recent progresses in the studies of biomaterials as NSCs

Author α: Department of Materials Engineering, Indian Institute of Science, Bangalore, India. e-mail: athiraiisc@gmail.com
Author σ: Centre for Neuroscience, Department of Biotechnology, Cochin University of Science and Technology, Cochin, India.

artificial niches to direct NSCs fate for brain tissue engineering (TE). We first summarized the components of the natural NSCs niche, as it is necessary to establish a solid conceptual framework for artificial niches design. We then outlined various biomaterial parameters both biophysically and biochemically directing NSCs fate in vitro, as well as functionalized scaffolds facilitating the in vivo transplantation of NSCs for brain TE. The adhesive properties of catecholamine, representatively as poly (dopamine), have helped to realize the efficient immobilization of biomolecules onto surfaces with various chemistries [7]. Importantly, the facile approach of using a catecholamine group as a coating agent not only allows flexibility in the selection of the substrate materials but is also an inexpensive and eco-friendly process [8]. The spatial arrangement of the surface adhesiveness may result in the patterned regulation of cellular behaviours, including differentiation, proliferation, and migration [9].

The discovery of NSCs, which have the ability to self-renew and differentiate into all types of neural lineages, offers promising prospect for the treatment of brain neurological disorders such as stroke/cerebral ischemia, TBI and neurodegenerative disorders. However, only limited number of NSCs could survive or propagate due to tissue inflammation or blood–brain barrier. Therefore, it is necessary to develop an appropriate culture system that highly mimics the natural NSCs niche to direct stem cell fate and behaviour for nerve regeneration. Both biophysical and biochemical properties of the NSC niche, including topology, mechanical properties, bioactive molecules, and their spatial and temporal presentations should be considered for the design of functionalized scaffolds, which could not only serve as the delivery vehicles of NSCs but also stimulate specific cellular responses at the molecular level, such as support endogenous or exogenous cells proliferation, migration and homing, even promote the growth of axon at the injured brain site.

II. Brain Injuries

Approximately 26 million people sustain traumatic brain injuries each year as a result of falls, motor vehicle accidents, being struck by objects or assaults. An additional 1.2 million individuals are affected by stroke, of which 80% are ischemic and are of varying severity. These numbers do not include major brain injuries caused by infections, tumours or other CNS diseases that account for another large population. Brain injuries are generally classified as mild, moderate or severe depending on damage sustained. Majority of TBIs are mild, resulting in a change in mental status or state of consciousness. Severe brain injuries may cause amnesia, long periods of unconsciousness, irreversible changes in cognitive (attention and memory), motor (coordination, balance, and limb weakness/paralysis) and sensorimotor function (vision, hearing, and touch), alteration in emotions (anxiety, depression and personality changes) and even death [10].

a) Pathophysiology

Individuals who do not die within the first few months after sustaining a severe brain injury are often left with disabilities and a poor prognosis for the duration of their lives. The acute affects can be observed within the first hours after injury and can be amplified within the first several weeks, generally attributed to the pro-inflammatory response to the injury that can last for months or years [11]. Neuronal damage and cell loss have been extensively documented and characterized in the cerebral cortex, the hippocampus and the thalamus in the acute phase following experimental brain injury [12]. The primary damage created by mechanical forces at the moment of the impact is irreversible. In response, immune cells are recruited to the damaged site, whereupon they release cytokines and chemokines triggering a neuroinflammatory reaction that produces a wave of secondary cell death. After a delay, the astrocytes surrounding the injury begin to produce a glial scar. Once formed, this scar tissue creates an inhibitory environment eliminating the possibilities of axonal regeneration due to the formation of a complex extracellular matrix (ECM) [13]. This prolonged and progressive pathologic cascade becomes the basis for the deficits in cognitive and motor function that begin in the first hours after TBI and may continue for years. Kuruvilla et al.[14] reported that serotonin and gamma amino butyric acid along with autologous bone marrow cells to 6-hydroxydopamine infused rats renders protection against oxidative stress mediated neuronal damage as in Parkinson's disease which makes them clinically significant for stem cell-based therapy. The alterations in dopamine D 1 receptor-binding parameters and gene expression during Parkinson's model were reversed by serotonin and gamma amino butyric acid supplementation [15]. Paul et al. [16] showed serotonin and norepinephrine functionally reversed Dopamine receptors significantly in rotenone induced Hemi-Parkinson's rat.

b) Treatment

After a person sustains an injury, the medical team will provide resuscitation procedures, and stabilize vital functions to minimize secondary damage to the brain. Mechanical ventilation is used to support respiration and to maintain lower intracranial pressure. Sensory devices may be surgically placed into the brain cavity to monitor or control intracranial pressure. Surgery may be required to repair haemorrhaged arteries or to eliminate blood clots. Blood, fluid and bone particles can be removed while damaged tissue, blood vessels or the skull can be surgically remodelled in severe cases

where there is extensive swelling. Patients are also kept sedated with medications to prevent them from causing any additional injury and to prevent seizures and spasticity. Doctors try to maximize cerebral perfusion pressure and blood flow (which includes oxygen and nutrients being supplied to the brain) while minimizing the swelling caused by pressure that may damage more cells [17]. Pharmaceutical agents also may be used to limit secondary damage to the brain which include: diuretics to reduce edema thus decreasing pressure; anti-seizure drugs to avoid additional brain damage; and coma-inducing drugs because a comatosed brain requires less oxygen to function [18]. Other medications such as analgesics, anti-anxiety agents, anti-depressants, anti-psychotics, muscle relaxants, sedatives and stimulants are also commonly utilized in patients sustaining TBI [19]. To date, there are no therapies capable of replacing the neurons lost to brain injuries, thus making full functional recovery after severe TBI impossible.

III. Tissue Engineering

Chronic limitations of traditional transplantation surgeries still exist due to the lack of appropriate donor tissues, risk of disease transmission, and potential for immune rejection. Tissue engineering, the multidisciplinary application of biology, chemistry, physics, engineering, and medical science, offers an alternative method to overcome these issues [20]. For therapeutic application of TE, engineered tissue is grown either within a patient or outside the patient and subsequently transplanted into the patient. Figure 1 provides a schematic representation of the process of neural tissue regeneration by engineering biomaterial scaffolds. Human cells are harvested from a patient and after in vitro cell culture, cells are seeded onto scaffolds with medium containing chemical stimuli, such as growth factors and differentiation-inducing factors. Scaffolds are three-dimensional (3D) matrices that support cellular growth processes, such as cell adhesion, migration, proliferation, and differentiation, by which cells are colonized onto the scaffold. The cell-colonized scaffold is then implanted into the patient, to regenerate bio-compatible, immunocompatible, and bio functional tissues or organs inside the patient body. Cells and scaffolds are essential to regenerate new tissues with TE. Cells become the primary component of engineered tissue and the scaffold provides cells with an appropriate physical and chemical environment where they can attach to the surface of the scaffold, migrate through the scaffolds' pores, and then proliferate. In some instances, such as stem cell therapy, collaboration of cells and scaffolds with differentiation-inducing factors is essential for stem cells to differentiate into engineered cell lineages and to develop new tissues.

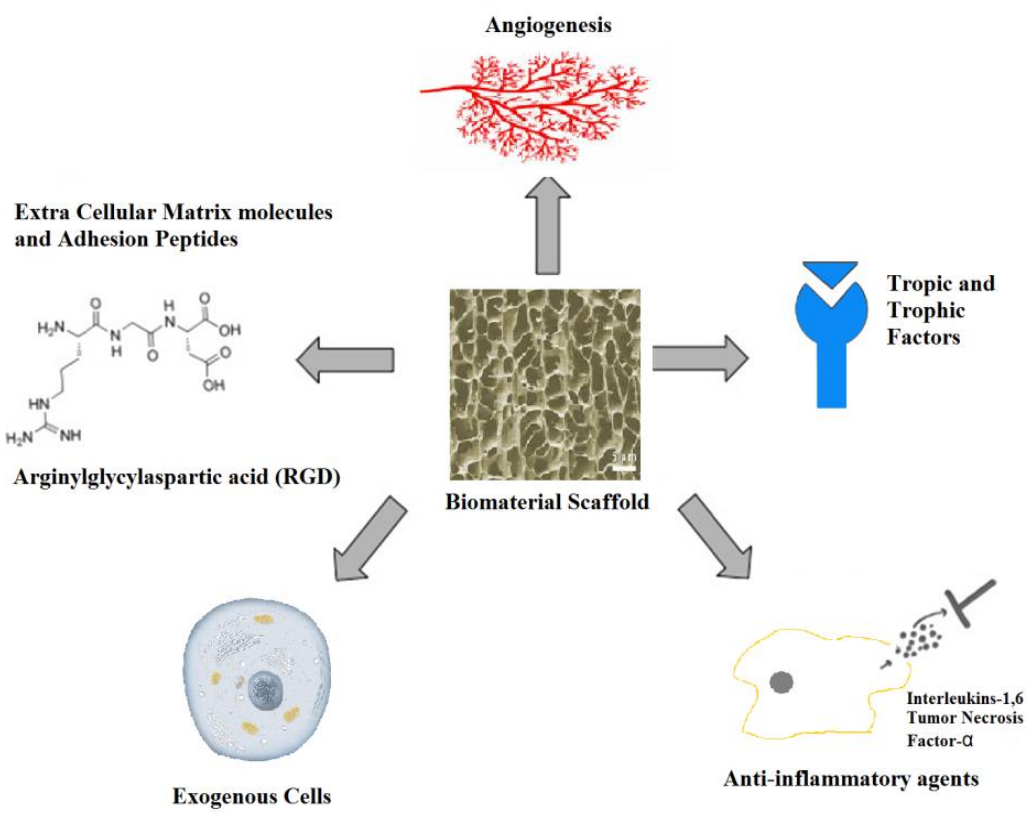

Figure 1 : Engineering Biomaterial Scaffold to Improve Neural Tissue Regeneration

