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Processing and Templating of Bioactive-Loaded Polymeric Neural Architectures: Challenges and Innovative Strategies

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Additional information is available at the end of the chapter

1. Introduction

The chapter highlights the current drug delivery strategies and phenomenon involved in the design of neural architectures employed for neuropharmaceutical applications attempting to replicate the nervous tissues. We seek to give an overview of the current drug delivery approaches such as degradable/diffusion-based delivery systems, affinity-based delivery systems, and immobilized drug delivery systems used to generate bioactive-release from scaffolds for neural tissue engineering applications. We talk about the combinatorial approach being employed in recent years in the form of "new generation of multifunctional biomaterials" which are - able to mimic the molecular regulatory characteristics by providing a three-dimensional architecture representing the native extracellular matrix and also able to sequester and deliver biomolecular moieties in highly specific manner. A special focus will be given to the micro- and nano-structured scaffolds which have been proved to be effective in axonal repairing, in guiding functional neurogenesis and in controlling stem cell differentiation. Emphasis will be given to the processing requirements for synthetic and natural polymers and biomaterials for producing potential scaffold materials as well as to the templating of the target drugs with their benefits and drawbacks. A detailed discussion will be provided for certain specialised and recently developed architectures in the form of injectable matrices, electrospun nanofibers, and hydrogels. Injectable scaffolds provide a very unique advantage of being a non-invasive approach for neural tissue engineering as further damage to the soft neural tissue due to surgery can be prevented. Current injectables in tissue engineering consists of gelation methods based on in situ chemical polymerization and crosslinking, photo-initiated polymerization and crosslinking, thermogelling injectable systems, ionic crosslinking, and self-assembling. Injectable scaffolds also provide opportunities

for drug delivery in the form of composite materials with embedded micro- and nanoparticles. Additionally, the easy customization of the injectable architectures by modification of solidification, porosity, biofunctionalization, mechanical properties, and biodegradation is an added attribute. The electrospinning process, on the other hand can be used to engineer neural architectures having micro to nanoscale topographical cues as well as micromeritic properties similar to the extracellular matrix. Enhanced cell attachment, drug loading, and mass transfer properties can be easily obtained by changing the surface-to-volume ratio and by controlling the bulk mechanical properties of electrospun scaffolds where the fibers can also be oriented or arranged randomly, to regulate the biological response to the scaffold. Drugs ranging from anti-inflammatory and antibiotic agents to growth factors, proteins, DNA, and RNA, and even living cells can be incorporated into electrospun scaffolds. Interventionally, the current tissue engineering and therapeutic approaches pertaining to neural injuries will be analysed and critiqued in detail with respect to various strategies, perspectives, challenges and expanding opportunities. Considerable efforts have been carried out to enhance the properties of tissue engineering architectures via surface engineering and surface functionalization for providing an extracellular matrix mimicking environment for better cell adhesion and tissue in-growth. These modifications can be carried out through plasma treatment, wet chemical method, surface graft polymerization, and co-fabrication of surface active agents and polymers. These modifications can further be designed and customised to release bioactive molecules, such as growth factors, DNA, or drugs, in a sustained manner to facilitate tissue regeneration (**Figure 1**) [1]. Target molecules are usually loaded on the surface of scaffolds by physical adsorption, nanoparticles assembly on the surface, layer-by-layer multilayer assembly, and chemical immobilization. Tissue engineering scaffolds can deliver bioactives via certain signals such as interspersed signals, immobilized signals and signal delivery from cells and hence act as specialised and desired controlled release matrices.

Material choices for the fabrication of neural architectures will be elucidated among, but not limited to, synthetic polymers such as poly(lactic acid), poly(glycolic acid) and co-polymers; poly(lactic acid-co-ethylene glycol); synthetic polyesters (PLA, PLGA) and collagen blends; aliphatic/aromatic degradable polyesters; polyfumarates; poly(ethylene terephtalate)–poly(butylene terephtalate); polyhydroxyalkanoates; poly(glycerol sebacate); hydroxyl group containing polymers; amine group containing polymers; poly(amido-amines)s; N-succinimidyl tartarate monoamine–poly(ethylene glycol)-block poly(D,L-lactic acid); poly(depsipeptide-co-lactide); poly(urethane)s; pluronic F-127 (PEO–PPO–PEO); tyrosine-derived polycarbonates; polyorthoesters; polyphosphazenes; polyanhydrides; polypyrrole; and poly ether ester amides and natural polymers such as alginates, chitosan, hyaluronans, and carageenans as well as self-assembling peptides.

Specific and representing examples cited, but not limited to, are as follows: Incorporation of protein-eluting microspheres (poly(lactide-co-glycolide) (PLGA 50/50)) into biodegradable nerve guidance channels (chitin tubes) for controlled release of growth factor; sodium tripolyphosphate cross-linked chitosan microspheres for controlled release of bioactive nerve growth factor; multifunctional, multichannel bridges that deliver neurotrophin encoding lentivirus (encoding the neurotrophic factors NT-3 or BDNF) for regeneration following spinal

cord injury (combining gene delivery with biomaterials); poly(2-hydroxyethyl methacrylate-co-methyl methacrylate) nerve guidance channels as nerve growth factor delivery vehicles; regrowth of axons in lesioned adult rat spinal cord: promotion by implants of cultured Schwann cells; and sustained release of dexamethasone from hydrophilic alginate hydrogel matrices using PLGA nanoparticles for neural drug delivery.
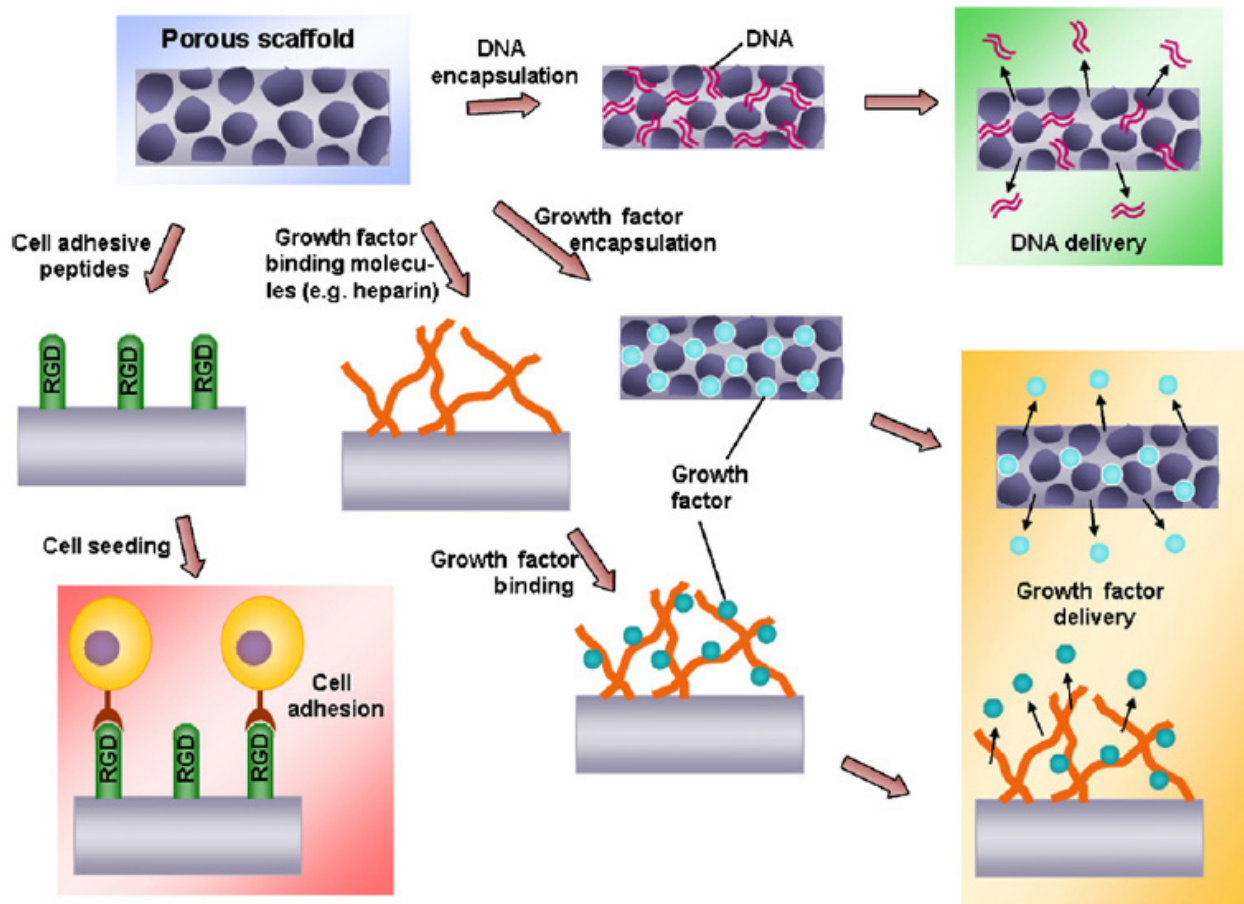

Figure 1. Surface engineered and growth factor releasing scaffolds for tissue engineering: Scaffolds can be either immobilized with cell specific ligands for cell adhesion, or encapsulated with growth factors or DNA to promote cell proliferation and morphogenesis [Adapted from Ref. 1 with permission © Elsevier Science BV].

2. Meta analysis of leading research reports

2.1. Local drug delivery for neuroprotection and tissue repair

In a series of studies, Shoichet et al., (2006, 2009a, 2009b) demonstrated the potential of hyaluronic acid-methyl cellulose (HAMC) blend as an injectable scaffold for the delivery of bioactives to an injured spinal cord, via an intrathecal injection, in conjugation with providing necessary support and microenvironment for the proliferation of neural cells. The studies were conducted in the backdrop of three most common strategies for localized intrathecal delivery viz. a bolus injection, a minipump delivery, and an injectable gel that localizes release to the site of injection. The first method pose a challenge in terms of the

effects being short-lived due to washing-out of the therapeutic molecule by cerebrospinal fluid and the second one being invasive and may lead to complication ranging from device blockage to infection. The novel HAMC blends' strategy provided a potential solution (**Table 1**) through the design of a shear-thinning based gel with an inherent property of increase in gel strength with an increase in temperature fast gelling [**Figure 2**].

S. No.	Design criteria	Advantage
1.	Fast gelling	localized delivery to the site of injection; no spread-out with the cerebrospinal fluid (CSF) flow
2.	Injectable through 30G needle	minimally invasive surgery
3.	Non-cell adhesive	minimization of scar formation in the intrathecal space
4.	Degradable	no need for scaffold removal afterwards
5.	Biocompatible	minimized foreign body reaction

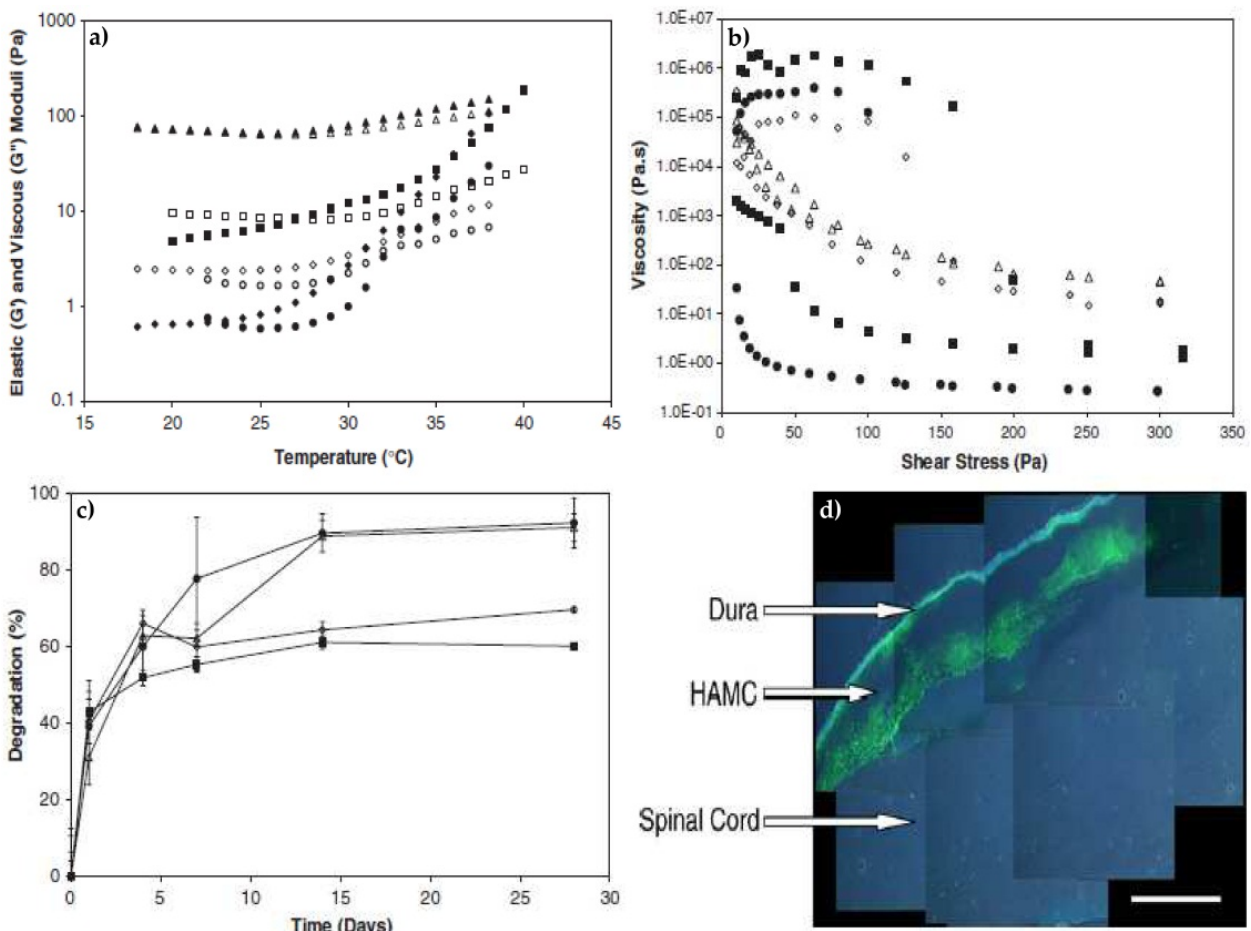**Table 1.** Design criteria for an injectable scaffold architecture

