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REVIEW ARTICLE

Scaffolds for Cell Transplantation in Neurology—The Suitability of a Thermoreversible Gelation Polymer: Our Perspectives

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ABSTRACT

Clinical translation of cell-based therapies in neurology, especially the spinal cord injury and damage to the brain, have been marred by several hurdles [Dedeepiya VD et al Expert Opinion on Biological Therapy (In print)] and one significant among them is the need for a suitable biocompatible scaffold, which can retain the transplanted cells, give an active or passive support to the cells, enable their proliferation, differentiation when needed and integration into the local niche until the restoration of the damage are complete, without any adverse reactions to the vicinity or to any of the systems of the animal or human being where it is applied. Scaffolds for neurological applications need to be biocompatible, biodegradable, non-immunogenic, must provide contact guidance for neurite outgrowth, should have porosity for vascularization and cell migration. Several natural scaffolds like collagen, alginate, silk fibroin, hyaluronic acid, chitosan, etc. and synthetic scaffolds like poly (lactic acid) (PLA), poly (glycolic acid) (PGA), poly (lactic-co-glycolic acid) (PLGA), polyethylene glycol (PEG), poly (lactide-co-caprolactone) (PLCL) have been employed for cell transplantation in neurology primarily for nerve injuries and stroke. In this review, we briefly outline the different studies utilizing these scaffolds employed for cell transplantation in neurology and we document the suitability of a unique poly (N-isopropylacrylamide-co-n-butyl methacrylate) (poly NIPAAm-co-BMA) and polyethylene glycol (PEG)-based thermoreversible gelation polymer for cell therapy applications in neurology.

Keywords: Scaffolds, Neurology, Regenerative medicine, Cell therapy, Thermoreversible gelation polymer (TGP).

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INTRODUCTION

Regenerative medicine is an evolving specialty of medicine, which has potentials in addressing the organ/tissue failures that occur due to cellular dysfunction, damage or loss, by regenerating, rejuvenating, repairing or replacing the lost or damaged cells. Accomplishing the goals of regenerative medicine require expertise in cell culture, tissue engineering and suitable biomaterials as both *in vitro* and *in vitro* handling and manipulation of the cells used as tools in regenerative medicine are indispensable. Tissue engineering can be defined as 'an interdisciplinary field that applies the principles of engineering and life sciences toward the development of biological substitutes that restore, maintain, or improve tissue function or a whole organ'.[1] Tissue engineering principles commonly utilize a combination of cells, scaffolds and biologically active molecules. Cells can be stem cells, progenitor cells or adult cells with regenerative capabilities. Scaffold can be defined a structure capable of providing a three-dimensional environment that attempts to recapitulate the native microenvironment of the cells for them to grow and function properly. The study by Engler et al in which matrix elasticity specified the lineage differentiation of cells with softer matrices making the mesenchymal stem cells grown on them to differentiate into neurogenic lineage,

stiffer matrices giving rise to myogenic differentiation and rigid matrices giving rise to osteogenic lineage stresses the importance of microenvironment and scaffolds in cell-based therapies.[2] This brief review attempts to provide an overview of scaffolds for cell-based therapies in neurological conditions and disorders including our perspectives on suitability of a thermoreversible gelation polymer as a scaffold for cell-based therapies in neurology.