a) Stem Cells in Tissue Engineering

Although it is difficult to grow some cell types such as cardiomyoctes and hepatocytes in large quantities, stem cells are undifferentiated biological cells that can produce more stem cells (self-renewal) and can differentiate into specialized cells (cell potency). Transforming growth factors beta 1 and 3 (TGF-β1 and TGF- β3), for example, have been reported to enhance the differentiation of Mesenchymal Stem Cells (MSC) to chondrocytes [21] and the chemical agent β-mercaptoethanol (BME) has been used for neural trans differentiation of MSCs [22]. TE may be used for tissue regeneration such as bone, cartilage and neural tissues using degradable biomaterial scaffolds. For example, tubular collagen nerve guides (Neuragen from Integra Life Sciences) were used clinically to treat peripheral nerve injuries and the critical gap length treated by nerve guides was longer than 10 mm in primates and could be further increased by adding fibers or hydrogel with cells [23].

b) Stem Cells in Neural Tissue Engineering

The CNS, consisting of the spinal cord and the brain, is a very unique tissue network with an unusual ECM structure and characteristic soft physical properties which is susceptible to damage, illnesses, and injuries, including traumatic brain injury, spinal cord injury, stroke, Parkinson's disease, and multiple sclerosis [24]. The mechanical properties, structure, and composition of the ECM are effectors of cell function, thus, soft hydrogel scaffolds are utilized for CNS applications to mimic the biochemical and mechanical properties of the CNS [24]. For instance, hydrogel scaffolds made of acrylamide and PEG with arginine-glycine-aspartic acid (RGD) can regulate cell behaviours, such as adhesion, cell renewal, and differentiation of NSCs [25]. Platelet-derived growth factor (PDGF)-AA immobilized agarose scaffolds have been reported to support differentiation of NSC and neural progenitor cells (NPCs) to oligodendrocytes [26]. Sakata et al. [27], preconditioned NPs with interlukin-6 (IL-6) before transplanting those 6–7 hours after transient middle cerebral artery occlusion. The preconditioned NPs were protected from death and they released Vascular endothelial growth factor (VEGF) resulting in increased angiogenesis within the target site. Hydrogel scaffolds made of RADA 16-1 IKVAV (isoleucine-lysine-valine-alanine-valine) have been shown to serve as a guiding cue to direct NSC adhesion and neural differentiation with in vitro and in vivo to direct stem cell differentiation toward neural lineages and to promote the signal transmission among neurons because of electrical conductivity. The hydrogel in a rat brain surgery model enhanced survival of NSCs, reduced the formation of glial astrocytes, and improved brain tissue regeneration after 6 weeks post-transplantation [28]. Electrical stimulation was shown to enhance the proliferation and differentiation of NSCs on thin film scaffolds made of laminin (LN) and single-wall carbon nanotubes (SWCNT) [29]. Bioelectricity has shown to affect intercellular signalling of the nervous system and extended neurite outgrowth compared to cells grown on non-stimulated scaffolds [30].

i. *Adult Neural Stem-Cell Niche*

NSC was discovered in adult nervous system, which broke the curse of brain as a quiescent organ that nothing may be regenerated. Therefore, NSCs for stem cell based therapies in the regeneration of adult brain have drawn much attention recently. Successful application of NSCs therapies clinically would require precise control over the cellular behaviour. The microenvironment of NSCs termed as NSCs niche was therefore extensively studied. In vivo, the sub ventricular zone (SVZ) of the forebrain and the sub granular zone (SGZ) of the hippocampus as two main resources of NSCs act as in vivo NSCs niche, which physically localizes NSCs and maintains their stem-cell fate. Niche could support following functions of NSCs: it maintains NSCs in a quiescent and undifferentiated state to avoid being depleted by aging; niche provide a neurogenic environment for NSCs because large amount of NSCs transplanted into the brain outside the niche are very prone to differentiate into glial cells; and niche is structured so that both the number and type of differentiated progeny can be modulated in response to a diverse array of physiological cues [31].

ii. *Cells in NSCs niche*

In the adult mammalian brain, the SVZ is composed of different types of cells, including a monolayer of ependymal cells lining the ventricle, NSCs, transit-amplifying cells, neural progenitors (neuroblasts), and astrocytes (Figure 2) [32]. All types of cells are not isolated from each other, but mutually connected. The NSCs are relatively quiescent cells that express markers reminiscent of embryonic radial precursors, as well as the glial fibrillary acidic protein. The NSCs give rise to transit amplifying cells, which in turn generate neuroblasts. The neuroblasts migrate in glial tubes to olfactory bulb and generate neurons that integrate into neural circuitry. Researches indicated that a rich plexus of blood vessels snake along and within neuroblast chains in the SVZ. Some of the NSCs and transit-amplifying cells are closely associated with blood vessel that they may receive important signals from the vasculature [33].

iii. *Extracellular matrix in NSCs niche*

The ECM is the most important non-cell component of NSC niche, which could provide anchorage for NSCs adhesion and manipulate the concentration and presentation of signalling molecules to regulate NSCs behaviours. Generally, the ECM is structurally composed of two major components, interstitial matrix and basement membrane, which

contains adhesive glycoproteins, glycosaminoglycan's (GAGs), and ions. The interstitial matrix of brain ECM is composed of a ternary network of the glycosaminoglycan hyaluronic acid, proteoglycans of the lectican family (brevican, aggrecan, neurocan, versican), and intermingled link proteins of tenascins connecting to cell surfaces. Hyaluronic acid (HA), which works as a ''backbone'' in brain, would bind with tenascins and proteoglycans forming an organized HA-proteoglycan network around the embedded cells [34]. LNs as the main component of the basement membrane in NSCs niche are a family of heterotrimeric proteins that contain one a, one b, and one g chain subunits, found in five a, three b, and three g genetic variants, respectively [35]. LNs not only play an important role as the framework but also have different functions in the signal transportation. For example, finger like processes of basal lamina called fractones extend from blood vessels to contact each stem cell in the niche. Consequently, each stem cell receives at least three different sources of LN signals: from interstitial LNs, from their processes attached to blood vessels, and from the contacting fractones. Other ECM molecules such as the glycoprotein tenascin C, chondroitin sulphate proteoglycans (CSPG), and heparin sulphate proteoglycans also play important roles in the migration, differentiation and proliferation of the NSCs.

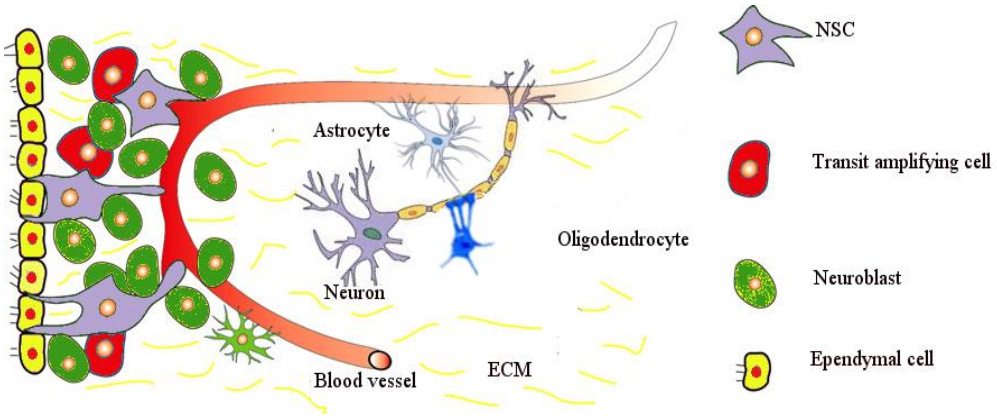

Figure 2 : NSC Niche in Sub Ventricular Zone of Adult Mammalian NSC

iv. *Endogenous stem cells*

Scientists have tried to expand the endogenous stem cells found inside the brain to repair damage after CNS injury. Despite significant work, several problems still exist with this approach. First, few neurons are generated in response to injury, as the vast majority of the new cells that are produced become glia. While infants have significantly larger numbers of NSCs than adults, and thus greater potential for repair [36], the NSCs of the immature brain simply do not produce many new neurons after TBI. Another barrier to regeneration from the endogenous stem cells of the brain is that the pools of NSCs are depleted with age [37]. Arvidsson et al. [38] researched the mechanisms of neuronal repair after stroke in an adult rat model and reported that less than 1% of the destroyed neurons are replaced from the endogenous NPs of the SVZ. Similar results were obtained in rat models of stroke in the immature animal where cell counts of immature neurons vs. mature neurons revealed that greater than 75% of the newly produced neurons failed to survive. Moreover, of those neurons that did survive were predominantly GABAergic inter neurons [39]. In a mouse model of TBI, same pattern was seen [40] and found that SVZ cells proximal to injured area produced a very small percentage of new neurons, while the majority became astrocytes. Whether the newly generated neurons died at injury site or failed to migrate from the SVZ to damaged region is unclear. In the adult brain, neural progenitors have a difficult time migrating to injured cortex due to dense white matter tracts [41].