Figure 2. A) Elastic and viscous moduli of injectable gels at 1Hz using a rheometer with a cone and plate geometry (●) 7%MC G', (○) 7%MC G", (■) 9%MC G', (□) 9%MC G", (▲) HAMC G', (Δ) HAMC G", (▼) acet-HAMC G', and (◊) acet-HAMC G". B) Thixotropic loop of injectable gels at 37°C (●) 7%MC, (■)

9%MC, (Δ) HAMC, and (◊) acet-HAMC. C) *In vitro* degradation in aCSF of (●) 7%MC, (■) 9%MC, (Δ) HAMC, and (◊) acet-HAMC determined by change of dry mass over time. D) Parasagittal section of rat spinal cord rostral to site of injection shows that the fluorescent HAMC is localized in situ in the intrathecal space where it was injected [Adapted from Ref. 4 with permission © Elsevier Science BV].

In a typical experiment, 2% HA was blended with 7% MC and the gelation mechanism, degradation profile, and cell adhesion were studied in vitro. Additionally, in vivo studies were carried out for testing the injectability, biocompatibility and therapeutic efficacy (2006). Furthering the study, the potential of HAMC blend for the delivery of neuroprotectant such as nimodipine and erythropoietin were investigated experimentally. Specifically, nimodipine-loaded gels yielded particle size-dependent biphasic release profiles suggesting the accelerated delivery of poorly soluble drugs with tuneable release rates. In an another report, in vitro erythropoietin release studies revealed a 16 h release profile with the implant showing reduced cavitation after spinal cord injury along with enhanced neuronal number proving the potential of HAMC in neuroprotection after neural injury [2-4].

2.2. Multifunctional, multichannel bridges that deliver neurotrophin encoding lentivirus

Tuinstra at al., 2012, addressed the neural injury regeneration challenge by proposing a "gene delivery-biomaterial combination" strategy to surpass the "multiple barriers limiting regeneration". Spinal cord injury induced neuron and oligodendrocyte cell death, demyelination, inflammation, and deposition of a glial scar are attributed to insufficient trophic factor support, and up-regulation of axonal growth inhibitors. To overcome the deficiency of trophic factors, peripheral nerve implants are preferred due to their biograft-architecture and secretion of trophic factors by cells such as Schwann cells. However, the source limitation and the ill-defined contribution of the architecture and cell-secreted factors led to the requirement of systems and strategies capable of recapitulating their effects. To overcome the challenges, the delivery of lentiviral vectors (encoding neurotrophin-3 (NT-3) and brain derived neurotrophic factor (BDNF)) from multiple channel bridges was proposed as a combinatorial approach to promote regeneration in the injured SC. Interventionally, the lentivirus was immobilized to hydroxyapatite nanoparticles and then loaded into poly(lactic acid-co-glycolic acid) (PLGA) bridges for implantation into a rat spinal cord lateral hemisection and the axonal growth and myelination were characterized as a function of time, treatment, and location within the bridge. The strategy was aimed at (a) providing mechanical stability to the injured tissue, (b) channels directing the axonal elongation, (c) supporting cell infiltration, (d) preventing cavity formation secondary to the initial injury, (d) limiting scar formation. Interestingly, the cells were aligned with the major axis of the bridge providing a directional signal for regenerating axons [5].

2.3. In situ gelling hydrogels for local delivery of BDNF after spinal cord injury

Over the last decade, a research group led by Ravi V. Bellamkonda at Wallace H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory

University, Atlanta, USA, has emerged as a leading authority working towards the development of neurological biomaterials and therapeutics especially focusing on neural injuries. Jain et al., 2006, developed an agarose in situ gelling three-dimensional (3D) scaffold capable of initiating axonal growth in 3D. To this 3D scaffold was added neurotrophic factor BDNF-loaded lipid microtubule (40μm in length and 0.5μm in diameter) for local release of the bioactive. The individual benefits incurred by the components are structured in **Table 2**. Spinal cords treated with agarose/BDNF displayed no axonal bulbed-up morphology and the axons crossed the interface into the hydrogel scaffold based on the NF-160kDa staining data analysis where NF-160 kDa intensity was significantly greater than the agarose-treated spinal cords at the interface [6].

Agarose scaffold	Brain derived neurotrophic factor
Biocompatible: does not cause an adverse reaction when implanted in vivo,	encouraged neurite growth into the scaffolds
can be optimized for maximum axonal outgrowth through manipulation of the porosity and mechanical properties,	reduced the reactivity of the astrocytes and the production of chondroitin sulfate proteoglycans (CSPGs)
Support cell migration	enhanced the ability of regenerating fibers to enter the permissive hydrogel scaffold
can be utilized as part of a trophic factor delivery system with embedded sustained release vehicles,	acted as a chemo-attractant for the axons to cross the interface
can be used to bind protein to its backbone for spatial control	reduces the minimal inflammatory response agarose gels generate in vivo

Table 2. Salient features of the in situ gelling hydrogel components

2.4. Neurite extension in anisotropic LN-1 scaffolds

An in vitro study by Dodla and Bellamkonda, the importance of directional cues for neural regeneration was proposed. The hypothesis was based on the performance outcomes of autografts where the researchers theorized that "anisotropic hydrogel scaffolds with gradients of a growth-promoting glycoprotein/extracellular matrix protein, laminin-1 (LN-1), may promote directional neurite extension and enhance regeneration". The scaffolds were fabricated via photochemical coupling approach wherein LN-1 was immobilized onto three-dimensional (3D) agarose scaffolds in gradients of differing slopes. A significant increase in the Dorsal root ganglia (DRG) neurite extension rates were observed in case of anisotropic scaffolds as compared to their isotropic analogues proving the importance and potential of built-in directional cues for guided tissue or nerve regeneration [7]. In line with the functionalized scaffold properties developed above, Crompton et al., 2007, described the potential of Polylysine-functionalized chitosan as an in vitro substrate, scaffold for cortical cells, and for neural tissue engineering as an injectable scaffold. The functionalized polysaccharide polymer was rendered thermoresponsive by ionic gelation mechanism of glycerophosphate. In first of its

kind study of chitosan-GP network gels on neural tissue, the 3D culture model showed that the survival of cells on chitosan/GP was less than the PDL-modified chitosan/GP justifying the inclusion of PDL as functionality for neural applications [8].

2.5. Topical delivery of Tx-MP-NPs into the contusion injured spinal cord

The delivery of bioactives on the site of neural injury can be achieved by various means including intravenous delivery eventually passing through blood brain barrier and reaching the site of action or through intrathecal/intracranial injection for localized delivery. Targeted delivery is an additional option but not much tried and tested for CNS injuries. The i.v. strategy encompasses the probability of inefficiency as compared to the localised delivery but invasiveness is a drawback for the later intervention. Chvatal et al., 2008, and later Kim et al., 2009, reported the therapeutic effectiveness of nanoparticle-mediated localized methylprednisolone (MP) delivery for spinal cord injury intervention. These studies tested the therapeutic effect of MP-loaded PLGA nanoparticles, incorporated into an agarose gel, in in vivo models of spinal cord injury. The first study by Chvatal et al, 2008, concentrated on the spatial distribution of MP along with the acute anti-inflammatory effect. In a typical fabrication, the drug was first conjugated with Texas-red cadaverine (Tx, Biotium) allow its visualization in and around an injured spinal cord. Thereafter, the PLGA nanoparticles were prepared using a double emulsion method followed by their incorporation into the agarose gel. Two different types agarose gel were used: denser gel without nanoparticles (SeaKem) and gel containing nanoparticles (SeaPlaque) as shown in **Figure 3**.

According to **Figure 4**; the drug-indicator conjugate revealed a slow release over six days and the data was used to determine the amount of Tx-MP released from the gel-embedded NPs showing a continual release pattern. The release was probably due to the gradual degradation of PLGA polymeric structures. In this way, a continual delivery of MP can be provided to the injured spinal cord thereby exerting its anti-inflammatory action with a single administration and hence can provide much needed lipid-peroxidation inhibition during the initial and acute phase of secondary injury. To further simulate the in vivo environment, the in vitro release studies of Tx-MP from NPs embedded in agarose hydrogels was examined by co-incubation with LPS-stimulated primary rat microglia. The quantification of nitric oxide (NO) release from the microglia at different time points, 24, 72, and 96 h, showed that the cells treated with Tx-MP-NPs had significantly reduced NO production as compared to the cells treated with Saline NPs (as a negative control) further proving that the hydrogel-based delivery system (i.e., NPs embedded in agarose gel) can be used as an MP depot.