Scaffolds—An Overview

Inside the human body, cells are in constant interaction with the structural elements of the extracellular matrix (ECM) that transmit biochemical and mechanical signals between the extra- and intracellular environments.[3] Thus, arises the need for scaffolds in tissue engineering and regenerative medicine to mimic this microenvironment *in vitro* and *in vivo* to provide the right cues for normal physiologic regeneration of the cells. For optimal regeneration, scaffolds should recapitulate ECM providing structural support, sufficient rigidity and elasticity to help cells to give rise to the right kind of tissues and also provide bioactive cues to the cells.[4] Scaffolds must also possess low antigenicity. The ECM is a complex network comprising of structural proteins like collagen and elastin, proteoglycans, e.g. chondroitin sulfate and functional/regulatory proteins like the growth factors, cytokines, etc.[3] Hence, scaffolds must either be ECM analogs or must be able to recapitulate the characteristics of this complex ECM. Scaffolds may be natural or synthetic based on their origin; conductive, i.e. provide a passive support for the cells to grow, or inductive, i.e. contain bioactive molecules. Further scaffolds may be biodegradable or nonbiodegradable.[3] Chan et al summarized the scaffolding approaches into the following four categories: (i) Pre-made porous scaffolds (ii) decellularized extracellular matrix, (iii) extracellular matrix secreted by the cells themselves during growth, (iv) cell encapsulated into self-assembling hydrogels. Premade porous scaffolds may be natural or synthetic.[4] Natural scaffolds include ECM from allografts and xenografts or may be in the form of the components of the ECM, such as proteins, polysaccharides, lipids and polynucleotides. Natural protein origin polymers include collagen, gelatin, silk fibroin, fibrin and other proteins like elastin. Among protein-origin polymers, collagen is the most widely used scaffold for tissue engineering applications. Polysaccharidic polymers include chitosan, alginate, hyaluronan, starch and chondroitin sulfate. Their advantages are that they are non-toxic; they have good hemocompatibility properties; and they are relatively inexpensive compared to scaffolds like collagen.[5] Naturally-derived polymers like collagen have the advantage of mimicking several properties of native ECM, but their disadvantages include batch to batch variation, difficulty in processing and sterilization.[5] Another potential drawback is the immunogenicity and pathogenicity when derived from animal sources.[4] Synthetic scaffolds are either made up of inorganic materials like bioglasses or organic materials like polymers. Synthetic scaffolds include materials like poly (lactic acid) (PLA), poly (glycolic acid) (PGA), their copolymer, the poly (lactic-co-glycolic acid) (PLGA), poly (ethylene oxide) (PEO), poly (vinyl alcohol) (PVA), poly (acrylic acid) (PAA), polyethylene glycol (PEG), poly (propylene furmarate-co-ethylene glycol) (P(PF-co-EG)), Poly (glycerol sebacate) (PGS), polypeptides, etc. Synthetic scaffolds are attractive biomaterials for tissue engineering as their chemistry and properties are controllable and reproducible. Further, they offer potential solution for the immunogenicity and pathogenicity associated with naturally-derived scaffolds.[6] Though cell adhesion and biocompatibility may be lesser compared to naturally-derived materials, the surface of these scaffolds can be modified to improve these properties.[4] Decellularized ECM scaffolds include allogenic and xenogenic ECM in which decellularization removes the allogenic or xenogenic cellular antigens from the tissues which are the sources for immunogenicity after implantation, but the properties of ECM are preserved. The disadvantages are the possibility of nonhomogeneous distribution of cells after seeding and incomplete removal of cells during decellularization, which may lead to immune reactions after implantation.[4] Cell sheets with self-secreted ECM are components in which cells when grown on thermoresponsive polymer surfaces secrete their own ECM during growth. These cell sheets can be detached by altering the temperature of the polymer once the cells reach confluence. The disadvantage with this method is it is difficult to construct thicker tissues. Cell encapsulation in self-assembled hydrogel matrices is an approach in which cells are embedded in hydrogels which are highly hydrated polymer materials with water content > 30% by weight. These hydrogels may be naturally derived or synthetic. Encapsulation in hydrogels enable injection of the cell scaffold during transplantation, as the biomaterials used for encapsulation can self-assemble from liquid monomers to solid polymer meshwork upon initiation by altering conditions like pH, temperature, ionic strength, etc.[4] Thus, the cells can be injected into the site of interest and the liquid monomer can then be initiated to form the polymer hydrogel *in situ*. Its advantages include homogeneous cell distribution in the liquid phase and good cell viability.[4]

Scaffolds for Cell Transplantation in Neurology

Cell transplantation in neurology comprises the approaches that are aimed at promoting nerve regeneration to repair damage caused to nerves by an injury or for enabling repair and regeneration in neurological conditions like stroke, Parkinson's disease, multiple sclerosis, Alzheimer's