c) *Brain Tissue Engineering*

A focal TBI results in a large number of dead cells and debris that are localized near the region of impact. Macrophages clear away remnants of dead or dying cells, but the injury creates a harsh, non-permissive environment that lacks nutrients, survival factors and most importantly, a habitable substrate and ECM that they once resided within [42]. This ECM is a scaffold that provides cells with structural and functional support. It is comprised of interconnected proteins and proteoglycans that create a framework that cells adhere to. Attachment to individual components of this matrix transduces mechanical signals that regulate both basic and complex cellular processes. The proteins and proteoglycans that comprise the ECM bind to a number of surface receptors found on cells that can affect proliferation, migration, differentiation, survival and other functions [43; 44]. Although reactive astrocytes produce ECM molecules in the process of generating the glial scar, this ECM is distinctly different from normal ECM in the brain functionally, chemically and mechanically [45].

IV. Biomaterial Scaffold Structures

Such biomaterial scaffolds can be used to promote the viability and differentiation of stem cells seeded inside-based on both the intrinsic properties of the material and the incorporation of specific cues into the material. NSCs have been isolated from various species such as mice, rats, and humans and from numerous regions in the developing and adult nervous systems including the SVZ, the SGZ of the hippocampus, the cortical neuroepithelium, and the spinal cord [2]. In vivo, the NSC is encompassed by a microenvironment or niche that presents it with a repertoire of diffusible factors, [46] cell-cell interactions, [47] and ECM ligands that bind to cellular receptors and thereby modulate signalling and gene expression [48]. These soluble and solid-phase components of niche collectively regulate cell behaviour and functions including mitosis, apoptosis, migration, and differentiation [49]. NSCs have therapeutic potential to treat disorders and injuries such as Huntington's disease, multiple sclerosis, Parkinson's disease, stroke, and diseases and injuries of the spinal cord [50]. In cell transplantation therapies, NSCs have survived in various regions of the CNS, including the striatum, hippocampus, ventricles, SVZ, olfactory bulb, and cerebellum, [51] and have shown promising results when implanted at the injured/diseased sites in animal models for numerous diseases and injury, such as Sly disease, myelin degeneration, Parkinson's disease, and spinal cord injury [52].

It is becoming increasingly clear that not only the biochemical but also the mechanical properties of microenvironment can modulate cytoskeleton, the adhesion and growth of cells, and even differentiation of stem cells [53]; therefore, it would be desirable to be able to finely tune mechanical properties of culture system. The biochemical and mechanical signals of proteins or materials mimicking the solid phase of native stem cell microenvironment will play a major role in controlling first expansion and then differentiation of stem cells for clinical applications. A scaffold is a 3D matrix that provides the framework and initial structural support for cells to attach, proliferate, and differentiate, facilitating the formation of an ECM [54]. For cell based TE, cells are usually seeded onto scaffolds which are made of materials such as acellular tissue matrices, naturally derived materials (natural biomaterials), and synthetic polymers (synthetic biomaterials). Synthetic biomaterials have tunable mechanical properties, however, the biocompatibility of natural biomaterials is better than synthetic materials, thus, hybrids of natural and synthetic materials are also used for scaffold fabrication. To support tissue regeneration for in vitro stem cell study, differentiation-inducing factors can be loaded into scaffolds to promote and to induce differentiation of stem cells, but these factors under specific circumstances remain indispensable. Achieving success in TE is attributed only to stem cells and scaffolds, suggesting that the effects of differentiation factors may be substituted with suitable scaffold structures [20].

a) Criteria to be used as scaffold

Scaffolds are 3D artificial structures that are created to recapitulate in vivo milieu providing cells with an appropriate microenvironment. Since brain injuries vary in shape and size, scaffolds that form after injection into wound cavity allow for a one size-fits-all solution. In an ideal biomaterial design, a list of desirable functions of a scaffold for a particular biological application should include nontoxicity, biocompatible with transplantable cells and brain tissue environment, maintain stemness of transplanted cells, controlled biodegradation, injectable, porous and remain local.

b) Natural Biomaterials

Tate and Shear, were some of the first investigators to use stem cells for brain TE in models of TBI. They produced collagen gels that contained either fibronectin and/or LN and showed that these scaffolds increased the survival of transplanted mouse NPs compared to NPs transplanted without the collagen matrix. They reported that the collagen-NP scaffold promoted tissue repair better than the NPs alone. Yu et al. [55], also reported that some NPs differentiated into neurons and formed synapses, which correlated with improvements in functional recovery. Elias et al. [56], used a similar approach repurposed for TBI. NPs transplanted on the scaffold showed increased survival and migration compared to cells injected without the scaffold; however, neuronal engraftment was not observed as only glial and endothelial cells (ECs) were observed amongst the grafted cells. They concluded that an additional growth factor or biochemical stimulus would be needed to achieve differentiated neurons in vivo. Another popular material being explored is HA, which is an abundant glycosaminoglycan in the brain. Survival rates for each type of precursor cell improved when encapsulated within the hydrogel.

i. *Protein-based biomaterials*

Different methods of purification exist depending on protein desired for scaffold fabrication and animal source. The most commonly used scaffold materials, collagen, can be isolated from a variety of tissues, such as skin, tendon, or bone. In vivo, fibrin, which is derived from fibrinogen, generates the blood clots that form after injury to the vasculature. Due this role and the ability to isolate fibrinogen from blood (both human and animal), it has been used as a sealant in clinical studies and as a biomaterial scaffold. Another protein that has been investigated for use in generating tissue engineered scaffolds is silk, which is secreted by insects and worms. Scaffolds made of silk or silk fibroin

have slow degradation rates and desirable mechanical properties, providing an alternative to the collagen and fibrin. Scaffolds made from silk fibers can be fabricated into a variety of structures, such as mats, sponges, meshes and membranes, expanding the possible applications. Silk can also be chemically modified to further enhance the properties of such a scaffold.

3D collagen scaffolds have been used to culture a wide variety of stem cells for different TE applications. One study demonstrated the monkey embryonic stem (ES) cells could differentiate into neural phenotypes as well as endothelial phenotypes when cultured as aggregates of cells known as embryoid bodies inside of collagen scaffolds [57]. Additionally, other types of stem cells have been used in conjunction with collagen scaffolds to produce engineered tissues. These approaches include seeding such scaffolds with NSCs [58]. These cells differentiate into neurons and form functional circuits inside of the scaffolds [59]. Although it has not been investigated as heavily as collagen, fibrin has also been studied as potential scaffold material for the culture of stem cells. A variety of stem cell lines can be cultured inside of fibrin scaffolds for many different TE applications. The properties of silk make it attractive for engineering bone, cartilage and ligament tissue and extensive research has been done using 3D silk scaffolds in conjunction with mesenchymal stem cells for these applications.

ii. *Polysaccharide-based biomaterials*

Agarose is isolated from red algae and seaweed, consists of a galactose-based backbone and is commonly used as a medium for cell culture in the form of agar. One of the attractive properties of agarose is that its stiffness can altered, allowing for tuning of the mechanical properties of the scaffold. Agarose scaffold have been investigated in combination with stem cells for generating a variety of applications, including cartilage, heart, and nerve. Studies have demonstrated that both mouse and primate ES cells can differentiate into dopaminergic neurons when encapsulated inside of agarose microcapsules [60]. This strategy could be used as a potential therapy for Parkinson's disease. In nutshell, agarose scaffolds provide a versatile platform for TE.