The quantification of spatial distribution of Tx-MP in the contusion injured spinal cord revealed that MP diffused up to 1.5mm deep and up to 3 mm laterally into the injured spinal cord within 2 days. Other significant observations of this study included decreased early inflammation, reduction in the number of ED-1þ macrophages/activated microglia, diminished expression of pro-inflammatory proteins such as Calpain and iNOS, and finally reduced lesion volume within 7 days after contusion injury [9].
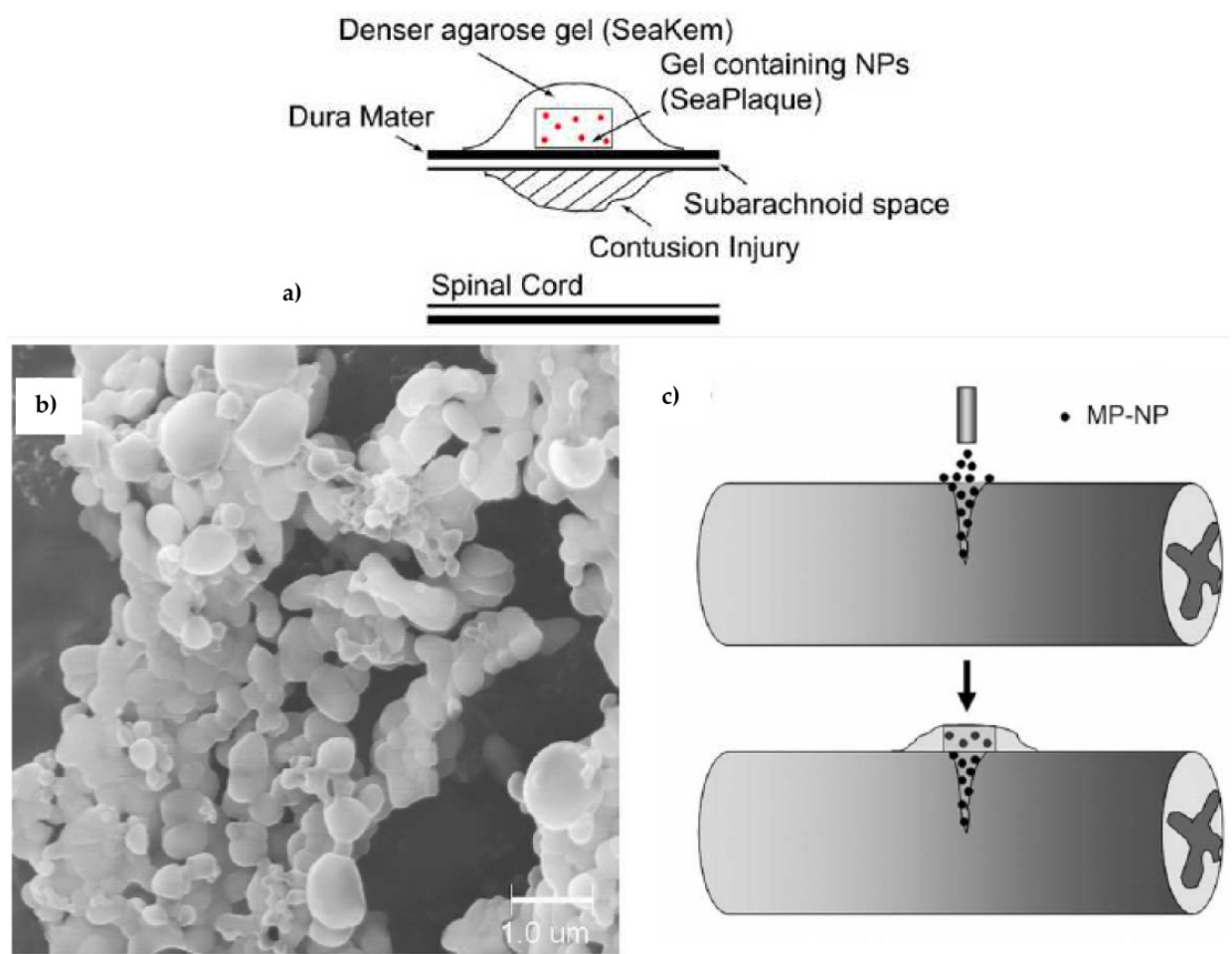
Figure 3. a) Schematic showing the Tx-MP-NP embedded gel is placed directly onto the injury site, on top of the dura. The denser gel is injected on top and quickly cooled to hold the NP embedded gel in place and minimize outward diffusion of Tx-MP. b) Methylprednisolone-encapsulating PLGA nanoparticles (MP-NP): SEM image of the lyophilized MP-NPs. c) Schematic of topical and local delivery of the MP-NPs onto dorsal over-hemisection lesioned spinal cord [Adapted from Ref. 9 with permission © Elsevier Science BV].
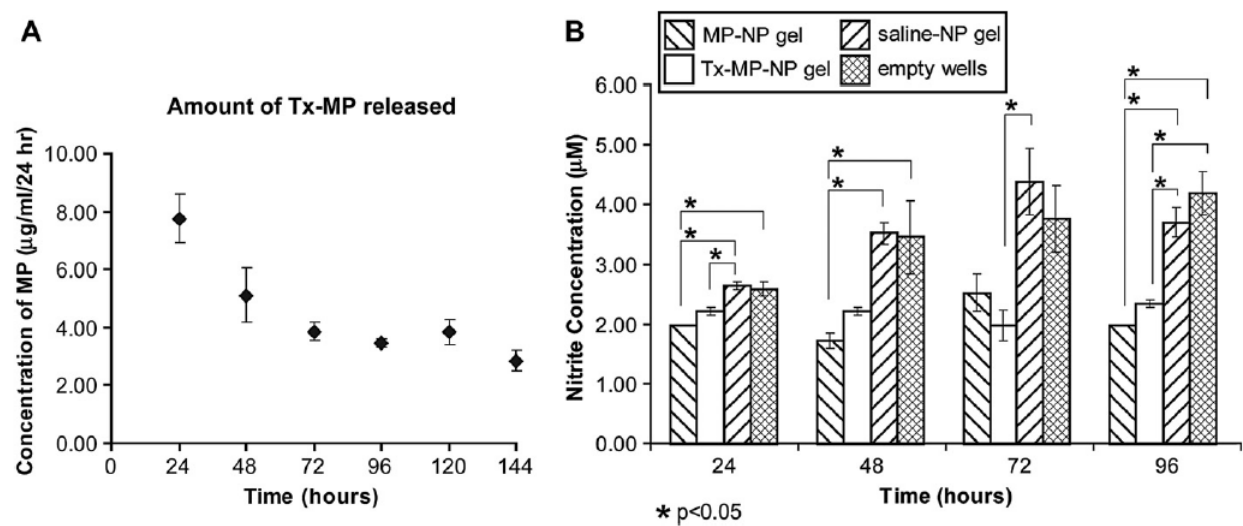

Figure 4. In vitro characterization of Tx-MP-NPs. (A) Release profile of Tx-MP over 6 days in vitro. Plotted is the amount of MP released every 24 h. (B) Bioassay showing the amount of nitrite in the

media surrounding LPS-activated microglia that had been treated with gels containing either MP-NP, Tx-MP-NP, Saline-NP, or nothing. This represents the amount of NO produced by microglia in each of these conditions for 4 days [Adapted from Ref. 9 with permission © Elsevier Science BV].

2.6. Nanoparticle-mediated local delivery of methylprednisolone

Further extending the above results, Kim et al., 2009, conducted a detailed histological and behavioural study of the developed local and sustained MP delivery through the use of biodegradable polymer-based nanoparticles. The following histological parameters were recorded:

1. reduced the reactivity of pro-apoptotic proteins (Calpain and Bax)
2. increased reactivity of anti-apoptotic protein (Bcl-2) at the lesion site
3. reduced iNOS (a key mediator of inflammation and neurotoxic effects expression
4. ratio of Bcl-2 to Bax, a key determinant of neuronal commitment to apoptosis, was increased indicating that MP treatment reduced the mitochondria-mediated cell death pathway.

These results suggest that sustained MP delivery by slow release MP-NP can reduce the reactivity of the injury-related proteins. Furthermore, MP-NP treatment produced significantly improved functional outcomes within one week after injury wherein MP-NP-treated groups performed significantly better in the beam walking test.

In conclusion, following advantages of MP-NP local delivery over conventional systemic delivery were proposed:

1. Better therapeutic effect as compared to systemic MP delivery;
2. More efficient, targeted delivery to the injury site as the MP delivery via systemic administration is influenced by the short pharmacokinetic half-life of intravenous MP (2.5–3 h) and P-glycoprotein-mediated exclusion of MP from the spinal cord necessitating a high-dose MP regimen. However, a significantly lower dose was required using nanoparticle-encapsulated MP (approximately 400 mg/ animal) and delivering this dose locally onto the target tissue. Additionally, this significantly enhanced the therapeutic effectiveness of MP by increasing the local dose levels at the target site;
3. Potential to adjust delivery rate or duration since the MP release profile from nanoparticles can be controlled through the composition of the biodegradable polymer, the rate, amount, or duration of delivery can be adjusted;
4. Stable formulation in form of injectable and lyophilized fine (submicron) powder formulation (unlike cell-based therapies). The formulation can be stored as a lyophilized fine powder and then easily suspended in saline or embedded in hydrogel and locally delivered onto the lesion site [10].

2.7. Sustained delivery of thermostabilized chABC

Furthering their study on the involvement of various axonal growth promoters and inhibitors, Bellamkonda and co-workers targeted Chondroitin sulfate proteoglycans, a major

class of axon growth inhibitors that are up-regulated after spinal cord injury and contribute to regenerative failure, via the use of Chondroitinase ABC (chABC) capable of digesting glycosaminoglycan chains on CSPGs thereby overcoming the CSPG-mediated inhibition. The formulation problem concerned with chABC is the loss of its enzymatic activity 37 °C, necessitating the use of repeated injections or local infusions for a prolonged period making it clinically inefficient. Lee et al., 2010, designed a thermostabilized chABC and developed a system for its sustained local delivery in vivo thereby resolving the problem to a great extent as the novel thermostabilized chABC retained its biological activity at 37 °C in vitro for up to 4 weeks [Figure 5].
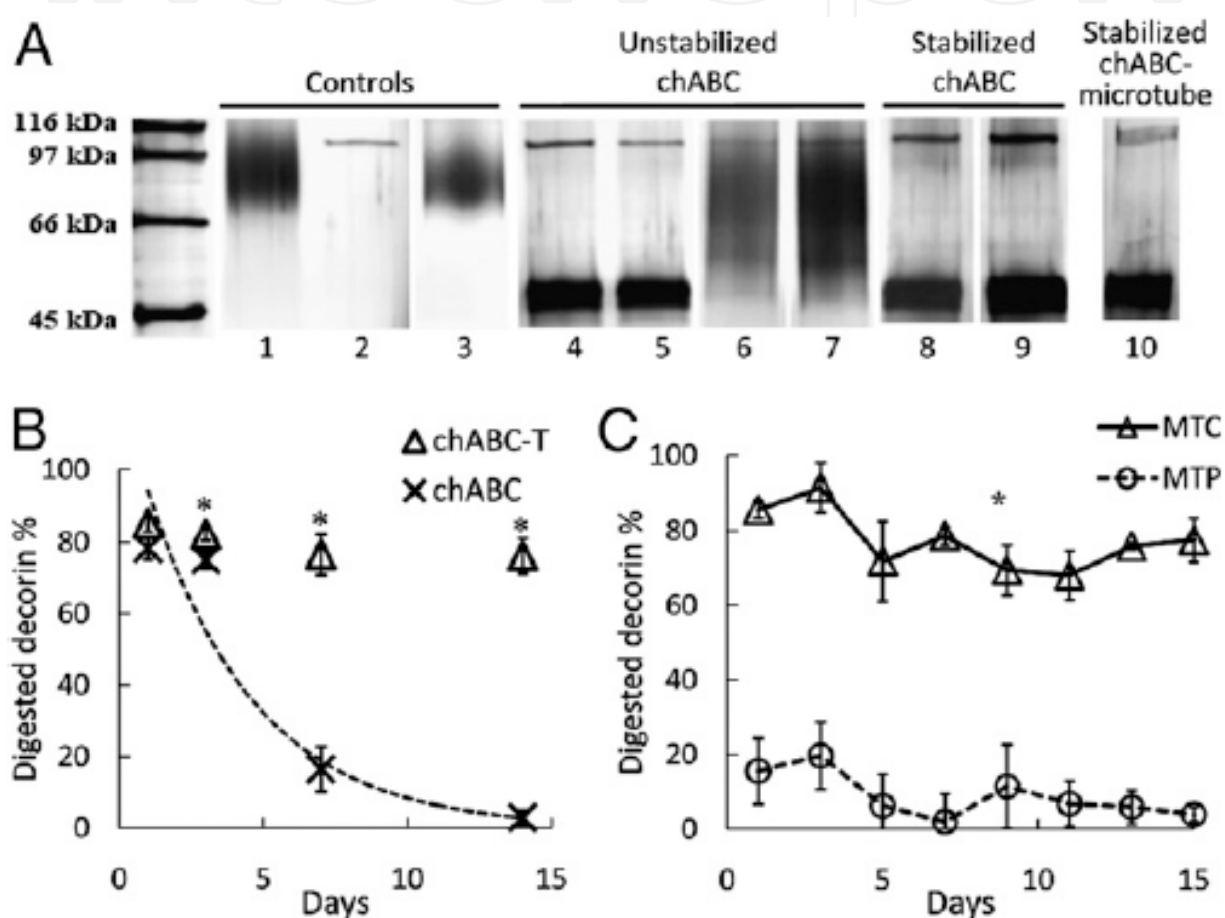

Figure 5. Enhanced thermal stability of chABC with trehalose, and sustained chABC release with lipidmicrotubes. (A) SDS-PAGE assay of enzymatic activity of chABC. (B) Kinetic analysis of chABC deactivation by DMMB assay. The dotted line represents the calculated deactivation curve of chABC in 1× PBS. (C) Enzymatic activity of post released chABC (Δ) and penicillinase (○) with trehalose/microtubes [Adapted from Ref. 11 © Proceedings of National Academy of Science (PNAS)].

Briefly, chABC was thermostabilize using the sugar trehalose and a hydrogel-microtube based delivery system was used for the sustained local delivery of chABC in vivo [11]. Key finding of the study included:

a. Trehalose Significantly Enhances chABC Thermal Stability and Prolongs Enzyme Activity

b. Temperature stabilization of chabc by trehalose was due to conformational stability

c. Thermostabilized chABC (TS-chABC) retains its ability to digest CSPGs in vivo 2 weeks post-injury,

d. CS-GAG levels remain significantly depleted at the lesion site for at least 6 weeks post-SCI.

e. Enhanced axonal sprouting and functional recovery is observed after topical delivery of agarose-microtube-chabc scaffolds after SCI

f. Animals treated with thermostabilized chABC in combination with sustained neurotrophin-3 delivery showed significant improvement in locomotor function and enhanced growth of cholera toxin B subunit– positive sensory axons and sprouting of serotonergic fibers.

g. chABC thermostability facilitates minimally invasive, sustained, local delivery of chABC that is potentially effective in overcoming CSPG-mediated regenerative failure.