disease, etc. Regenerative approaches in neurology should be highly meticulous compared to other tissues in the body owing to the highly complex nervous system. Also, mechanisms of nerve injury and repair have not been fully unraveled yet.[7] The ideal properties of a scaffold for use in nerve regeneration are biocompatibility, less inflammatory potential, controlled biodegradability, ability to provide contact guidance for neurite outgrowth, have porosity for vascularization and cell migration.[8] Nerve injury may be peripheral or central nervous system (CNS) injury. CNS injuries include spinal cord injury (SCI) and traumatic brain injury (TBI). In CNS repair, it is essential that the scaffold must help reduce glial scar formation while permitting cell adhesion for regenerating neurons to extend axons into the injury site. Also, the scaffold must serve as a bridge to guide the axons to restore connections with the already existing innervations for promoting functional recovery.[9] Several scaffolds have been employed in various translational studies that investigated their suitability for cell transplantation in nerve injury. However, in this review, only selected studies of specific scaffolds are being documented as reviewing all the scaffolds employed so far will be beyond its scope. Considering natural scaffolds, collagen scaffolds, which are highly biocompatible, have been impregnated with cells and applied in animal models of SCI,[10,11] TBI[12,13] and peripheral nerve injury[14,15] with positive outcome on axonal regeneration and functional recovery. Qu et al's study reported that culturing human marrow stromal cells (hMSCs) with collagen scaffolds caused upregulation of genes involved in angiogenesis, neurogenesis and signal transduction. These in turn, the authors state, may contribute to tissue repair and functional recovery after TBI.[12] Inclusion of therapeutic agents like Nogo receptor, chondroitinase ABC (ChABC), collagen binding neuroprotective protein, EGFR neutralizing antibody, etc: have shown to improve the efficacy of these collagen scaffolds.[10,11] Collagen has also been combined with synthetic scaffolds like polymer poly (lactic-co-glycolic acid) (PLGA) to improve the strength.[16] The disadvantage with collagen is the risk of immune response if cross species transplantation is employed.[9] A study that compared the ability of methylcellulose, collagen, and a commercial available laminin-containing ECM preparation (Biomatrix), to deliver drug in peripheral nerve repair showed that methylcellulose was the most suitable of these three materials.[17] In a study that combined collagen with Schwann cell, the strategy has not been able to show a successful regeneration compared to controls with autologous grafts.[18] Thus, collagen, though, has excellent biocompatible properties further studies are needed to improve its efficacy as a scaffold for nerve regeneration. A study by Liu et al on human umbilical cord blood-derived mesenchymal stem cells (hUCB-MSCs) seeded in acellular spinal cord (ASC) scaffolds promoted long-distance axon regeneration and functional recovery in spinal cord injured rats.[19] Next are the studies on application of Matrigel for cell transplantation in nerve injury. A combination of Matrigel and neural-induced mesenchymal stem cells (NMSCs) has shown to improve hindlimb function in dogs with SCI.[20] In another study transplantation of human neural cells with the Matrigel scaffold improved the outcome from focal cerebral ischemia in rats even after delayed application.[21] Chitosan is a biodegradable scaffold in use in various biomedical applications, but does not adequately support neural cell attachment and proliferation. Immobilizing poly-D-lysine (PDL) onto chitosan has been proposed as a potential strategy to promote cell adhesion and neurite growth for nerve tissue engineering.[22] Chitosan-coated conduit has been reported to perform multiple functions like inhibiting inflammation, inducing neurosphere cells from adipose-derived stem cells and combining these cells with the chitosan conduits has shown to facilitate nerve regeneration in rat sciatic injury.[23] Bone marrow stromal cells (BMSCs) loaded chitosan conduits have been able to enhance motor functional recovery and remyelination in complete transection injury in the rat spinal cord.[24] In Cho et al's work, Chitosan has been reported to seal compromised nerve cell membranes, thus, acting as a neuroprotector following acute spinal cord trauma.[25] In 14 patients with chronic paraplegia caused by spinal cord injury, peripheral nerve grafts combined with chitosan-laminin scaffold along cotransplantation bone-marrow-derived mesenchymal stem cells containing them in a chitosan-laminin paste has been shown to cause neurological improvement. However, chitosan disintegration causing postoperative seroma formation was reported as a complication.[26] In the transected spinal cord of rats, alginate scaffold has been shown to contribute to the reduction of the barrier caused by connective tissue and reactive astrocytic processes. Also, alginate has been shown to serve as a scaffold for the regenerating axons and elongating astrocytic processes.[27] Calcium alginates though biocompatible and non-immunogenic were unable to support longitudinally-oriented growth and this was overcome by developing alginate-based highly anisotropic capillary hydrogels (ACHs). The ACH hydrogels after implantation into acute cervical spinal cord lesions in adult rats integrated into the spinal cord parenchyma without eliciting major inflammatory responses and induced directed axon regeneration across the scaffold. Including adult neural progenitor cells (NPCs) with such ACH scaffolds has been reported to be a promising strategy for nerve repair.[28] Silk from the silkworm, Bombyx mori, which has been in use for biomedical suture applications for centuries, has been investigated as a biomaterial scaffold for regenerative applications.[29] The advantages of