Alginate, which is derived from the cell walls of brown algae, forms scaffolds through the use of ionic cross-linking, allowing for encapsulation of cells. Alginate has also been used for neural TE applications. One study demonstrated that adult NPCs seeded inside of alginate scaffolds survived in vivo for two weeks after implantation into a spinal cord injury model [61]. A different study developed tunable alginate scaffolds by incorporating microspheres that released enzymes over time to degrade the scaffold. These scaffolds were successfully used to culture NPCs and increased their proliferation rate compared to when such cells were cultured in alginate scaffolds without microspheres [62]. Hyaluronan, also known as HA, is one of the major components of the ECM. It contains sites for cell adhesion and hyaluronan expression is upregulated during embryogenesis, suggesting its suitability as a scaffold material for the culture of ES cells. Hyaluronan is also expressed in many different tissues, including cartilage and nerve, suggesting it could also be used for the culture and differentiation of adult stem cells. Another polysaccharide that has been explored for TE applications is chitosan. It is derived by deacetylation of chitin and consists of glucosamine units. Chitosan has been used extensively as material for regenerating skin, bone and nerve tissue and is recently studied for use in combination with stem cells.

iii. *Natural Surfaces and Gels*

Numerous surfaces and gels have been generated from natural components such as collagen, other ECM proteins, and calcium alginate. However, natural components can face several challenges. It can be difficult to tune the mechanical properties of natural materials, and it is generally not possible to independently tune the mechanical and biochemical signals of these systems. Natural components, such as ECM proteins, also have problems with purity and the availability of large-scale sources of the materials, particularly if human proteins are involved. Numerous efforts have used 3D type I collagen, which can form gels, to culture rat embryonic cortical NSCs [59]. In one study, O'Conner et al. [63] cultured neurospheres on the top of collagen I gels and found that cells were able to migrate and disperse from the spheres and subsequently extend neurite processes. Most cells remained attached to and proliferated on gel surface during first week of culture, and cells that did differentiate during this initial time gave rise primarily to neurons that showed capacity to form synapses. During second week of culture, remaining NSCs differentiated into glial cells [63]. ECM molecules other than collagen have also been used to prepare surfaces for culture and differentiation of NSCs. Results demonstrated that precursor cells propagated with same mitogen can exhibit a different behaviour as a function of substrate.

Neurospheres of postnatal human cortical NSCs and mouse embryonic cortical NSCs have been analysed on various ECM proteins adsorbed to glass surfaces. The R 6 integrin was shown to be functionally important for cell attachment to LN [64]. Studies showed the importance of tuning the mixture of soluble factors and substrates to elicit specific cellular behaviours. ECM and other factors combine to regulate cell behaviour, which raises the experimental difficulty of exploring many possible combinations of factors. However, complex combinations of factors, including ECM, may be necessary to achieve tight control over cell function. Microarrays can yield substantial information on the

combinatorial effects of substrate and soluble factors on cell function, results that will aid the development of bioactive, synthetic microenvironments. In addition to high-throughput screens, surface patterning can be used to analyse the effects of spatially organized signalling factors on cellular behaviour. Because alginates are both biocompatible and inexpensive, they have been broadly explored in cell encapsulation and tissue-engineering applications [65]. Studies show the potential of calcium alginate for engineering microenvironments for NSCs and results indicate that when encapsulated in some materials, cells can presumably provide their own signals and therefore do not require the addition of ECM molecules, although adding exogenous signals may afford more control over cell behaviour.

iv. *Semisynthetic Surfaces and Gels*

Surfaces and gels have also been developed using a blend of synthetic and natural components. The natural component in these blends is typically an ECM protein that is adsorbed to the synthetic component and presents signals to modulate cell attachment, growth, and differentiation. Moreover, the addition of a synthetic component enables control over the architecture and mechanics of the materials. These bioactive, modular materials can therefore be viewed as an intermediate step toward developing completely synthetic materials, although the ECM protein still poses challenges for purity, immunogenicity, scalability, and other considerations. Studies collectively demonstrate that natural components can provide biochemical signals necessary to support cell attachment, proliferation, and differentiation when presented from a synthetic substrate. Promising semisynthetic materials also provide a promising basis for the development of fully synthetic materials that avoid some challenges of using isolated proteins, as these can potentially be replaced with recombinant or synthetic signals.

v. *Fully Synthetic Surfaces and Gels*

Natural ECM proteins offer the important advantage of presenting both identified and likely unidentified motifs that bind to cellular receptors and thereby regulate cell behaviour. However, natural components have the potential to elicit an immune response if implanted, can transfer immunogenic molecules to stem cells, [66] can pose a risk of pathogen transfer, and often do not offer the capacity to readily control the mechanical properties of the material. By comparison, materials composed of primarily synthetic components offer advantages including low immunogenicity, reproducible and scalable synthesis, and the ability to tune mechanical and biochemical properties, an important consideration for stem cells [53].

vi. *Self-Assembling Peptides and Peptide Amphiphiles*

Specific polypeptide sequences have the capacity to self- assemble into various structures, ranging from assembly of β-sheets via hydrogen bonding to cylindrical micelles via hydrophobic interactions [67]. To build upon these capabilities for creating bioactive matrices, self-assembling peptide sequences can be synthesized as fusions to motifs found in ECM proteins, including RGD and IKVAV from fibronectin and LN [68] respectively to create self-assembled structures that can engage cellular adhesion receptors. These synthetic peptides also offer the advantage of being able to display a broad diversity of natural and even unnatural side chains from the peptide backbone, enabling creation of multifunctional assemblies. A study using peptides that assemble into fibrous structures via β-sheet formation showed that this scaffold encouraged putative neural stem or precursor cells from adult rat hippocampal slices to migrate away from tissue explants laid on top of the scaffold [69].

c) *Synthetic biomaterials*

Although not as commonly employed as natural materials, synthetic materials also have been used in brain TE applications. Bible et al. [70] determined that transplanting MHP36 NPs into intact tissue lead to further damage. Some groups are encapsulating NPs into self-assembling peptide hydrogels. Peptides readily self-assemble and they can form nano-fibrous networks that mimic native ECM. Moreover, like hydrogels they can be injected in soluble form and subsequently solidify to form gels in situ. Li et al. [71] reported utilization of graphene foam, a 3D porous structure, as a novel scaffold for NSCs in vitro. It was found that 3 graphene foams can not only support NSC growth, but also keep cell at an active proliferation state with up regulation of Ki67 expression than that of 2D graphene films. 3D-GFs can enhance the NSC differentiation towards astrocytes and especially neurons.

i. *Polymer-based biomaterials*

The Polymer-based scaffolds have specific mechanical properties and can be modified to contain cues using various chemistries. There are some issues with these polymer-based scaffolds including a lack of sites for cell adhesion and the potential for toxic by-products after degradation. Poly (lactic-co-glycolic acid) (PLGA) is a copolymer that consists of monomers of glycolic acid and lactic acid connected by ester bonds. Neural TE represents another area where PLGA scaffolds seeded with stem cells shows promise as therapy for disorders of the nervous system. Work done by the Langer lab has shown the potential of such strategies. One study that showed that PLGA scaffolds designed to mimic the spinal cord and seeded with murine NSCs produced an increase in functional recovery after traumatic spinal cord injury in preclinical testing [72]. An additional study demonstrated that

human ES cells seeded inside of PLGA scaffolds could be directed to differentiate into neurons when treated with the appropriate cues [73]. The same study also showed that these cells could differentiate in cartilage and liver tissue inside of such scaffolds when exposed to the appropriate cues. A follow up study further characterized the differentiation of human ES cells treated with neurotrophins when seeded inside PLGA scaffolds for engineering neural tissue [74]. Seeding retinal progenitor cells into PLGA scaffolds provided an effective method of cell delivery in vivo, and the cells were able to differentiate into neurons and astrocytes [75]. PLGA has also been demonstrated to be a suitable scaffold for the culture of progenitor cells isolated from the hippocampus in terms of cell viability and differentiation [76].

Poly (ethylene glycol) (PEG), with high molecular weight versions being referred to as poly (ethylene oxide), is a commonly used polymer for biomaterial applications due to its ability to resist protein absorption. Other examples in the literature show the suitability of PEG scaffolds for engineering nerve tissue for the treatment of CNS disorders, such as Parkinson's disease or spinal cord injury. Work by Mahoney and Anseth demonstrated that NP cells could be cultured inside of PEG scaffolds and investigated the effects of adding bFGF (basic fibroblast growth factor) and collagen to such a system [77]. PEG scaffolds functionalized with poly-L-lysine to add sites for cell adhesion, and the NSCs seeded inside these scaffolds survived and were able to differentiate into mature phenotypes [78]. They used macro porous PEG scaffolds for the co-culture of NPCs and ECs to engineer nerve tissue. The addition of the ECs allowed for formation of a microvasculature inside of the nerve tissue when tested in vivo [79].

ii. *Peptide-based biomaterials*

Peptide-based biomaterials consist of short sequences of amino acids, which can produce self-assembling scaffolds. These scaffolds can potentially combine the functionality of protein-based scaffolds by using motifs derived from naturally occurring proteins with the reproducibility of synthetic scaffolds. Many of the peptide-based biomaterials can self-assemble into 3D scaffolds through the use of amphiphilic peptides, which form aggregates in aqueous solutions. The Stupp lab was one of the first groups to use such self-assembling scaffolds for promoting the differentiation of murine NPCs into neurons [67]. These scaffolds contained the peptide sequence IKVAV derived from LN and this sequence had been shown previously to promote neurite outgrowth [80]. The importance of selecting appropriate peptide sequence for promoting survival and differentiation of stem cells seeded inside of such a scaffold is also illustrated.