2.8. Sustained delivery of activated Rho GTPases and BDNF

In the next stage of their Chondroitin sulfate proteoglycans targeting, Bellamkonda and co-workers fabricated constitutively active (CA) Rho GTPases, CA-Rac1 and CA-Cdc42, and BDNF loaded 1,2-bis-(tricosa-10,12-diynoyl)-sn-glycero-3-phosphocholine lipid microtubes for the sustained release of bioactives to the lesion site for the alleviation of CSPG-mediated inhibition of regenerating axons. The lipid tubules were further loaded into an *in situ* gelling agarose-protein architecture similar to previous studies where nanoparticles were incorporated into an agarose injectable gel. The sustained release characteristics of the developed system were characterised both in vitro and in vivo BDNF was conjugated to rhodamine for its in vivo quantification. As depicted in **Figure 6**, 3 mg of BDNF was released within the first 24 h followed by a cumulative release of 4mg within the first 3 days. After this initial burst release, approximately 3ng/day of BDNF was released for the subsequent 11 days. For in vivo release study, microtubes loaded with BDNF/Rhodamine embedded in the agarose were injected into the spinal cord cavity. Spatial quantification of BDNF/Rhodamine displayed its presence in the spinal cord tissue between 1 to 2 mm proximal to the lesion site (Fig. 1B). An overlap of ED-1+ cells (green) with the BDNF-Rhodamine demonstrated co-localization and subsequent engulfment of the BDNF-Rhodamine by the ED-1+ cells. Additionally, BDNF-Rhodamine was double labelled with DAPI to visualize cell nuclei confirmed the presence of neurotrophins [12]. In conclusion, the study utilized the hydrogel/microtubule scaffold delivery system to deliver BDNF, CA-Cdc42, or CA-Rac1 to decrease the sensitivity of growth cones to CSPGs, and promote axonal growth through CSPG-rich regions at the lesion site based on following three phenomena:

1. The use of the in situ gelling hydrogel allows the lesion cavity to be conformally filled.
2. The lipid microtubes allow slow release of therapeutics from the hydrogel over time.
3. This strategy allows neuroprotective or axonal migration stimulators to be delivered locally over time.
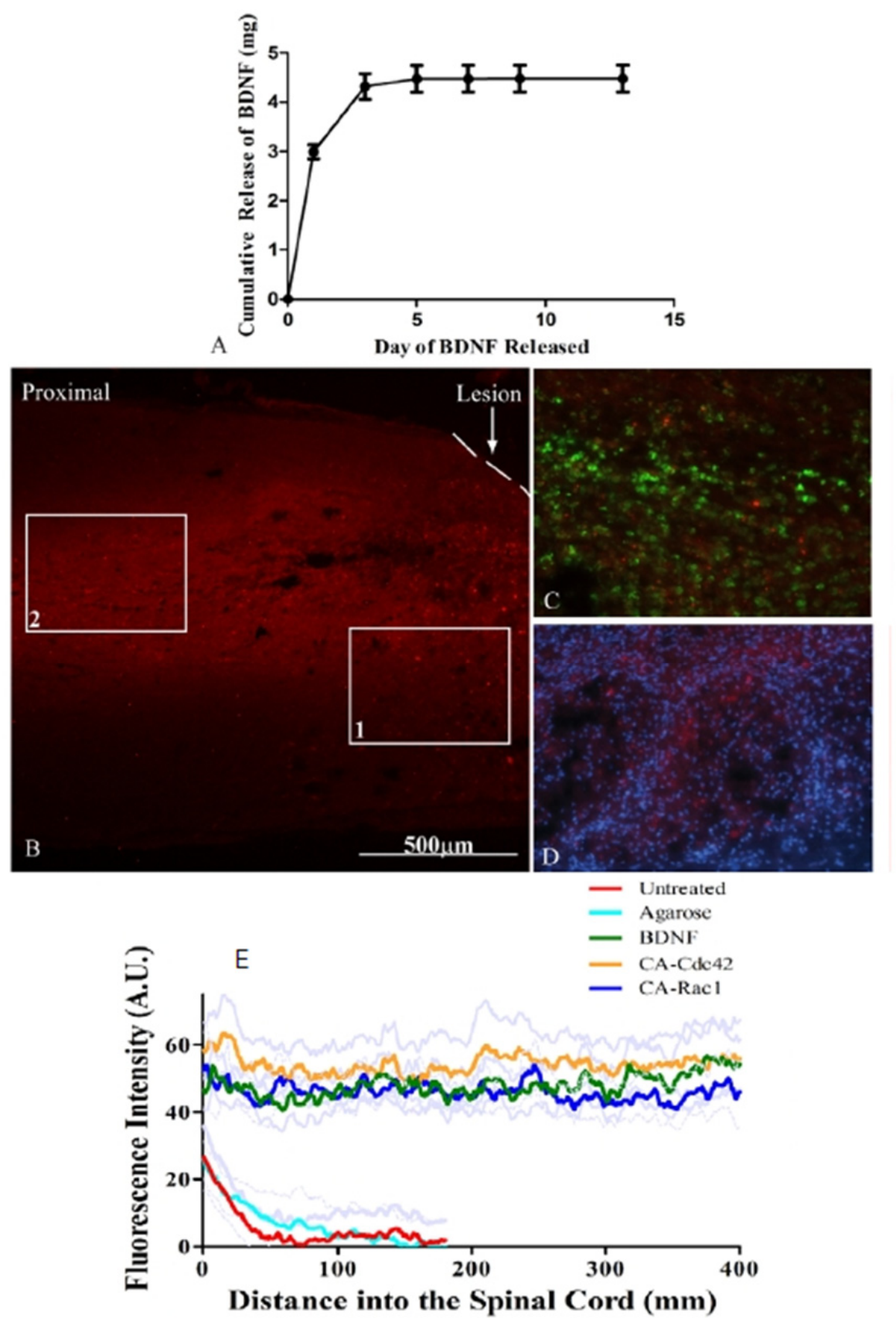
Figure 6. In Vitro and In Vivo Diffusion of BDNF. A. In vitro release assay of BDNF over the first 2 weeks. The graph shows that an initial burst released 4 mg of BDNF within the first 3 days. An average

of 3 ng/day of BDNF was released for the following 11 days. The data represent mean 6 SEM B. An image of the proximal region of a spinal cord section after delivery of BDNF/Rhodamine at 46. The 206images labelled 1 and 2 are outlined with white boxes in A and demonstrate that BDNF/Rhodamine diffused approximately 2 mm proximal to the lesion site. The dashed line represents the interface between the spinal cord and scaffold. White arrows indicate BDNF/Rhodamine. C. A 206image of ED-1+ cells (green) and BDNF-Rhodamine (red). The image shows some overlap demonstrating engulfment of the BDNF-Rhodamine by the ED-1+ cells. D. A 206image of DAPI (blue) and BDNF-Rhodamine (red) demonstrates that not all of the BDNF-Rhodamine is taken up by resident and migrating cells. Quantitative analysis of NF-160 intensity for the stained spinal cords. The spinal cords treated with CA-Cdc42, CA-Rac1, and BDNF had significantly higher fluorescent intensity and also had extended further into the scaffold filled spinal cord cavity than the untreated and agarose controls [Adapted from Ref. 12 © PLoS ONE].

2.9. Multiple-channel scaffolds to promote spinal cord axon regeneration

Moore et al., 2006, introduced a proposition regarding the use of a conjugation of molecular, cellular, and tissue-level treatments for spinal cord injury to elicit functional recovery in animal models or patients. In a tri-component study, multiple-channel biodegradable scaffolds were tested as a platform to investigate the effects scaffold architecture, transplanted cells, and locally delivered molecular agents on axon regeneration. Biodegradable polymers such as PLGA can provide a tissue scaffold, a cell delivery vehicle, and a reservoir for sustained drug delivery. Therefore, PLGA scaffolds were fabricated using injection moulding with rapid solvent evaporation.

The scaffolds so formed have characteristic plurality of distinct channels running parallel along the length of the scaffolds with various customized channel sizes and geometries. Inherent to the channels were void fractions (as high as 89%) with accessible void fractions as high as 90% through connections 220mm or larger. Scaffolds degradation and bioactive release in vitro accounted for over a period of 30 weeks and 12 weeks, respectively. The degradation profile of PLGA scaffold demonstrated typical polyhydroxy acid characteristics as the scaffold molecular weight decreased steadily from 100% of the initial molecular weight down to 5% by 26 weeks of degradation. Interestingly, minimal mass loss was observed for the first 20 weeks. The next ten weeks culminated into a more precipitous mass loss generating a sigmoidal mass loss behavior typical for PLGA degradation. The negligible change in mass during initial phase corroborated well with the slight changes in morphology. However, the morphology of the degrading scaffolds changed drastically during the precipitous phase. The model drug, Fluorescein isothiocyanate (FITC-D), was incorporated using, mechanical mixing within the polymer scaffolds. However the method was quite effective for scaffold loading, an inhomogeneous dispersal appeared in the final fabricated scaffolds. A drug release-scaffold degradation comparison in **Figure 7** compared the mass and molecular weight degradation profiles with model drug release. An initial burst over the first 48 h was followed by a period of more steady release for 4 weeks. The sustained release continued for next 8 weeks after which little or no release was detected for the remainder of the degradation period.
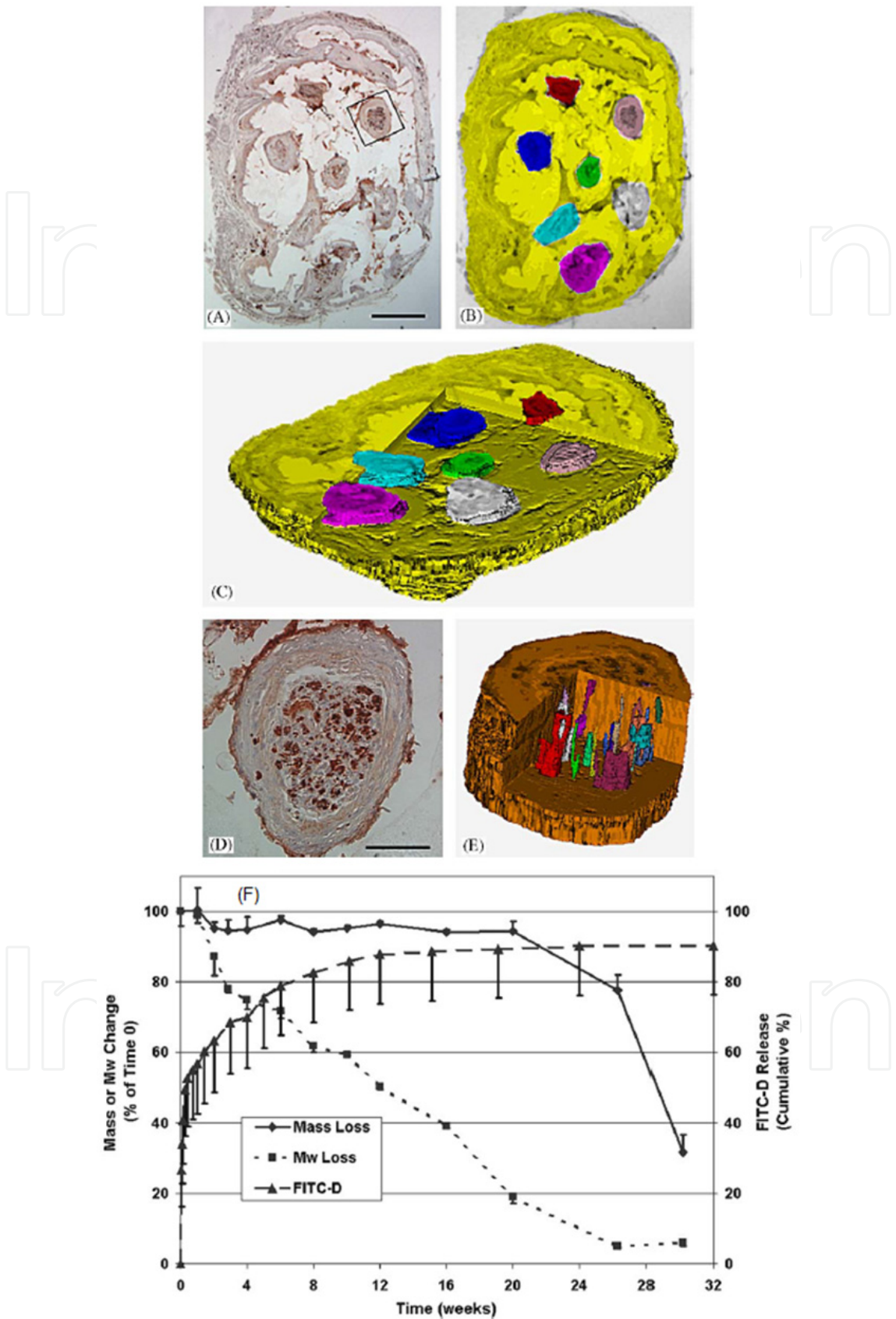
Figure 7. Histological images of tissue cables within scaffold channels. (A) Histological cross section near rostral end of scaffold showing tissue cables within all seven channels. Boxed region is shown in

greater detail in D. (B) Histological cross section shown in A with channels segmented and displayed in color. (C) Orthographic, wedged view of 16 serial slices registered, segmented, and reconstructed to reveal 3-D structure of channels. (D) Histological cross section of boxed region in A; (E) Orthographic, wedged view of reconstructed channel shown in D, with segmented axon bundles displayed in color. (F) Degradation and FITC-D release profile. Mass or molecular weight change is indicated as a percentage of initial value at time zero. FITC-D release is indicated as a cumulative percentage of loaded drug [Adapted from Ref. 13 with permission © Elsevier Science BV].