silk are their very high strength and excellent elasticity. Undesirable Immunological reactions associated with silks reported in studies diminished the interest in silk, but later identification of sericin as the source of immunological problems revived the interest in silk-based scaffolds.[30] Silk fibroin conduits have been reported to promote neurite growth, enhance restoration of nerve continuity and functional recovery in nerve injury.[31-33] Hyaluronic acid (HA) hydrogel with good biocompatible properties has been reported in Hou et al's study to have similar mechanical properties and rheological behavior to the brain tissue and implantation of HA hydrogel into cortical defects mechanically created in rats demonstrated cell infiltration and angiogenesis along with inhibition of scar formation.[34] A potential disadvantage with HA is the limited cell adhesion and blending HA hydrogels with other mate-rials to promote cell adhesion has been devised as a solution.[35] HA hydrogels modified with laminin showed promotion of neurite extension.[34] Transplantation of adult brain-derived neural stem/progenitor cells (NSPCs) in a hydrogel blend of hyaluronan and methyl cellulose (HAMC) modified with recombinant rat platelet-derived growth factor-A (rPDGF-A) into a subacute SCI rat model has demonstrated significant reduction in cavitation with improvement in graft survival.[36] Moving on to synthetic polymer scaffolds for cell transplantation in nerve injury, synthetic polymers have the advantage of improved mechanical properties, less immunogenicity compared to natural polymers, controllable and reproducible manufacturing processes, but their biocompatibility is relatively lesser. Synthetic scaffolds may be biodegradable or nonbiodegradable. Nondegradable materials used to fabricate nerve guidance channels include silicone, polyacrylonitrile/polyvinylchloride (PAN/PVC), poly (tetrafluoro-ethylene) (PTFE), and poly (2-hydroxyethyl methacrylate) (PHEMA). Some degradable scaffolds are poly (glycolic acid) (PGA), poly (lactic acid) (PLA), poly (lactic acid-co-glycolic acid) (PLGA), and poly (lactide-co-caprolactone) (PLCL).[37] For neurological applications, degradable synthetic polymers are preferred as long-term effects like inflammation and scar may compromise nerve function. The kinetics of degradation depends on factors like molecular weight, crystallinity, etc. and in case of poly (lactic acid) (PLA), poly (glycolic acid) (PGA) co-polymer, the poly (lactic-co-glycolic acid) (PLGA), degradation rate depends on ratio of glycolic acid to lactic acid, etc.[38,39] Synthetic scaffolds used in neurology include PLA, PGA, PLGA, PGS, PEG, etc. On PLGA scaffolds, which have been employed in several studies of cell transplantation for nerve repair[40-43] the study by Xia et al reported that neural stem cells (NSCs) can survive in PLGA and cografting them with Schwann cells (SCs), in hemitransected rat spinal cord made the NSCs to differentiate toward neurons *in vivo*.[40] The authors further reported that this strategy might have contributed to formation of synaptic connections. However, the study concluded that the regenerating axons had limited contribution to motor function recovery. The disadvantage with these PLA, PGA and PLGA polymers is their brittle nature, lack of functionalities other than end-groups for chemical modification, bulk rather than surface degradation and decreased cell adhesion. Modification of their structure by incorporating amines from which peptides can be tethered to modulate cell behaviors and adhesion has been suggested as a potential solution.[38] A long-term *in vivo* experiment has proven the regeneration of axons after transplantation of poly ε-caprolactone (PCL) conduit in a rat sciatic nerve injury model.[44] In another study, MSC transplantation using a PCL nerve guide in mice model of sciatic nerve injury has exhibited sciatic nerve regeneration and neuronal survival.[45] Next is PEG whose administration has shown to be useful for improving behavioral outcomes, and distal axonal density after autografting in rat sciatic nerve injury model[46] and application of PEG has been reported to rapidly repair nerve membrane damage associated with severe spinal cord injury in adult guinea pigs.[47] In rats, treatment with PEG after traumatic brain injury has been observed to reduce beta-amyloid precursor protein accumulation in degenerating axons[48] as beta-amyloid precursor protein accumulation is detrimental which might lead to cell death after such injuries. Conova et al's study on injectable hydrogels based on poly (N-isopropylacrylamide) (PNIPAAm), lightly cross-linked with PEG or methylcellulose (MC) showed them as an effective vehicle for cell delivery, supporting graft survival in SCI.[49] These scaffolds also showed no inflammatory response which is highly advantageous for nerve repair. A study that compared Schwann cell-loaded scaffolds made up of PLGA, poly (ε-caprolactone fumarate) (PCLF), oligo (polyethylene glycol) fumarate (OPF) hydrogel or positively charged OPF (OPF+) hydrogel in transected rat thoracic (T9/10) spinal cord model concluded that all polymers supported the axonal growth, but axons regeneration was relatively higher with OPF+ polymers.[50] Thus, various natural and synthetic scaffolds have been employed to contain the cells in the lesion site and provide an optimal microenvironment for regeneration and repair in nerve injuries. Considering conditions like Alzheimer's disease, Parkinson's disease and multiple sclerosis, studies of scaffolds for cell transplantation have not been reported to our knowledge as these conditions are generalized and affect the neurons in various parts of the body and transplanting cells with scaffolds might not be feasible. However, scaffolds can be employed to enhance the neuronal regeneration *in vitro* and these neurons grown in scaffolds can then be transplanted for regenerative therapy