iii. *Ceramic-based biomaterials*

Ceramics are inorganic materials formed through treatment with heat and are often porous and brittle. They have crystalline structures and are used for a wide variety of applications.

iv. *Synthetic Polymers*

NSCs have also been cultured on numerous synthetic polymers, many of which have previously been used with other cell types for many applications including TE and controlled drug delivery [81]. Optimizing these materials may lead to the development of reproducible, scalable, nontoxic, and nonimmunogenic materials for in vitro expansion or differentiation, as well as in vivo implantation, of NSCs. In summary, fully synthetic, bio functionalized materials can support cell proliferation, and the addition of differentiating media leads to multipotent differentiation. Future work may explore the extent to which the substrate can guide cell lineage commitment. Furthermore, the use of thick gels can enable studies of the effects of matrix mechanics on NSC proliferation and differentiation [82].

d) Incorporating growth factors

Many of the aforementioned studies reported that cell survival was often poor and neuronal differentiation difficult to achieve from transplanted neural stem and progenitor cells. Therefore, investigators have found that they need to increase the complexity of their scaffolds to incorporate survival and/or differentiation factors. Neurotrophic factors have been incorporated into biomaterial based drug delivery systems to promote nervous tissue repair. For the past several years scientists have endeavoured to produce a multifunctional microsphere scaffold optimized for transplanting NPs into the TBI brain. NPs transplanted without a scaffold often remain clustered at the site of injection [83]. It is possible that the Fibroblast Growth Factor-2 that is delivered on the scaffold is facilitating the migration of the NPs [84]. Several of the groups listed above also have reported greater migration of transplanted NPs when delivered using a biomaterial scaffold.

e) Type of Biomaterial Scaffold Structures

From this list we may narrow down the types of scaffolds and the compositions of biomaterials optimal for use. Since an injectable scaffold is desired, this significantly limits the biomaterials available. Two common designs that would apply would be hydrogel systems and micro or nanoparticle systems. Hydrogels are liquid, but undergo gelation upon injection into the brain. Often times this is achieved through the change in temperature from ambient air temperature of ~ 21°C to the body temperature of ~ 37°C. Alternatively, micro or nanoparticles could be produced varying in configuration from microscopic spheres, irregular

particles or as fibers that are subsequently suspended in a liquid or gel for transplantation.

i. *Hydrogels*

Hydrogels are water-soluble polymer chain networks. They can absorb up to 99 percent water, which makes them a strong candidate for brain scaffolding. They have excellent nutrient and oxygen permeability, allowing cell survival in the scaffold [85]. Hydrogels can also be modified with proteins, GAGs, cytokines, drugs and other factors that will stimulate cell adhesion and/or growth [86]. Cells are readily encapsulated into hydrogels to replace missing autologous cells. Most importantly, hydrogels form in situ. As their name suggests, they gel following injection into tissues [87]. Furthermore, hydrogels possess elastic properties that are similar to those of natural brain tissue. Hydrogels can be created with low compressive moduli that tend to direct stem cell differentiation toward neural lineages [88]. A downside to hydrogels is that cellular migration and outgrowth is often poor due to its weak mechanical structure. In the CNS migration is essential for the initial formation of cortical architectural, for axonal growth and synaptogenesis and for white matter colonization by oligodendrocyte progenitors prior to myelination. Moreover, cells, and in particular neurons, do not extend their neurites through three-dimensional matrices efficiently [89]. Neurite outgrowth is best observed on 2-D rigid structures. This is due, in part, because neuronal growth cones require stiff substrates to pull on in order to grow or stretch. The filo podia of many cells have similar properties. Cells placed onto softer substrates are often round and maintain very short processes. Thus a hydrogel will not likely create a suitable environment for radial glial cells that naturally extend their processes long distances to the pial surface of the brain during embryonic development. Another disadvantage in using hydrogels is that their biodegradation is hard to control [90]. Because the majority of hydrogel systems focus on gelation and cytocompatibility, degradation rates are often sacrificed or difficult to manipulate.

ii. *Microspheres and micro particles*

Microspheres and micro particles on the other hand, possess a rigid surface structure, as opposed to the soft structure of hydrogels. Due to their rigidity, the tension that neuronal growth cones require can be created and maintained more easily on microspheres than on hydrogels. Furthermore, microspheres can be transplanted by syringe, whereupon they can mould to the injury dimensions. In addition, microspheres can be fabricated to encapsulate, immobilize and deliver specific growth or trophic factors to aid engraftment and survival of the transplanted cells [91]. A downside in using microspheres is that they may be more difficult to inject than hydrogels, since hydrogels are liquid within the injection syringe and gel upon contact with the brain (usually due to temperature differences) whereas, micro particles typically need to be suspended in an additional solution. Another limitation is the weak elasticity of micro particles. Stiffness might increase neurite outgrowth, although it might also decrease differentiation. Studies have shown that materials constructed with elastic properties similar to that of natural brain tissue are more likely to favour neuronal differentiation [92]. Microspheres are inferior in this regard.

V. Biomaterial Parameters for Directing Neural Stem Cell's Fate

The natural NSCs niche provides a model for designing a powerful artificial microenvironment to regulate the NSCs fate, which is essential for the CNS regeneration (Fig. 3). The cells, blood vessels, and the ECM in the NSCs niche work together to determine the fate of NSCs [5]. According to their different properties, biophysical and biochemical parameters can be concluded as two main stem-cell-regulatory cues in the NSCs niche. The biophysical parameters contain the mechanical properties and architecture of the ECM. The biochemical parameters are composed of the chemical and bioactive cues originating from the soluble cytokines and growth factors released by the adjacent cells, cell adhesion molecules, and ECM molecules. A functionalized scaffold for CNS TE and regeneration should be designed to bio mimic the NSCs niche to regulate fate of NSCs.

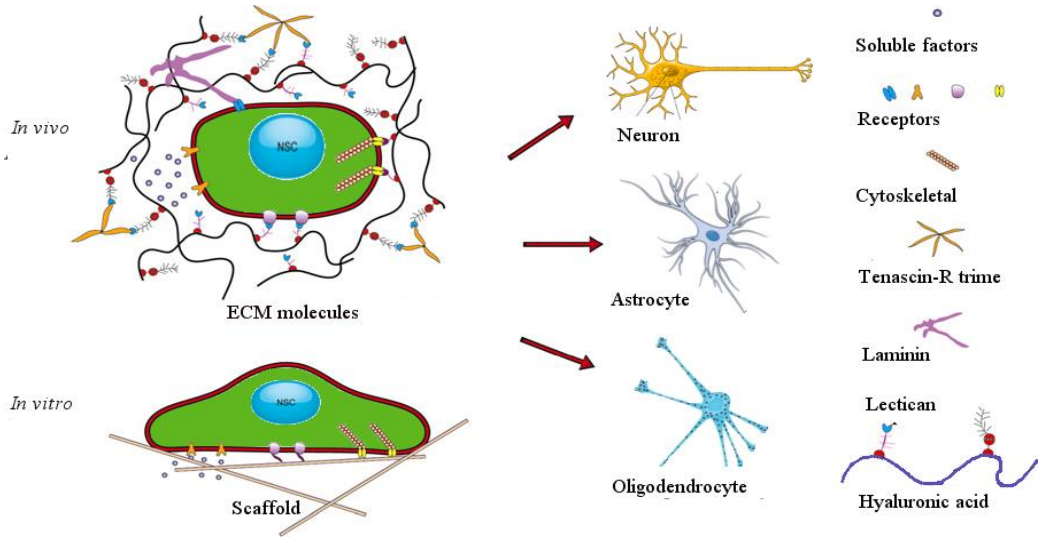

Figure 3 : NSC's Niches (in vivo or in vitro) Directing its Fate for CNS Regeneration

a) Biophysical parameters of designed biomaterials

During the development and throughout the life, the NSCs may be exposed to a variety of biophysical signals, such as tensile, compressive, shear, osmotic, fluid stresses, and so on so forth [93], that often directly provoke the dynamically remodelling of NSCs cytoskeleton networks. The topography and the mechanical property of the substrate are two major biophysical cues influencing NSCs state. For example, tension of the ECM can induce stretch of the cytoskeleton and nucleus through focal adhesions, while compression of the ECM can significantly alter local charge density and ion concentrations, potentially activating osmotically sensitive ion channels [94]. The topography of the substrate provides geometric cues to NSCs in the form of fiber diameter, length, and aligned/interwoven patterns, as well as surface micro/Nano topography. The cell shape was changed in response to different topography, which in turn influenced cellular signalling path-way and cell functions. Similarly, during the cell culture of NSCs in vitro, the biophysical property also momentously affects the adhesion, differentiation and proliferation of the NSCs [89].