The release of the drug progressed in corroboration with scaffold degradation. However, the inhomogeneity played its role as a very high variability was observed. The polymeric architecture was designed to act as a template for the arrangement of cells that may attract and guide regenerating axons rather than for guiding the regenerating fibers by providing an oriented surface. The delivery system was proposed to be flexible enough to provide incorporation of various drugs/biomolecule/bioactives that may add tropic attraction, neuroprotection, or reduce the inhibitory effects of secondary injury. Another important aspect of the study was to attest the effect of incorporation of Schwann cell in the scaffold as their harvesting, suspension, and distribution into scaffold channels is of critical importance in this field. Results indicated that Schwann cells were successfully harvested, suspended in Matrigel, and loaded into distinct channels, and that cells survived in culture under physiologic conditions within the scaffold. Axon regeneration was visualized by 3-D reconstruction of serial histological sections, an approach that appears useful for evaluation of regenerating tissue architecture as shown in **Figure 7**. The 3-D reconstructions demonstrated the arrangement of the seven channels containing regenerating axons, as well as live axon bundles within the channels. This novel depiction corroborated well with the channels which were found distributed as arranged by the scaffold, with scaffold and tissue in the spaces separating the channels, approximately. An important finding revealed that channels generally contained a centralized core of tissue containing axons and capillaries, surrounded by circumferential fibrous tissue. Furthermore, macrophages could be identified, due to their engulfment of neurofilament-stained material [13].

2.10. Laminin and nerve growth factor-laden three-dimensional scaffold

Yu et al., 1999, engineered Leminin-functionalized agarose hydrogel scaffolds for the stimulation of neuronal process extension based on the hypothesis that "substrate-bound neurite promoting extracellular matrix (ECM) proteins and chemo-attractive diffusible trophic factors influence and stimulate axon guidance and neurite extension". The study indicated that the effect of LN-modified agarose gels on DRG/PC12 cell neurite outgrowth involves receptors for YIGSR/integrin/31 subunits, respectively. Additionally, 1,2-bis(10,12-tricosadiynoyl)-sn-glycero-3-phosphocholine based lipid micro-cylinders were loaded with nerve growth factor (NGF), and embedded into agarose hydrogels which resulted in a directional neurite extension from DRGs in agarose hydrogels due to the presence of trophic factor gradients. The NGF-loaded lipid micro-cylinders released physiologically relevant amounts of NGF for at least 7 days *in vitro* based on a PC12 cell-based bioassay confirming

that the agarose hydrogel scaffolds can be used as biosynthetic 3D bridges capable of promoting regeneration across severed nerve gaps [14].

2.11. Agarose and methylcellulose hydrogel blends for nerve regeneration applications

Taking leads from the research done by Shoichet and co workers and from Bellamkonda and co workers, Martin et al., 2009, designed injectable scaffold consisting of agarose and methylcellulose (MC) as the responsive polymers. The following rationale was cited in support of using a blend of MC and agarose instead of using agarose alone: the low melting point of agarose makes it difficult to use *in vivo* and hence need to be cooled after injection wherein blowing of gases onto nervous system tissue could potentially be harmful to neurons that survived the initial injury. The release of bioactive from such gelling mixtures was related to the dissolution of the scaffold itself under physiological conditions. In an interesting experiment the percentage of hydrogel dissolved was measured on day 1, 4, 7, 14 and 28. Plain agarose dissolved completely when left in PBS in a 37 ∘C incubator for only 7 days, making it difficult to record the percentage dissolved due to hydrogel instability. However, the blends were approximately 70% dissolved by the end of 28 days in the cycled PBS solution. Pristine 7% methylcellulose proved to be a weaker hydrogel that dissolves more rapidly than the hydrogel blends, dissolving over 75% in 28 days. This confirmed the applicability of the blend in comparison to individual polymers. Furthermore, the authors pointed that "the ease of creation, simplicity of delivery and use of natural biocompatible components that solidify naturally without the use of ultraviolet light or cross linkers add to the advantages of utilizing this blend" [15].

2.12. An injectable, biodegradable hydrogel for trophic factor delivery

Piantino et al., 2006, devised a new method for an injectable based drug delivery system for the delivery of trophic factors to the injured neural tissues. The method utilized an injectable liquid polymer solution that crosslinks into a biodegradable, water-swollen hydrogel when photo-activated under visible light. The investigators described the uniqueness of their system over other systems as follows:(1) the system can provide constant and tailorable delivery of one or more growth factors to the precise site where needed; (2) reduce the possibility of host–graft rejection, as may occur with the use of live tissue or cell preparations; (3) does not involve viral vectors, which may induce an inflammatory response and which may require prolonged delays in achieving high levels of gene expression; and (4) does not involve the use of devices that can malfunction or cause infections (e.g., pumps or catheters). Briefly, degradable poly(ethylene glycol) hydrogels are formed from the radical polymerization of acrylated poly(lactic acid)-b–poly(ethylene glycol)-b–(poly lactic acid) (PLA-b–PEG-b–PLA) macromers. The network degradation and subsequent molecular release properties can be controlled by altering the network chemistry of these injectable hydrogels such as the number of degradable units and network crosslinking density by changing the macromer concentration). The degradation of the

hydrogels was postulated to be due to hydrolysis in the network crosslinks. The release profile of NT-3 *in vivo* was determined using ELISA and a peak was reported at the site of injury as early as 3 h after hydrogel administration. High concentrations over a distance of 1 cm and more were cited site on day 6 followed by a decline to approx. one-third of maximum after 14 at 14 days. The NT-3 levels within the first few millimetres of the lesion site were in the range of 50 ng/ml/g of tissue when averaged over the entire tissue sample. In vitro release studies corroborated well with the inv vivo data with a burst release of ~40% over first 24 hours and a sustained release thereafter. Interventionally, NT-3, delivered via hydrogel in the vicinity of injured nerve terminals, was presumed to be transported retrogradely to cortical pyramidal cells thereby promoting axonal branching along with promoting plasticity of the raphespinal tract and finally promoting the outgrowth of descending pathways involved in locomotor control leading to the lesion size being slightly smaller in NT-3-treated vs. control animals as early as 1 day and 1 week post-lesion [16].

2.13. Hydrogel scaffold and microspheres for supporting survival of neural stem cells

To develop a biomaterial composite for promoting proliferation and migration of neural stem cells (NSCs) and to rescue central nervous system (CNS) injuries, Wang et al., 2011 constructed a NSCs-cultured delivery system based on crosslinked hyaluronic acid (HA) hydrogels, containing embedded BDNF and vascular endothelial growth factor (VEGF)-loaded poly(lactic-co-glycolic acid) (PLGA) microspheres for controlled delivery as well as angiogenesis on the materials. The study was designed on the drawbacks of previous studies that have shown an intrinsic ability of neural stem cells (NSCs) to regenerate in various neurological diseases' intervention. The most important among these were the low viability and undesired differentiation of the grafted NSCs and also the glial scar formation. In addition to these, the deficiency of neurotrophic factors and growth factors following the CNS may also contribute to failure of neural regeneration. The in vitro release kinetics study by ELISA measured the loading of BDNF and VEGF in microspheres as 0.10 and 0.34 μg/mg microspheres, respectively, corresponding to 52% and 84% loading efficiency making the loading efficiencies of HA gel composites in the range of 17.4 and 56.6 ng/mg, respectively, when embedded with microspheres. Interestingly, no burst release characteristic to the PLGA microspheres was within the period of 6 days wherein bioactives were released from the microspheres constantly and cumulatively. The thorough washing of the microspheres was believed to overcome the burst release of the proteins from the surface and provided a slow and linear release profile during the test period. During the first week of the study, about 20–30% of the loaded proteins were release possible by diffusion from areas near the surface as the degradation of PLGA was insignificant during initial phases. In contrast, the release of growth factors from the hydrogel embedded with microspheres revealed an initial "burst" followed by a stable release phase. This was attributed to the fact that released factors might have remained in the hydrogel during the cross-link processing leading to the diffusion through the hydrogel matrix. However, the cumulative amount of bioactive release was reduced to 12% and 13% of total loading as compared to 20-30% in case of

microspheres alone which was postulated to be due to the presence of the surrounding HA hydrogel as a delayed or deposited effect on the release profile of the biofactors [17].

2.14. Controlled release of Neurotrophin-3 from fibrin-based tissue engineering scaffolds

Pathologically, the ability of chronically injured supraspinal axon tracts to regenerate decreases if intervention is delayed for too long. The barriers to axonal regeneration in case of a chronically injured spinal cord are reported to be overcome interventionally by using a combination of diffusible growth stimulating molecules and a favourable growth stimulating extracellular matrix such as the use of a continuous transgenic delivery of neurotrophic factors from engineered cells that also secrete extracellular matrix (ECM) is conducive to regeneration [18]. However, there is a limitation of this strategy as that the growth is limited to the regeneration of axons into the transplant site, the source of the growth stimulating neurotrophins, and the axons fail to regenerate back into host tissue. Sakiyama-Elbert and co workers, 2009, coined the need for temporary controlled release of growth-stimulating neurotrophins that not only promote axon growth into the lesion but also foster growth into healthy spinal cord tissue thereby connecting the newly grown tissue to the neural tissue that survived the injury. The intervention consisted of delayed treatment of SCI with controlled release of neurotrophin-3 (NT-3) from fibrin scaffolds providing a supportive regenerative environment, reducing the accumulation of reactive astrocytes surrounding the lesion and enhancing the presence of neural fibers within the lesion. The heparin-based delivery system (HBDS) developed earlier by the investigators [19], was utilized as it forms non-covalent interactions between neurotrophins, heparin, and a covalently linked bi-domain peptide (ATIII peptide), which can be incorporated into the fibrin scaffold and allow for the controlled release of growth factors from the scaffolds. The controlled released of the biofactor from the fibrin scaffold was assessed in terms of quantification of neural fiber density, astrocyte density, and chondroitin sulfate proteoglycan density along with macrophage/microglia immune profile analysis. The final results demonstrated that controlled release of NT-3 promotes increased neural fiber density within the lesion and decreased reactive astrocyte staining around the lesion of subacute SCI (2 weeks after the injury) at a dose of 500 ng/mL of NT-3 with HBDS [20]. These results were in corroboration with the earlier reports where the NT-3/HBDS was employed in an acute SCI (immediately after the injury) model wherein the it promoted a significant increase in the density of neural fibers sprouting [21-23].

2.15. Nerve guidance channels as drug delivery vehicles

However, safe and effective use of pro-regenerative molecules requires a localized, controlled and sustained delivery to the site of neural injury. However, there are many challenges pertaining to the various approaches available in the neural arena as described in Table 3.

To overcome the challenges shown in the table above, Piotrowicza and Shoichet, 2006, developed a liquid–liquid centrifugal casting process which enables the formation of nerve

guidance channels (NGCs) from acrylate-based hydrogels such as poly(hydroxylethylmethacrylate-co-methylmethacrylate) or P(HEMA-co-MMA). The NGCs themselves were fabricated by a liquid–liquid centrifugal casting process and the protein incorporation strategies were compared in terms of protein distribution and nerve growth factor (NGF) release profile. The centrifugal casting process is based on the following phenomenon: Phase separation of the polymer phase from the monomer formulation during polymerization in a rotating cylindrical mould and hence the denser polymer phase is pushed to the periphery by centrifugal forces, where it gels forming a tube. The NGCs thus formed were semipermeable, soft and flexible, and matched the modulus of the nerve or spinal cord.