for these conditions. In cerebral ischemia or stroke, studies on cell transplantation using scaffolds, though limited compared to nerve injuries, nevertheless have been reported. Implantation of a gelatin-siloxane hybrid scaffold derived from the integration of gelatin and 3-(glycidoxypropyl) trimethoxysilane has shown integration with CNS tissue, cell migration, angiogenesis and dendrite elongation.[51] Bible et al reported that plasma polymerized allylamine (ppAAm)-treated PLGA scaffold particles can provide structural support for neural stem cells transplanted into stroke-induced brain cavities.[52] In Yasuda et al's study survival, migration, and differentiation of the BMSCs transplanted into the cortical lesion in rats was enhanced by an injectable fibrin matrix.[53] Having briefly explained about the various scaffolds for cell transplantation in neurology, we document the suitability of a thermoreversible gelation polymer (TGP) hydrogel composed of thermoresponsive polymer block [poly (Nisopropylacrylamide-co-n-butyl methacrylate) (poly NIPAAm-co-BMA)] and the hydrophilic polymer block [polyethylene glycol (PEG)] (commercial name: Mebiol gel) as a scaffold for cell transplantation in neurological applications in this review.

Thermoreversible Gelation Polymer (TGP)

This thermoreversible gelation polymer (TGP) hydrogel was reported by Yoshioka et al in the 90s as a unique hydrogel of an aqueous solution of a co-polymer composed of thermoresponsive polymer block [poly (Nisopropylacrylamide-co-n-butyl methacrylate) (poly NIPAAm-co-BMA)] and the hydrophilic polymer block [polyethylene glycol (PEG)] (commercial name: Mebiol gel). They reported that the aqueous solution of the co-polymers turned into hydrogel upon heating and back into fluid upon cooling with the sol-gel transition temperature being 35º C. Such thermoreversible sol-gel transformation has been reported for methyl-cellulose systems, but what makes this TGP unique is that this PNIPAAm-PEG system exhibits the sol-gel transition while being transparent and without syneresis, i.e. separation of water and the transformation occurs rapidly without hysteresis.[54,55] The sol-gel transition helps cells to be embedded in TGP when they are in the liquid state at lower temperatures and then cultured 3-dimensionally in a hydrogel state at 37°C.[56] Madhavan et al studied the growth of continuous culture cell lines of Vero, HEp-2, HeLa, BHK-21, McCoy and CHO cell lines embedded in this TGP and showed that the cells grown were healthy, without showing any signs of degeneration or cytotoxicity in the presence of the TGP and the pH was maintained during the 7 days study period. Their study concluded that TGP was not toxic to any of the cell lines studied based on the observation that nearly 90% of cells in each cell line migrated out of the gel compared to increased number of dead cells in the control.[57] The TGP has been reported to support the three-dimensional growth of many cell types like corneal limbal stem cells,[58] hepatocytes,[59] chondrocytes,[60] embryonic stem cells, induced pluripotent stem (iPS) cells[61] and bone marrow mononuclear cells.[62] TGP has also been used for microencapsulation of islet cells[63] and as a wound dressing.[64] Growth of neuroblastoma cell line SH-SY5Y in TGP was compared with type I collagen and Matrigel. The results showed that in type I collagen gel or in Matrigel, the cells exhibited an elongated shape with many processes while in TGP, the colonies formed were round and there was no interaction between the matrix and cells. Neural stem cells have been reported to form single-cell-derived homogeneous spheroids in TGP.[61] TGP has shown to maintain stem cells in an undifferentiated manner for a longer period of time.