i. *Topographic cues of the substrate*

The well-defined matrix architecture of stem cell niche, such as unique nano-fibrous characteristics of basal lamina membrane in different tissue relates to the specialized cellular functions, suggesting the importance of substrate topography in stem cell niche [95]. Previous studies demonstrated that micro-to-nanoscale substrate topography plays an important role in controlling the adhesion, proliferation and differentiation of NSCs. For instance, the rat NSCs (rNSCs) were cultured on polyether sulfone fiber meshes with average fiber diameter of 283 nm, 749 nm and 1452 nm respectively under the differentiation condition (1 mm retinoic acid and 1% foetal bovine serum) [96]. It was shown that rNSC had an oligodendrocyte differentiation on smaller fiber mesh (283 nm) and neuronal differentiation on larger fiber mesh (749 nm) compared with tissue culture plate. And the rNSCs showed lower viability on fiber mesh with diameter 1452 nm. Besides, when the rNSCs were cultured in serum free medium, higher degree of proliferation and cell spreading and lower degree of cell aggregation were observed as the fiber diameter increased. Besides the fiber diameter, the aligned substrate topography has also been proven to influence NSCs morphology and neuronal differentiation [97]. The random and aligned Polycaprolactone fibers with average diameter of 260 nm, 480 nm and 930 nm were yielded by electrospinning. NSCs elongated along the major fiber axis and a higher fraction of cells exhibited neuronal differentiation marker (Tuj1) compared with random matrix of similar dimensions. Aligned fibers could guide Tuj1þ cells to extend neurites several times of the length of the cell body along the axis of fiber alignment. While, such cells on the random fiber meshes showed randomly extended neurite pattern. Among the fiber meshes, 480 nm diameter aligned fibers supported highest fraction of neuronal differentiation.

In addition to the geometrical features at the nanoscale level, the higher level of organization of the substrate was also proven to be important for cell growth. Three kinds of chitosan scaffolds with different topologies (film, porous scaffold and multi microtubule conduit) were introduced to influence the fate of rat NSCs [98]. In the presence of Foetal bovine serum, chitosan film supported rNSCs to differentiate more easily into astrocytes, while rNSCs preferred to differentiate into neurons in chitosan multimicrotubule conduit and porous scaffold. In serum free medium with 20 ng/ml bFGF, rNSCs showed an increased

differentiation trend on all the types of chitosan scaffolds.

ii. *Mechanical properties of the substrate*

NSCs may encounter different mechanical microenvironments in adult brain, including blood vessels, layered cells structure, glial scars and grey and white matter, which may present variable modulus (#10 2 –10 3 Pa) [99]. Recent work indicates that primary neural cells exhibit mechanic-dependent neuronal morphological differentiation and glial survival [100]. NSCs differentiation into neurons increases when they are cultured on softer substrates with modulus similar to that of native brain. Saha et al. [82] developed a hydrogel culture system to assess the adhesion ligand presentation and material modulus from 10 to 10000 Pa on adult NSCs behaviour. In serum-free neuronal differentiation medium, scaffold with the physiological stiffness of brain tissue (500 Pa) favoured NSCs to differentiate into neuron. Under the mixed differentiation medium, soft gels with modulus of #100–500 Pa greatly favoured neurons, whereas harder gels with modulus of #1–10 kPa promoted glials. Besides, substrates with modulus of #10 Pa inhibited cell spreading, self-renewal and differentiation. Similar phenomenon was also observed on the behaviours of encapsulated NSCs in different mechanical properties of alginate hydrogels [92]. Different alginate hydrogels with modulus from 180 to 20000 Pa were obtained by varying the concentrations of alginate and calcium chloride. The rate of proliferation of NSCs decreased with the increase of the modulus of alginate hydrogels. The softest hydrogels which had similar modulus of brain tissues greatly enhanced the expression of neuronal marker b-tubulin III.

In addition to moduli of biomaterials, neural crest stem cells (NCSCs) were subjected to cyclic uniaxial strain to determine whether vascular mechanical strain modulated the differentiation of NCSCs into smooth muscle lineage [101]. Mechanical strain enhanced NCSCs proliferation and smooth muscle cell differentiation, and suppressed the differentiation into Schwann cells (SCs). Besides, sinusoidal inertia force (at 12.5 Hz, 25 Hz or 50 Hz of frequency, and 0.25 G or 0.5 G of acceleration amplitude) was also applied to cultured NSCs and could have effects on NSCs [102]. The mechanical vibration at 25 Hz is most effective on cell proliferation at 0.25 G. The enhancement of cell proliferation is probably caused by the suppression of apoptosis. The differentiation of the NSCs depends on acceleration amplitude and the mechanical vibration may maintain some properties of stem cells.

b) Biochemical parameters of designed biomaterials

In the NSCs niche, lots of biochemical factors work together or signally regulate the fate of NSCs. Recently, many in vitro studies showed that the NSCs were sensitive to their surrounding biochemical factors, such as different surface chemical groups and bioactive cues. NSCs exhibited morphological changes in response to different chemical groups at single cell level. In the downstream differentiation, –SO3 H favoured NSCs to oligodendrocytes, while –COOH, –NH2, –SH and –CH3 support the cells to differentiate into neurons, astrocytes and oligodendrocytes [103]. A type of mouse NPCs were also used to evaluate the effect of different chemical groups on cell behaviours and functions.[6] The chemical functional groups of –N2, –COOH and –SH could promote the secretion of glutamate decarboxylase.

Water soluble factors as a kind of small molecule proteins play an essential role in neural development, differentiation, survival, regeneration and function in both in vivo and in vitro [104]. Stem cell characteristics such as self-renewal and differentiation potential could be maintained by fibroblast growth factor (FGF). Neural and astrocytic differentiation can be induced by PDGF and cardiotrophin-1/ciliary neurotrophic factor, respectively [105]. Oligodendrocytic differentiation can be induced by thyroid hormone, T3. In addition, NSCs can also differentiate to mesenchymal cell lineages with the stimulation of bone morphogenic protein-4 [106]. Similarly, inhibitory factors produced by the reactive astrocytes at the site of injury could inhibit neurite extension outgrowth [107]. Scar or lesion-associated inhibitors encompass CSPGs, myelin associated glycoprotein and members of the ephrin and semaphorin families [108]. Nogo A is up-regulated or accumulated at the human lesion sites [109] and enhances the cognitive defects after experimental brain injury in rodent. Other myelin-associated molecules with neurite growth inhibitory activity play important roles in early development of neuronal maps [108]. To reduce the effect of inhibitory factors in neurogenesis, antibodies of these inhibitors were used to modify scaffold or injected into lesion sites directly. Nogo-66 receptor antibody was used to modify the HA scaffold to block Nogo-66 and simultaneously inhibit the formation of glial scar [110].

In the NSCs niche, the ECM is another vital factor for NSC growth and differentiation, in which intrinsic organization is necessary for influencing NSCs to guide restructuring in host tissues [111]. The neurotransmitters; acetylcholine [112] and dopamine [113] are also reported as biochemical cues of NSCs niche. Collagen is one of the most prevalent ECM molecules in human and other mammalian tissues [114] and is commonly used for immobilization of NSCs [115]. The successful culture of NSCs in three dimensional (3D) collagen gels was previously reported [59]. Little et al. [116] has summarized the use of collagen gels with NSCs in an excellent review. Neurosphere-forming NSCs had good survivals and proliferations in collagen hydrogels with addition of epidermal growth factor [115], and a part of the cells differentiated into neuronal

and glial lineage. A layer-by-layer printing of double-layered 3D collagen scaffold with NSCs and VEGF-containing fibrin gel was introduced to study changes of murine NSCs morphological and migration [117]. Cells migrated towards fibrin gel with VEGF and showed growth factor-induced morphological changes.

HA is another essential organization and structural component in the ECM of native tissues [118]. HA is particularly abundant in the foetal brain and surrounds immature neurons during differentiation in the spinal cord [119], which has been shown to significantly influence nerve regeneration, neuronal and glial development [120]. Recently, NSCs were photo-encapsulated into HA hydrogels and remained viable after encapsulation [88]. HA modified with polylysine and anti-NgRs were developed as scaffold for both neurospheres and single NSCs [121]. After 5 days cell culture, single dispersed NSCs were observed to differentiate into neurons and astrocytes, while neurosphere-forming NSCs migrated from their original aggregate and maintained the NSC phonotype. Incorporation of PLGA microsphere encapsulating brain-derived neurotrophic factor was further explored to promote NSC adhesion and proliferation [122]. NSCs would differentiate into neurons and astrocytes, and neurites extended along the wall of scaffold and formed extensive network. Other natural polymer biomaterials, such as alginate [123] gelation, chitosan and fibrin [124], have also been used to prepare 3D culture models for NSCs/NPCs. Alginate composition (the ratio of D - mannuronic and L -guluronic acid) will affect the NSCs survival and proliferation [123]. Fibrin will support NSCs to neuron differentiation and inhibit proliferation of astrocytes [124].