S. No.	Approaches	Challenge
1.	Systemic administration	short half-lives and high potency of many biomolecules, and delivery to the CNS is further limited by the blood–brain barrier
2.	osmotic mini-pumps	infections or tissue damage, pump failure due to catheter dislodgement or occlusion, as well as drug instability in the pump reservoir
3.	local administration as growth factor solutions	factors lose bioactivity in solution
4.	local administration as matrices saturated with growth factors	easily leak from the channel during the implantation procedure
5.	NGCs prepared by dip-coating or solution casting	the creation of a non-symmetric growth factor concentration profile within the lumen of the NGC

Table 3. Challenge inherent to various bioactive administration approaches

The investigators formulated three different approached for the delivery of nerve growth factor (NGF) using Nerve guidance channels (NGCs constituted with of poly(2-hydroxyethyl methacrylate-co-methyl methacrylate), P(HEMA-co-MMA) as the polymeric platform to facilitate regeneration after transection injury to the spinal cord: (1) NGF was encapsulated (with bovine serum albumin (BSA)) in biodegradable poly(D,L-lactide-co-glycolide) 85/15 microspheres, which were combined with a PHEMA polymerization formulation and coated on the inside of pre-formed NGCs by a second liquid–liquid centrifugal casting technique; (2) pre-formed NGCs were imbibed with a solution of NGF/BSA and (3) NGF/BSA alone was combined with a PHEMA formulation and coated on the inside of pre-formed NGCs by a second liquid–liquid centrifugal casting technique. The channels imbibed with NGF showed no sustained release of NGF as was achieved from NGCs with either NGF-loaded microspheres or NGF alone incorporated into the inner layer with values of 1040 pg/cm, 220 pg NGF/cm, and 8624 pg/cm, respectively, after 28 days [**Figure 8**]. As a key finding, the release of the bioactive from the conduits was dependent on the slow-degrading characteristic of the microspheric system as well as on the "maximum amount of microspheres" that could be incorporated into the cylindrical structure. Another

important finding of the report was that the liquid–liquid centrifugal casting process demonstrated potential towards localized and controlled release of multiple factors key to tissue regeneration after neural injuries [24].
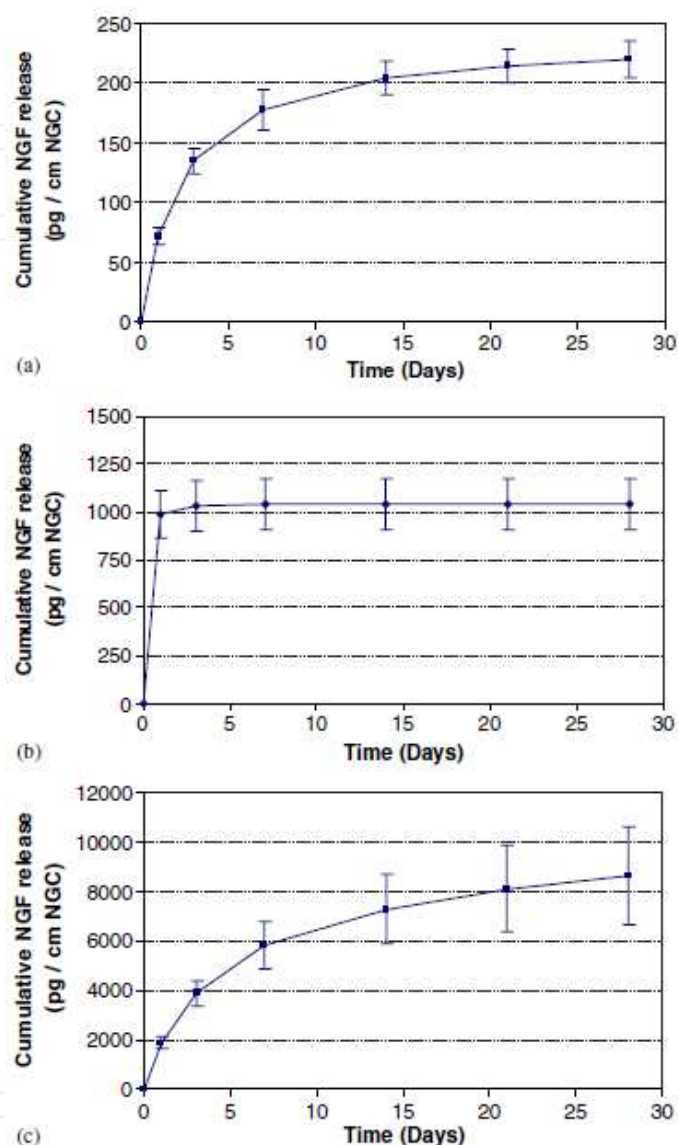

Figure 8. Cumulative release profile of NGF (pg/cm of NGC) over a 28-day period from NGCs (a) coated with an inner layer of PHEMA containing NGF-loaded PLGA 85/15 microspheres; (b) imbibed with NGF; and (c) coated with an inner layer of PHEMA containing directly entrapped NGF [Adapted from Ref. 24 with permission © Elsevier Science BV].

2.16. Self-assembling peptide nanofiber scaffold enhanced with RhoA inhibitor

In a recent study, Zhang et al., 2012, focused on two major targets for axonal regeneration after a spinal cord injury:

a. The reconstruction of the gap in the injured spinal cord
b. Inactivation of RhoA to ameliorate the hostile microenvironment perilesion

To achieve these targets, Zhang et al., 2012, transplanted self-assembling peptide nanofiber scaffold (SAPNS) based local delivery of RhoA inhibitor to the lesion sites after acute spinal cord injury centred on their previous reports wherein the SAPNS had shown multiple regeneration-facilitating properties to improve the recovery of the injured spinal cord and brain and that SAPNS has potential for controlled bioactive release. The study was based on the hypothesis that this novel combination might act by providing regrowth-promoting scaffold reducing the physical obstacles after injury (characteristic SAPNS capability of filling the cavities and alleviating glial scarring) and the RhoA inhibitor released from the combination implants may exert a therapeutic effect conducive for axonal regrowth.

As depicted in **Figure 9**, the SAPNS implants, with or without CT04 incorporation, reconstructed the injured spinal cord whereas a significant gap was developed in the lesion site in the saline group. Additionally, the implants integrated closely with the surrounding host tissue with no cavities or gaps occurring between the implants and host tissue. Another striking finding was the presence of the cells such as fibroblasts, which mainly clustered in the centre of the lesion area and formed collagen fibers, along with lymphocytes, macrophages, astrocytes, and microglias scattered among the fibroblasts. The growth of such cellular mass may further hindered the cavity or cysts formation- a major cause of anatomical disconnection after SCI. On the drug delivery note, SAPNS delivery of CT04 significantly improved the axonal regrowth. Based on fluorescent characteristics of dextran, it was incorporated into SAPNS to track the spread-out and diffusion of the bioactive in the lesion site. Seven days after the transplantation of the SAPNS incorporated with Dextran, significant fluorescence was observed into the surrounding host cord from the SAPNS, while the implant still retained high fluorescent signal confirming the release of chemicals from SAPNS and indication that SAPNS can effectively serve as a platform for further controlled release of exogenous therapeutic molecules of interest. In conclusion, the SAPNS delivery system proved to be capable of minimal risk of carrying biological pathogens or contaminants, providing 3-dimensional environment constructed for cell growth and migration, minimal cytotoxicity to the host, no apparent immune response, easy conformity to the various shape of lesion cavities, and immediate haemostatic and controlled drug release properties [25].

2.17. Sustained delivery of chondroitinase ABC from hydrogel system

As explained earlier in this report, chondroitin sulfate proteoglycans (CSPGs) are among the principal factors responsible of axon growth inhibition and they contribute to regenerative failure, promoting glial scar formation in an injured spinal cord. Chondroitinase ABC (chABC), although capable of digesting these proteoglycans leading to the degradation of glial scar and favoring axonal regrowth, suffers from delivery drawbacks such as administration being invasive, infection-prone and clinically problematic. Rossi et al., 2012, developed an agarose-carbomer (AC1) hydrogel, which they previously used in SCI repair strategies [26-28], as a delivery system capable of an effective chABC administration based on following considerations: ability to include chABC within its pores and the possibility to be injected into the target tissue; and release kinetics and the maintenance of enzymatic activity can be
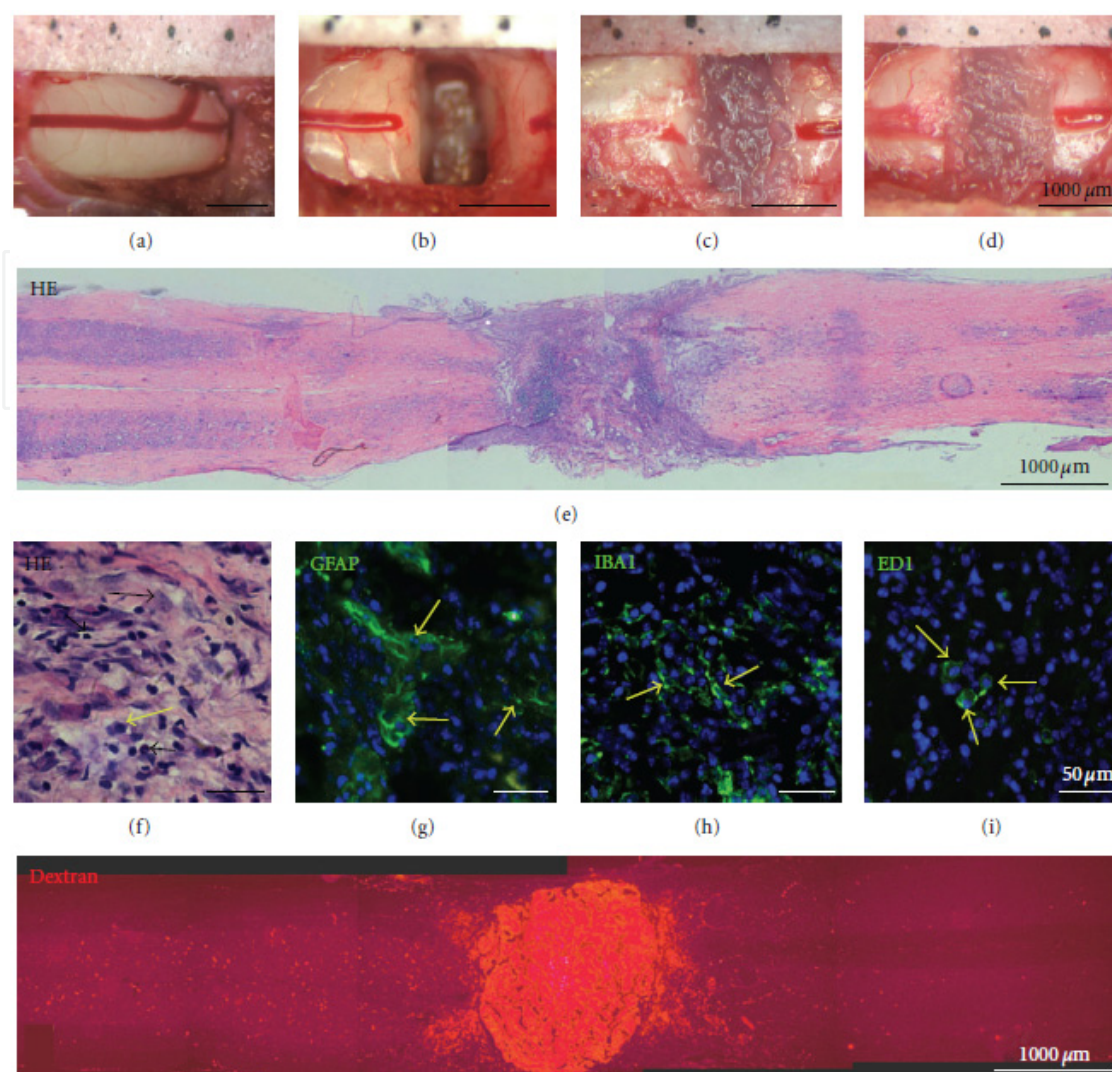
Figure 9. The implants of various SAPNS reconstructed the injured spinal cord. (a–d)Macroscopic observations of lesion sites immediately after surgery. (a) Sham surgery; (b) saline group; (c) SAPNS with vehicle group; (d) SAPNS+CT04 combination group. (e) HE staining shows the lesion area was reconstructed 12 weeks after SAPNS transplanted. (f) HE staining shows various cells in the grafted scaffold, which include fibroblast (black arrow), macrophage (yellow arrow), and lymphocyte (arrow head). (g–i) Immuno-staining shows the GFAP-positive astrocytes (g), IBA1 positive microglias (h) and ED1 positive macrophages (i) in the grafted scaffold. (j) shows the Dextran, a red fluorescent dye, was partially diffused from the mixture of SAPNS and Dextran at 7 days after transplantation [Adapted from Ref. 25 © Hindawi Publishing Corporation].