[65] Nagaya et al's study on TGP poured into a penetration lesion of a rat liver concluded that TGP induces the emergence of hepatic stem cells in the partially injured rat liver.[66] Sudha et al demonstrated the growth of cadaver human corneal limbal epithelial cells cultivated in Mebiol Gel and they showed that the cells grown expressed both limbal and corneal phenotype.[67] TGP does not allow the growth of fibroblasts. This ability of TGP allowing corneal epithelial cells to grow without allowing fibroblasts made Sitalakshmi et al to study expansion of autologous corneal epithelial cells derived from limbal biopsies (before experimentally inducing limbal stem cell deficiency (LSCD)) from rabbits, in TGP and the cells were harvested three weeks later by reducing the temperature. These cells were then transplanted to the rabbits with the LSCD and the results proved that autologous limbal epithelial cells grown in TGP could help successfully reconstruct the surgically damaged ocular surface and the corneal epithelium in these LSCD rabbits.[58] Thus, TGP allows growth of corneal limbal stem cells without employing biological materials or animal 3T3 feeder layer that have the risk of biological contamination.[58] Also, cells grown can be harvested by just lowering the temperature without the need for enzymatic digestion. With reference to neurological applications, TGP has been intralesionally applied along with stem cells in animal models. William et al's study on intralesional transplantation of autologous bone marrow mono nuclear cells (BMMNCs) seeded TGP combined with intravenous cell transplantation in canine with traumatic spinal cord injury is unique as it was not an experimentally induced SCI, but rather was an injury due to an automobile accident, thus, recapitulating injury in natural settings. The results showed that recovery of motor and sensory functions occurred on the 53rd day after cell transplantation and animal had satisfactory ambulation on the 133rd day.[62] Thereafter, the life style of the animal was gradually restored to normalcy and this status was main-

tained for nearly 2 years of follow-up. In a study in which mice were subjected to permanent middle cerebral artery occlusion, a comparison of three groups, group A (pouring of phosphate buffer saline (PBS) alone in the lesion cavity); group B (transplantation of BMSCs in PBS); and group C (transplantation of BMSCs embedded in TGP) revealed that the engraftment of transplanted cells were significantly higher in the group C (BMSC-TGP construct) as compared to the group B (BMSCs in PBS). Further, the study also reported that the TGP hydrogel disappeared almost completely and there was no inflammatory reaction on the brain surface at four weeks after transplantation.[56] In a recent study, it was reported that TGP culture enables long-term, serial expansion of human pluripotent stem cells (hPSCs) including human embryonic stem cells and iPS cells, with a high expansion rate and purity. Karyotyping revealed that the hPSCs retained pluripotency and normal karyotype even after long-term culture in TGP.[68] Also, 3D-directed differentiation of the hPSCs into several cell lineages including dopaminergic neuron progenitors with high yield was possible using the TGP culture method.[68] TGP has been applied clinically in human in a study where autologous bone marrow mononuclear cells (BMMNCs) embedded in the TGP was applied for periodontal regeneration in a patient with advanced periodontitis and severe horizontal bone loss. The 6 months follow-up revealed improvement in clinical parameters. Three years follow-up established the safety and efficacy in terms of improvement in vertical and horizontal bone height.[69] Thus, the safety and efficacy of TGP as a valuable scaffold material for cell therapy have been established in the various *in vitro* and translational studies.