Although natural scaffolds offer important advantages for cellular receptors binding to regulate cell fate, they may transfer pathogen immunogenic molecules to stem cells [66]. In comparison, synthetic poly-peptide composed of biological building blocks offer advantages including none or little immune response, reproducible and scalable synthesis, and amenable to design and modification to achieve specific needs [125]. Many types of self-assembling peptides are designed to undergo spontaneous assembly through weak interactions into well-ordered interwoven nanofibers in water and rapidly form a gel-like 3D network, which is similar to the structure of natural ECM [126]. Since the building blocks are natural L -amino acids, these peptide scaffolds are chemically compatible with aqueous solutions and physiological conditions. Most importantly, specific cell interaction bioactive motifs could be conjugated into the peptides to enhance their interaction with NSCs. For example, IKVAK motif has been shown to encourage differentiation of NSCs into neurons [67]. RADA16-I is one of such poly-peptide biomaterials, which contains alternating amino acids that contain 50% charged residues [127]. The peptide could undergo spontaneous assembly into well-ordered interwoven nanofibers in water and rapidly form hydrogel with #10 nm fiber diameter, 5–200 nm pore size, and over 99% water content under physiological conditions.

VI. In Vitro Testing of Biomaterials to Improve Cell Survival

Extensive in vitro studies have developed 2 D surfaces or 3D gels for culturing either relatively uniform NSC populations or to a lesser extent CNS tissue explants. These efforts have focused on engineering substrates, sometimes in conjunction with growth or other soluble factors, which support or regulate specific cellular behaviours such as proliferation, differentiation into either neurons or glia, or neurite growth from neurospheres. The development of materials for in vitro cell culture is important for stem cell expansion and differentiation and can also serve as a first step towards design of materials that can support the survival and engraftment of stem cells in vivo upon implantation. Numerous studies have been performed in vitro to compare the efficacy of scaffolds for neuronal differentiation and survival [128] reporting the efficacy of stem cells transplanted together with a biomaterial matrix in TBI models.

VII. Mechanisms of Cell Death Following Cell Transplantation

Many factors contribute to cell death following cell transplantation including: time after injury; [129] distance from the transplantation site to the epicentre of injury; 10 state of the cells transplanted—differentiated or undifferentiated; [130] developmental state of cells transplanted—embryonic versus adult; [131] mode of cells delivered—single cells vs. neurospheres; [132] host immune response; [133] and phagocytocytic response of host [134]. The mechanisms of cell death following transplantation were investigated by Hill et al. [135]. The percent of surviving cells was found to be consistent irrespective of the number of cells injected. Necrosis was the leading cause of death for transplanted cells during the first 24 hours after transplantation, resulting in 6 times more cell death than apoptosis. Since apoptotic SCs diminished after the first 24 hours following transplantation, the authors postulated that apoptosis may have been initiated prior to transplantation in response to removal of serum, mitogens or ECM. During cell culture prior to transplantation, there are two main mechanisms that contribute to cell death: detachment of cells from their adherent surface and the removal of optimal growth factor concentrations. Therefore, when cells are prepared for transplantation as single cells, integrin–ECM interactions are lost and apoptosis is initiated. Cell

survival is further limited by the additional cell death induced by the environment at the injury site.

a) In vitro testing of biomaterials to improve cell survival

To increase survival, cells have been delivered in biomaterial scaffolds that are designed to provide the cells with a permissive microenvironment. This microenvironment includes chemical and physical cues designed to guide cell growth and integration with the host tissue [136]. In order to identify a suitable biomaterial for cell delivery, it must be first tested for cytotoxicity. For example, Puramatrix, which is a peptide hydrogel, was found to be cytocompatible at 0.25% but cytotoxic at 1% to human foetal NSCs, demonstrating that gel concentration is as important as gel composition [137]. Importantly, the effect observed with NSCs may be different for another cell type or even the same cell type from another species, thus the biomaterial has to be designed and tested for a specific cell type and injury. Combination strategies of biomaterials and growth factors have been studied for cell delivery. It is well understood that cell survival is improved in the presence of growth factors. However, when designing a biomaterial it is important to consider how the growth factors can be co-delivered with the transplanted cells to provide a sustained and localized release.

Synthetic materials such as PLGA have also promoted cell survival in vitro cultures of neural cells. PLGA has been investigated as it has good biocompatibility; is easily manufactured; and is believed to reduce scarring and cyst formations in models of SCI. NSCs grafted into PLGA slices of 2 mm depth were viable after 14 days of culture [138]. Electrospun poly (3-caprolactone) (PCL) nanofiber scaffolds promoted the in vitro survival of cortical cells. Similar to PLGA, PCL is biocompatible and has been investigated as a biomaterial to increase cell survival. Electrospun nanofibers can be modified to control the fiber alignment, diameter of the fibers and interfiber distance. Due to these tuneable parameters, it is proposed that electrospun nanofibers can provide a 3D environment to stimulate neural cells. To maintain a local supply of BDNF, PCL scaffolds were chemically modified with BDNF. Significantly greater cell survival was observed on PCL scaffolds immobilized with BDNF vs. PCL scaffolds with soluble BDNF or PCL scaffolds alone. However, despite increased cell survival, the proportion of apoptotic cells was not significantly reduced compared to 2D culture on PDL-coated glass coverslips [139]. While chemical modifications of scaffolds with growth factors can improve cell survival, methods to decrease cellular apoptosis on scaffolds must also be addressed. Poly (D-lysine) (PDL) is known to attract neurons and promote neurite outgrowth, and for this reason it has been used in numerous cell culture experiments. While the interaction with neurons is non-specific, PDL provides generically cell-adhesive substrates.

b) Improvement of cell survival using biomaterials in vivo

In vivo studies shows the promise of PGA, PLGA, and alginate materials in TE for the spinal cord and brain. Donor NSCs were able in some cases to aid in recovery from the injury and differentiate in vivo into different proportions of glial and neuronal cells. In addition, these observations were dependent on the chemical microenvironment created by the material, as well as its topological structure. Furthermore, host neuronsand glial cells were even able to incorporate into one of the scaffolds. Results were generally better when both the cells and the scaffold were used, showing the combined promise of biomaterials and NSCs in tissue regeneration.

Transplantation of alternative cell types has been proposed as a method to increase cell survival. Immune rejection decreases cell survival after transplantation. To minimize immune rejection of transplanted cells, the immunosuppressant cyclosporine was co-delivered with SCs [133] or neural stem progenitor cells (NSPCs) [140]. Co-delivery of 10 mg kg ῖ1 and 20 mg kg ῖ1 cyclosporine, respectively, enhanced cell survival in both cases; however, prolonged immunosuppression is problematic for the patient's overall health and thus this strategy is limited. While the ultimate goal of designing biomaterials in nerve regeneration is to control the endogenous and exogenous NSCs in vivo with the biomimetically artificial niche and achieve complete functional recovery of injured nerves. These extracted biophysical and biochemical parameters will actually elicit synergistic effects on directing NSCs lineage commitment, as well as the behaviours of many other types of tissue cells. More than that, peptide amphiphiles were another type of impressive synthetic polymers for nerve TE. Diblock copolypeptide amphiphiles have great promise as highly versatile and finely tunable hydrogels for potential therapeutic applications in CNS regeneration.

As reported that the SVZ was rich in a plexus of blood vessels that snaked along [141], and NSCs closely apposed to the LN-containing ECM surrounding vascular ECs. It was proved that normal SVZ cells in vivo tend to proliferate adjacent to blood vessels because the ECs can stimulate self-renewal and expand neurogenesis of NSCs by releasing soluble factors. The activated NSCs give rise to transit amplifying cells, which in turn generate neuroblasts in the SVZ niche. Neuroblasts differentiated from NSCs may migrate away from the niches and then underwent differentiation into certain lineages in a specific destination. It has been observed that the complex and far-reaching form of neural migration occurred even in the adult brain [142]. The neuroblasts migrate from the walls of lateral

ventricles to the olfactory bulb where they differentiate into local interneurons. While it is not very clear what the driving forces of neuroblasts migration away from SVZ are. One of the possible reasons is ependymal flow arisen by the formation of chemo repulsive gradients in SVZ.