positively assessed. Additionally, being a hydrogel, AC1 had its own advantages in terms of (I) the ability to retain water; (II) mimicking living tissues; (III) high biocompatibility; and (IV) the possibility to allow precisely controlled release rates. As demonstrated in Figure 10, AC1 appeared to be a chemical gel synthesized through a statistical block polycondensation between Carbomer 974P and agarose. chABC was loaded before the sol/gel transition resulting in its physical entrapment within the three-dimensional polymeric network of the gel. Macroscopically, a highly entangled nanostructure was confirmed by ESEM analysis proving its ECM mimicking nature. In brief, Carbomer 974 P provided the main cross-linking

properties via its carboxylic groups which in turn reacted with hydroxyl groups from agarose leading to the formation of a three dimensional matrix. The in vitro release kinetics displayed typical hydrogel characteristics wherein a rapid initial release of Tx was observed during the initial phases due to a high concentration gradient possible caused by: molecules placed near solvent-hydrogel interface that may rapidly escape into the supernatant solution; molecules diffusing through the large pores of the hydrogel in comparison to the molecules diffusing through smaller pores. The burst release was followed by slower release and eventually forming a plateau. After this initial burst, the release became slower and reached a plateau - steady state condition - after 7 days. Importantly, the whole amount of Tx loaded was release eventually confirming the absence of any stable bonds between hydrogel polymeric network and loaded Tx. Furthermore, it confirms the hydrogel's capacity to deliver even those molecules that are deeply entrapped in its core [29].
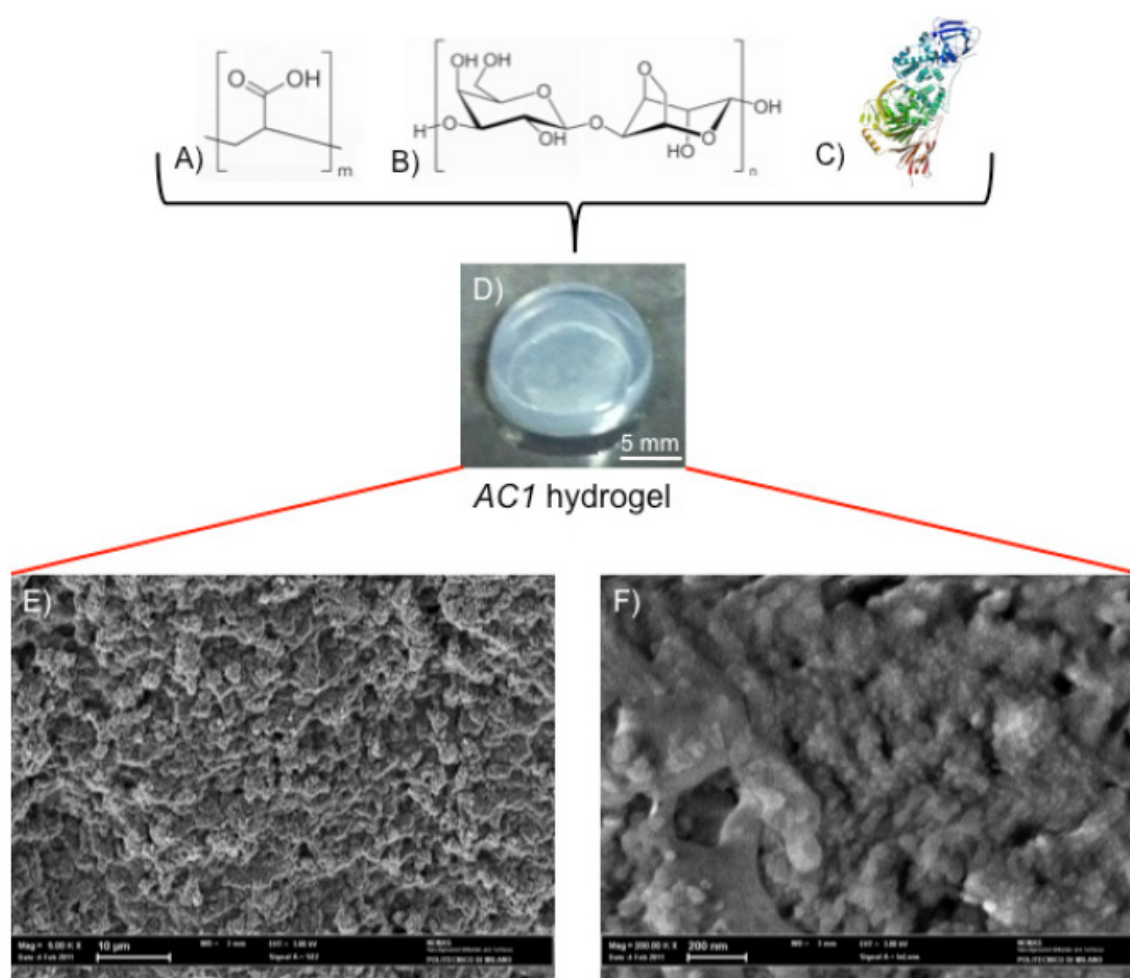

Figure 10. Carbomer 974P (A); agarose (B) were chemically cross-linked to form hydrogel in phosphate buffer saline solution. Esterification, hydrogen bonding and carboxylation bring polymer chains statistically closer, thus creating a stable heterogeneous structure. The gelling solution was homogenized together with chondroitinase ABC (chABC); (C) above sol-gel transition temperature. From a macroscopic point of view the resulting material appears as in (D). From a microscopic point of view the hydrogel is densely structured, as observable by ESEM analysis. [Adapted from Ref. 29 © MDPI Publishing]

2.18. Injectable functionalized self-assembling scaffold

Self-assembling peptides (SAPs) perfectly mimic the extra-cellular matrix's structural and conformational characteristics; can be injected into the lesion site; are reabsorbable; and have sites for biofunctionalizations making them a perfect nanomaterial for application in regenerative. Cigognini et al., 2011, explored the biofunctionalizability of self-assembling peptides to enhance the *in vitro* neural stem cells survival and differentiation. This injectable nanofibrous gel was composed of RADA16-I functionalized with a bone marrow homing motif (BMHP1) which was further optimized via the insertion of a 4-glycine-spacer for amelioration of scaffold stability and exposure of the biomotifs. The scaffold was injected immediately after contusion in the rat spinal cord was evaluated for its putative neuroregenerative properties in terms of early effects by semi-quantitative RT-PCR and the late effects by histological analysis. The axon regeneration/sprouting across the cyst was quantified by the presence of GAP-43 positive fibers and the relative value of GAP-43 immunopositive area was represented as percentage of the total cyst area (Figure 11Bi). Interestingly, a significantly greater synthesis of GAP-43 in 4G-BMHP1 group (12.9462.03% of the whole cyst area) in comparison with saline (6.3361.7% of the whole cyst area) and SCI control (5.8461.29% of the whole cyst area) groups was originated (Figure Bii). The chronic inflammatory response, evaluated by counting macrophages into the lesion site (Figure 11Ci), revealed that several infiltrating CD68 immunopositive cells in all groups, indicating that at the late phase of the injury the scaffold didn't significantly affect the host immune response (Figure Cii). Similarly, cyst and cavities extent, measured on hematoxylin-eosin stained sections (Figure 11Ai), was similar in all groups (Figure Aii) wherein no significant differences were observed among treatment and both control groups when the cavities size within the cyst excluding strands of connective tissue (trabeculae) were measured (Figure Aiii). Conclusively, the injectable SAP based scaffold demonstrated potential for enhanced matrix remodelling, release of trophic factors, cell migration and basement membrane deposition leading to an increased number of regenerating/sprouting axons after incomplete SCI. Apart from the neuro-compatibility, the scaffolds were able to fill the cavities at SCI site with implications leading to a good matrix for the in vivo delivery of growth factors and/or stem cells into the injured CNS [30].

2.19. Effects of dibutyryl cyclic-AMP on survival and neuronal differentiation of neural stem/progenitor cells

Enthused by the poor control over transplant cell differentiation and survival, Kim et al., 2011, introduced a combinatorial approach wherein the cell transplantation therapy was adjoined with a bioactive release platform. Firstly, dibutyryl cyclic-AMP (dbcAMP) was encapsulated within poly(lactic-co-glycolic acid) (PLGA) microspheres and embedded within chitosan guidance channels. Secondly, neural stem/progenitor cells (NSPCs) were seeded in fibrin scaffolds within the channels (Figure 12). The idea behind the study was to determine whether dbcAMP, which can influence the in vitro differentiation of NSPCs into neurons, will be able to enhance survival of transplanted NSPCs through prolonged exposure either in vitro or in vivo through its controlled release from microspheres in
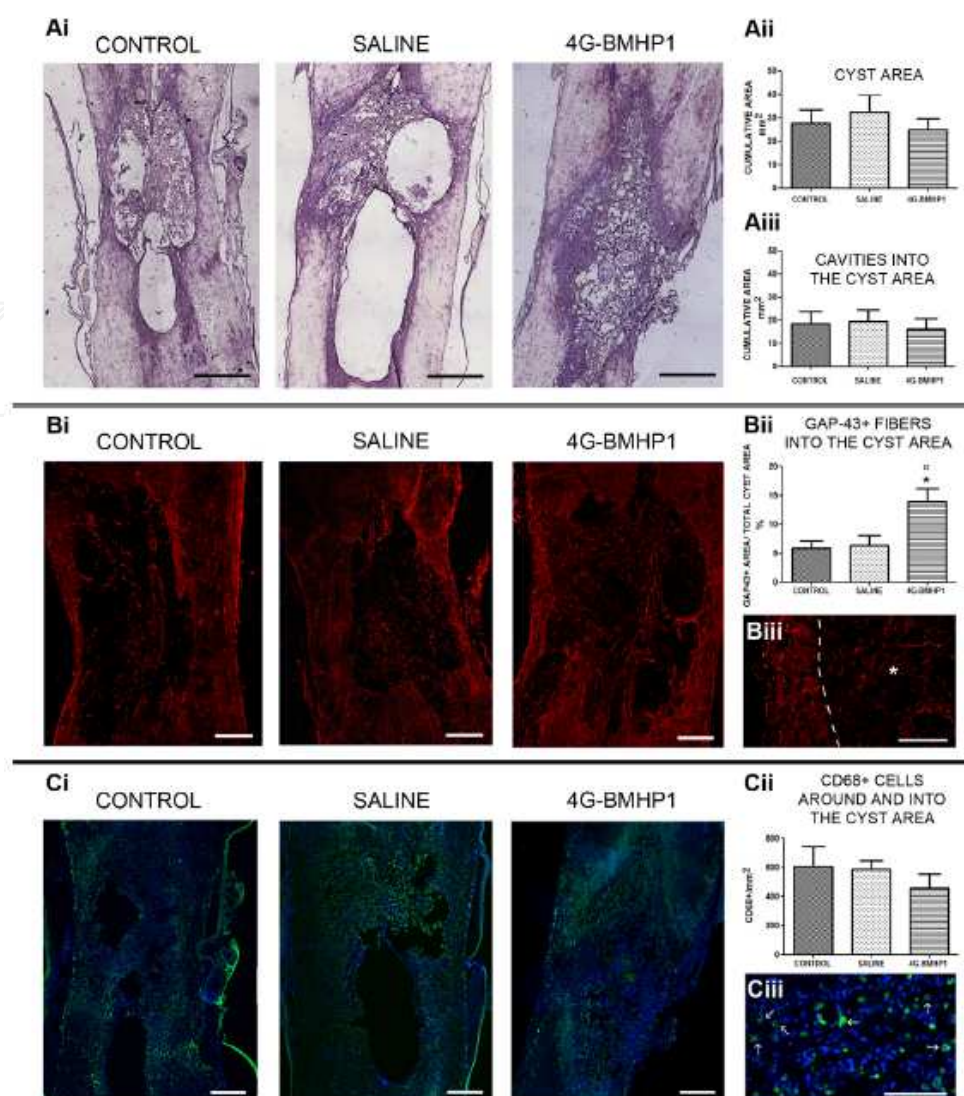
Figure 11. Quantitative histological analysis in the chronic phase of SCI. (A): lesion size was quantified on spinal cord longitudinal sections stained with hematoxylin/eosin (Ai) and it was reported as cumulative area (mm2). No significant differences among groups were found when measuring both the whole cyst area (Aii) and the cavities into the cyst area (Aiii). (B): GAP-43 positive fibers (Bi and Biii, red) were measured on six longitudinal sections after immunofluorescence staining and the values were expressed as percentage of the total area of the cyst. The GAP-43 immunopositive area was significantly higher in biomaterial-treated group (4G-BMHP1) than both control groups (saline and SCI control) (Bii). In Biii the positive GAP-43 signal is showed at higher magnification (asterisk and dotted line indicate the cyst and its border, respectively). (C): CD68 positive cells (Ci and Ciii, green) were counted on three longitudinal sections after immunofluorescence staining and reported as cumulative number per mm2. Nuclei were counterstained with DAPI. Macrophage infiltration was observed in the tissue surrounding the cyst and into the cavities of all groups (Cii). In Ciii, at higher magnification, an image representative of the CD68 positive cells (arrows) was observed in all groups [Adapted from Ref. 30 © PLoS ONE].