DISCUSSION

In vivo retention of transplanted cells in the lesion site is an important parameter that determines the outcome of cell transplantation. *In vivo* retention becomes even more a diffi-cult task in neurological applications owing to the highly complex nature of the nervous system. Animal models have demonstrated long-term *in vivo* retention of cells in CNS injury, even up to 15 months.[70] However, the situation is different clinically and hence strategies to promote *in vivo* retention and to provide appropriate micro-environment for the cells to grow and enhance regeneration become essential. Scaffolds serve to provide this optimal micro-environment for cells to grow and promote repair. Several naturally-derived scaffolds like collagen, alginate, matrigel, silk fibroin, hyaluronic acid and synthetically-derived scaffolds like PGA, PLA, PLGA, PCL, PEG, etc. have been employed in various neurological applications as described earlier in this manuscript. Natural scaffolds have good biocompatibility but poor mechanical properties and the risk of immunogenicity when derived from animal sources. Synthetic scaffolds have good mechanical properties, but relatively less biocompatibility and cell adhesion properties. TGP is unique among synthetic scaffold by virtue of its excellent biocompatibility as observed from the various studies in which different kinds of cells could be grown in TGP without any interaction or toxicity. In Hishikawa et al's study collagen altered the gene expression profile of mesenchymal stem cells, while TGP did not.[71] This property was also proven in Lei et al's study in which hPSCs maintained normal karyotype even after long-term culture in TGP.[68] TGP allows us to culture cells without incorporating animal-derived biological materials, which pose the risk of biological contaminants. TGP is transparent, which allows visualization of cells *in vitro*. TGP is biodegradable,[56] which is an important requisite for neurological applications and it is non-immunogenic.[56] The ability of TGP to maintain the viability of the cells for a longer period of time[65] along with its ability to direct the differentiation of cells into various cell lineages under appropriate conditions[61,68] signifies it as a highly versatile scaffold which can be used for growing the different kinds of cells for the various neurological applications. TGP is porous and allows diffusion of nutrients. TGP has cleared intraperitoneal toxicity studies in rats, reverse mutation studies, *in vitro* chromosomal aberration tests and antigenicty tests (data unpublished). Takeuchi et al have reported that tissue engineering using cell sheets show relatively higher survival *in vivo* than cell suspensions as proven by *in vivo* bioluminescence imaging.[72] Cell sheets can be fabricated using temperature-responsive culture surfaces in which cells grown on them can be detached by merely changing the temperature.[73] Regeneration using cell sheet has been demonstrated to be feasible in the regeneration of esophageal mucosa,[73] for cardiac disease,[74] periodontal regeneration,[75] bladder augmentation[76] regeneration of the corneal epithelium,[77] reconstruction of the endometrium,[78] to mention a few. As TGP is a thermo-responsive gel and cells can be detached by lowering the temperature,[58] it can be tried in appropriate combinations with cell sheet based methodologies of cell culture and tissue engineering. TGP supports cell proliferation, allows cell differentiation in the desired direction, is biodegradable, does not have the risk of biological contamination with animal proteins, non-teratogenic and nonmutagenic, which are characteristics outlined by Dedeepiya et al as requisites for an ideal scaffold for cell therapy applications in SCI.[79] Relatively more neural precursors being present when BMSCs are transplanted with TGP in Osanai et al's study[56] further emphasizes the role of TGP for cell transplantation applications in neurology. Jones et al's[80] suggestion that it is the large animal models that may actually serve as human equivalents to study treatment strategies for SCI makes us to mention at this juncture the study by William et al on BMMNC transplantation in

TGP in Canine which had a road traffic accident making the injury equivalent to what happens in humans.[62] The follow-up of two years establishing safety and efficacy further makes us to suggest the TGP as a highly suitable scaffold for neurological applications. Though TGP has been applied clinically in humans with safety established,[69] it has not yet been applied for neurological conditions in humans. Studies in the future are essential in this regard as application in humans will throw light on the various known-unknowns that still needs to be addressed for making TGP a scaffold for routine cell therapy applications in neurology.

CONCLUSION

This review has briefly outlined the various studies done on different scaffolds employed for cell transplantation in neurology. Further, we have documented the suitability of a an unique PNIPAAm-co-BMA-PEG-based hydrogel, the TGP for cell transplantation in neurology based on earlier studies, which have established the safety, efficacy and characteristics of TGP to support the regeneration of cells *in vitro* and *in vivo*. After appropriate validations, further clinical studies in large number of patients are required to make the transplantation of cells embedded in TGP for neurological applications, a routine clinical procedure in the future.

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