Given the limitations of the endogenous NSCs, transplanting exogenous NPs into the injured brain has gained traction as a more appropriate solution to promote CNS regeneration. Yet this raises the issue of which cell type to transplant. Since brain injuries result in the demise of a range of different neuronal cell types as well as the astrocytes and oligodendrocytes that support them, the ideal cell would be one that has the capacity to produce a large repertoire of different neurons and glia. To date, several types of CNS progenitors as well as several NSC lines have been transplanted into the injured brain. Generally speaking, studies that transplanted progenitors or more differentiated cells have been less successful than studies using NSCs in replacing or rebuilding a neural circuit. Although there is no study directly comparing neuron, progenitor and stem cell transplantations, the vast majority of research on CNS regeneration focuses on the use of stem cell or early progenitor therapies. Lineage progression from a stem cell to a mature neuron is a process in which proliferation, migration and multipotential capacity decreases. Bliss et al. [143], transplanted human post-mitotic neurons (from hNT cell line derived from human teratocarcinoma) into a rat model of stroke and noticed low donor cell survival [144]. Although they saw neurite extension from hNT neurons, there was no migration. Poor cell survival in the cell preparation and during the transplantation process has been noted, especially when transplanting more committed cells into the unwelcoming milieu of a focal neocortical injury. Thus stem cell transplantation studies are more commonly observed in CNS therapeutics, whereas neurons and more differentiated cell types are generally avoided. Bone marrow stromal cells have been shown to improve outcome after brain injury and stroke [145], but the evidence suggests that the functional improvements obtained are not a result of cell replacement but are due to secreted factors that are neuroprotective.

These experimental studies suggest that many obstacles have been overcome in the grand quest to heal TBI with exogenous cell transplants, but the extent of neuronal cell replacement has still been variable and few of transplanted cells are retained [146]. Most of the transplanted cells either do not survive [147] or differentiate into glial cells instead of neurons [148]. This is a concern that the stem cells transplanted do not differentiate into reactive astrocytes that can contribute to glial scarring. Shear et al. [149] and Boockvar et al. [150], found that NG2 positive glial cells were produced upon transplanting NPs and Sun et al. [151], observed that the majority of the precursors that they transplanted became Olig2 positive cells (pre- sumably glia). Ma et al. [148], transplanted NPs (comprised of 4% NSCs) and reported that only 11% of the differentiated cells expressed a neuronal marker. Poor survival of NPs were observed when transplanted directly into the parenchyma following TBI [152].

i. *Improvement of cell survival using biomaterials for cell transplantation into the brain*

Synthetic biomaterials have better defined chemical structures and origins than naturally derived materials which can be advantageous. Polyglycolic acid (PGA) was investigated as a scaffold for NSC transplantation into injured brain. NSCs were transplanted alone or on PGA scaffolds (on which they had been cultured in PGA for 4 days) into brain 7 days post injury. The injury induced cavity filled with new parenchyma and there was minimal monocyte infiltration into the NSC–PGA complex at the interface between the complex and the host cortical penumbra whereas there was significant monocyte infiltration in the untransplanted infarct controls. Astroglial scarring was also reduced in PGA–NSC transplanted groups relative to non-transplanted infarcts. It was proposed that the reduction in astroglial scarring was due to either inhibitory factors produced by the NSCs, the mechanical features of the scaffold or the actions of the NSC–scaffold complex upon the host's injury response [111]. To support NSC survival following transplantation into brain, NSCs have been delivered on fibronectin-coated PLGA particles which provide sites for cell adhesion [70].

ii. *Improvement of cell survival using biomaterials for transplantation into the spinal cord*

Non-ECM derived natural materials have also been used in cell transplantation strategies to improve cell survival. Chitosan and chitin films were shown to promote cell survival in vitro [153] and investigated as cell guidance channels to promote survival of transplanted NSPCs. Three million brain-derived or spinal cord derived NSPCs were seeded in chitosan tubes coated with LN and implanted in the injured rat spinal cord after transection of the cord. Brain-derived NSPCs showed a significantly greater survival than spinal cord-derived NSPCs 12 weeks after transplantation, yet the increased cell survival did not translate to improved functional recovery or axonal regeneration [154]. In a combinatorial approach, ECs and NSPCs were co-delivered in a two-component biomaterial composed of an outer PLGA scaffold and an inner PEG/poly-L-lysine macro porous hydrogel to the injured rat spinal cord in a hemisection model of SCI. ECs were included to promote vascularisation within the transplant to increase cell survival. At eight weeks post-transplantation, the number of functional blood vessels at the lesion site for NSPC/EC + implant animals was

significantly greater compared to the NSPC + implant. Interestingly, the NSPC/EC + implant was the only group that reformed the blood–spinal cord barrier on the lesioned side of the injury epicentre. Surprisingly, increased vascularisation did not result in increased NSPC survival: at 8 weeks post-transplantation, NSPC survival was 8% in the NSPC/EC + implant group vs. 20% in the NSPC + implant group. The authors attributed this unexpected result to the different number of NSPCs originally transplanted. Since NSPCs produce a number of survival factors, [155] which promote cell survival and 4.5 times more NSPCs were transplanted in the NSPC + implant group than the NSPC/EC + implant group, the difference in cell survival may be attributed to the greater number of NSPCs secreting more survival factors [156].

VIII. Conclusions

Advanced biomaterials can provide a more biomimetic micro environment and significantly contribute to impaired nerve repair and regeneration, which have been an indispensable element in CNS regeneration. Although the discovery of NSCs opens the possibility to achieve CNS regeneration, it is still far from successfully clinical applications, since several challenges, such as precise control of NSCs self-renewal and lineage commitment, structural remodelling of differentiated NSCs, neural reconnection, and correct transmission of nerve signals, are still major obstacles to achieving functional recovery. Therefore, recreating NSCs regeneration niche by designing bioactive materials with complexity of biophysical and biochemical parameters is an important and fundamental prerequisite of CNS TE and regeneration. Each single biophysical or biochemical property of biomaterials will have direct regulatory effect on NSCs fate and should be considered when designing the applied scaffolds. Biomaterial scaffolds allow essential growth factors and other beneficial molecules to be delivered resulting in improved NP survival and repair. All above data indicate that this pNE coating can be a powerful tool to broaden the range of material choices for ex vivo expansion of hNSCs, an important goal for cell therapy.

In order to fully repair a brain lesion, the architecture of the regenerated neural parenchyma must recapitulate the structure of the adjacent host tissue. This is especially true in the case of the neocortex, a region of the brain that is frequently damaged by trauma. The neocortex is a laminar tissue with 6 layers where the neurons located within each layer have specific neurochemical properties and they receive inputs from specific brain regions. Moreover, they send their axons to other, highly specified targets. Thus, in regenerating the neocortex, the neurons that reside in the deeper layers of the cortex (layers 5 and 6) cannot be located in more superficial regions (layers 1 through 4), and vice versa. It has been documented that NPs have the ability to sense their surroundings and reorganize to appropriately fit a cortical layer [157], though it is not likely that transplanted NPs will do the same. Therefore, new biomaterial techniques will be required to ensure the appropriate differentiation and location of NPs within the specific brain region of interest. For the neocortex, we can envision creating a multi-layered scaffold, in which the different biomaterial layers govern the migration, differentiation and survival of appropriate laminar neurons. Alternatively, it might be possible to inject a biomaterial that would organize into a gradient and within this gradient, plasmids, proteins or other bioactive molecules would be organized to promote the systematic migration and differentiation of engrafted NPs [158]. Although it may be more difficult to achieve such a highly organized structure as required to repair neural circuits compared to other organ systems, utilizing TE applications to heal the injured brain remains a promising discipline for future studies.

NSCs are very promising for the treatment of neurodegenerative disorders and injuries of the CNS. Engineered materials containing natural and/or synthetic components can support the expansion and potentially in the future induce the lineage-specific differentiation of NSCs in vitro, with a variety of applications ranging from cell replacement therapy to in vitro diagnostics and screens. Furthermore, highly modular systems that enable the independent variation of mechanical and multiple biochemical signals have strong potential for the application of reductionist biology approaches to understand fundamental mechanisms of stem cell behavioural regulation. However, a number of challenges remain in the design of materials that are nonimmunogenic, scalable, mechanically tunable, and bioactive in their presentation of key regulatory signals to cells. Synthetic materials have considerable promise for offering these capabilities, although challenges remain in the development of synthetic analogues of complex biochemical signals such as ECM proteins. If these challenges can be overcome, however, bioactive materials can be designed to present a microenvironment that can not only support cells in vitro but also protect them in the harsh environment of a diseased or injured region of the CNS and thereby greatly aid stem cell-based regenerative medicine.

Although the combination of stem cells and TE is currently in the research phase and still far from clinical application, it has greatly enhanced the possibility of tissue regeneration. However, many different biomaterials such as nano- biomaterials that have not adapted for use with stem cell culture could be studied in near future. Stem cell transplantation presents a viable strategy for the repair of CNS injury. However, following transplantation cell death is prevalent and limits the efficacy of this technique. Two of the factors

that contribute to poor cell survival are anoikis and growth factor withdrawal. Biomaterials can be modified with cell adhesion proteins or motifs to improve cell attachment and minimize cell death caused by anoikis. Furthermore, survival factors, such as growth factors, can be encapsulated into the biomaterial to enhance cell survival. By using biomaterials to minimize cell death and promote cell integration with host tissue, more regenerative medicine strategies will be successfully translated to the clinic.

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