vicinity of NSPCs. The in vitro differentiation if NSPCs to betaIII-tubulin positive neurons was evaluated by immunostaining and mRNA expression. The in vivo studies were conducted by transplantation in spinal cord injured rats wherein the survival and differentiation of NSPCs was evaluated. For the greatest neuronal differentiation in the

presence of constant dbcAMP, the dbcAMP release from PLGA microspheres was optimized to release the bioactive for a period of 1 week based on the preliminary in vitro data. As depicted in Figure 12, drug release from native microspheres was linear over 11 days while the release of dbcAMP occurred over approximately 5 days after incorporation into channels. The mass balance results cleared this anomaly in release profiles which showed that the there was less drug content in dbcAMP microsphere loaded channels than expected microsphere quantity which was further due to drug losses during the process of embedding microspheres into the channel. Two major results were reported for the NSPC survival and differentiation as follows:

a. NSPC survival was highest in the dbcAMP pre-treated group, having approximately 80% survival at both time points as compared to that of stem cell transplantation results of less than 1% survival at similar times.
b. The dbcAMP pre-treatment resulted in the greatest number of in vivo NSPCs differentiated into neurons (37±4%), followed by dbcAMP-microsphere treated NSPCs (276±14%) and untreated NSPCs (15±67%).
c. The reverse trend was observed for NSPC-derived oligodendrocytes and astrocytes, with these populations being highest in untreated NSPCs.
d. Chitosan channels implanted in a fully transected spinal cord resulted in extensive axonal regeneration into the injury site, with improved functional recovery after 6 weeks in animals implanted with pre-differentiated stem cells in chitosan channels [31].
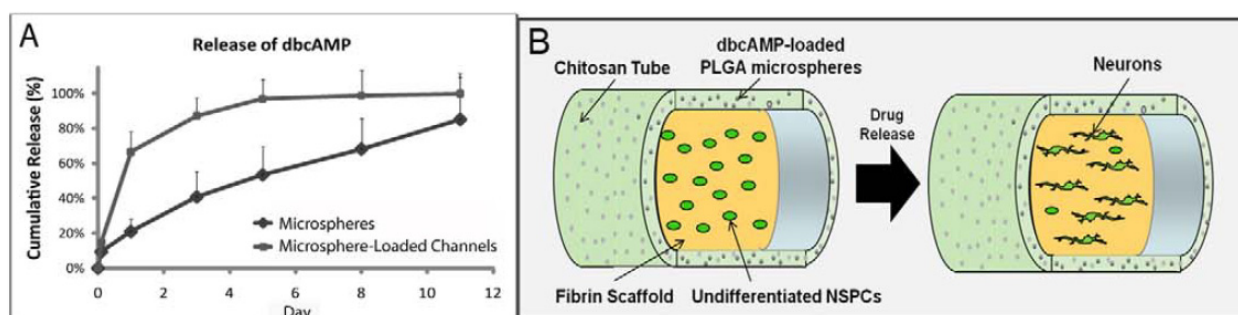

Figure 12. Microsphere-loaded channels effectively release dbcAMP in vitro. A) Cumulative release profiles of dbcAMP from free-floating microspheres and microsphere-loaded channels. The process of embedding microspheres into channel walls is likely responsible for early degradation of PLGA and faster drug release from channels. B) Schematic of the entubulation strategy. NSPCs are seeded on fibrin scaffold within a chitosan channel. Drug-loaded PLGA microspheres release the differentiation factor dibutyryl cyclic-AMP in a local and sustained manner, influencing NSPCs to preferentially differentiate into neurons [Adapted from Ref. 31 © PLoS ONE].

2.20. Local gene delivery from ECM-coated poly(lactide-co-glycolide) multiple channel bridges

In order to promote transgene expression in the injured spinal cord, Laporte et al., 2009, investigated the potential of surface immobilization to deliver complexed DNA (lipoplexes) from a multiple channel bridge in order to address the barriers related to cell survival, scar

tissue formation, and axonal elongation and guidance by targeting a range of cellular processes. The report was based on the inherent low expression levels of lipoplexes due to the lipoplex instability upon injection in vivo. The authors postulated that the "local delivery of lipoplexes from a biomaterial may have the ability to maintain lipoplex stability, and therefore increase the number of transfected cells and transgene expression". Based on their previous study, multiple linear guidance channels were able to support cell infiltration and integrate effectively into the spinal cord wherein the channels induced cell orientation along its major axis and supported and directed axon elongation across the channels [32]. Strategically, lipoplexes were immobilized to the surface of the bridges as follows:

i.   incubation of DNA with extra cellular matrix (ECM)-coated PLGA surfaces (incubation),
ii. drying of ECM onto PLGA and then drying of DNA onto ECM (2-step drying), and
iii. drying a mixture of DNA and ECM proteins onto PLGA surfaces (1-step drying).

A series of in vitro studies were carried out to investigate the surface properties of the polymer, three ECM proteins (collagen, laminin, and fibronectin), and the immobilization strategies for their ability to bind and stabilize the vector, and to transfect cells. In vivo studies were performed with a rat spinal cord lateral hemisection model using conditions identified from in vitro studies. In conclusion, the combination of the multiple channel bridge and gene delivery provided the required physical and chemical guidance cues for spinal cord regeneration [33].

3. Conclusion

The current drug delivery and tissue engineering techniques discussed in this chapter demonstrate that a only a combinatorial approach can provide the desired characteristics required for an efficient repair, regeneration, restoration, reconstruction, and reorganisation of the neural tissue after traumatic CNS injuries. A synergistic therapeutic approach can be designed by incorporating scaffolds with directional cues, bioactives to promote regeneration and repair, neural/progenitor cells for release of growth factors, and functionalized polymers for better neurocompatibility. Most importantly, the design of polymers capable of releasing the drugs, cells and biofactors at a predetermined and controlled rate is essential for the success of these tissue engineering approaches (Table 4). The chapter provides a comprehensive account of recent as well as long established approaches for the delivery of bioactive of neural interest. For further reading, the readers are encouraged to read various enlightening reviews in this area as tabulated in Table 5.

S. No.	Architecture	Biomaterials employed	Bioactive incorporated	Reference
1.	Injectable gel	Hyaluronic Acid-Methyl cellulose (blend)	Nimodipine; Erythropoietin	2-4
2.	Nanoparticles-incorporated multiple channel bridges	PLGA (scaffold); Hydroxyapatite (nanoparticles)	lentiviral vectors encoding NT-3 and BDNF	5

S. No.	Architecture	Biomaterials employed	Bioactive incorporated	Reference
3.	Lipid microtubules-incorporated In situ gelling hydrogels	1,2-bis-(triscosa-10,12-diynoyl)-sn-glycero-3-phosphocholine; Agarose	brain derived neurotrophic factor (BDNF)	6
4.	Photocrosslinked 3D scaffold	Agarose; Sulfosuccinimidyl-6-[4'-azido-2'-nitrophenylamino] hexanoate	Laminin-1	7
5.	Nanoparticles-incorporated injectable gel	PLGA (nanoparticles); agarose gel	Methylprednisolone	9,10
6.	hydrogel-microtube delivery system	Agarose; 1,2-bis-(tricosa-10,12-diynoyl)-sn-glycero-3-phosphocholine microtube	Chondroitinase ABC; Rho GTPase, CA-Rac1 and CA-Cdc42; and BDNF	11,12
7.	3D Scaffolds	PLGA	Schwann cells	13
8.	micro-cylinders embedded hydrogels	1,2-bis(10,12-tricosadiynoyl)-sn-glycero-3-phosphocholine; Agarose	nerve growth factor (NGF)	14
9.	injectable scaffold	Agarose; methylcellulose	-	15
10.	Injectable hydrogel	acrylated poly(lactic acid)-b–poly(ethylene glycol)-b–(poly lactic acid)	NT-3	16
11.	crosslinked hydrogels containing embedded microspheres	hyaluronic acid (HA); poly(lactic-co-glycolic acid) (PLGA)	BDNF and VEGF-loaded	17
12.	scaffolds	Fibrin; heparin; bi-domain peptide (ATIII peptide)	neurotrophin-3 (NT-3)	20
13.	Nerve guidance channels combined with microspheres	poly(hydroxylethylmethacrylate-co-methylmethacrylate); PLGA	Nerve growth factor	24
14.	nanofiber scaffold	Self assembling peptides	RhoA inhibitor	25
15.	Injectable hydrogel	Agarose; carbomer	Chondroitinase ABC (chABC)	29
16.	Self assembling peptide gel	RADA16-1	bone marrow homing motif (BMHP1)	30
17.	microspheres embedded within guidance channels	poly(lactic-co-glycolic acid) (PLGA); Chitosan; fibrin	dibutyryl cyclic-AMP (dbcAMP)	31
18.	disks and multiple channel bridges	poly(lactic-co-glycolic acid) (PLGA)	DNA complexes	33

Table 4. Summary of various bioactive-loaded platforms for spinal cord injury intervention

S. No.	View point discussed in the review	Reference
1.	The current approaches to drug delivery from scaffolds for neural tissue engineering applications and the challenges presented by attempting to replicate the brain, spinal cord, and peripheral nerve tissues were summarized	34
2.	The complex processes of cell guidance occurring within native ECM; strategies to design biomimetic scaffolds able to recapitulate these processes; approaches in controlling the release of the relevant factors; challenges to design novel scaffolds; time and space orchestrated exposure of biomacromolecular moieties	35
3.	Tissue engineering and novel therapeutic approaches to axonal regeneration following spinal cord injury. Axonal growth is supported by inherent properties of the selected polymer, the architecture of the scaffold, permissive microstructures such as pores, grooves or polymer fibres, and surface modifications to provide improved adherence and growth directionality.	36
4.	The state of the art work in electrospinning and its uses in tissue engineering and drug delivery	37
5.	Electrospun fibers mimic the nanoscale properties of native extracellular matrix; the fiber morphology is affected by changing the process parameters	38
6.	Tissue-engineered implant is a biologic-biomaterial combination in which some component of tissue has been combined with a biomaterial to create a device for the restoration or modification of tissue or organ function; Specific growth factors, released from a delivery device or from co-transplanted cells aid in the induction of host paraenchymal cell infiltration and improve engraftment of co-delivered cells for more efficient tissue regeneration; polymeric device development (micropaticles, scaffolds, and encapsulated cells) for therapeutic growth factor delivery in the context of tissue engineering was outlined.	39
7.	The tissue engineering paradigm includes a matrix or scaffold to facilitate tissue growth and provide structural support, cells, and the delivery of bioactive molecules; injectable materials are designed using processing techniques inherent to both tissue engineering and drug delivery.	40
8.	Basic technology of controlled protein delivery using polymeric materials; techniques under investigation for the efficient administration of proteins in tissue engineering	41
9.	An overview of strategies using natural and artificial substrates to present active biomolecules in the development of vascular structures; the replacement and augmentation of arteries using vascular grafts or stents; the recruitment of microvasculature secondary to an ischemic event or for the purpose of developing perfused, large volume tissue-engineered constructs	42
10.	Polyblend nanofibers and nanostructures can act as proxies of the native tissue, while providing topographical and biochemical cues that promote healing; they are prepared with mixtures of synthetically and naturally derived polymers that can behave cooperatively to demonstrate unique combinations of mechanical, biochemical and structural properties.	43
11.	Synthetic polymers used for tissue growth scaffold fabrication and their applications in both cell and extracellular matrix support and controlling the release of cell growth and differentiation supporting drugs.	44

S. No.	View point discussed in the review	Reference
12.	In the future, engineered tissues could reduce the need for organ replacement, and could greatly accelerate the development of new drugs that may cure patients, eliminating the need for organ transplants altogether.	45
13.	Biodegradable and biocompatible scaffolds have a highly open porous structure and good mechanical strength to provide an optimal microenvironment for cell proliferation, migration, and differentiation, and guidance for cellular in-growth from host tissue; natural and synthetic polymeric scaffolds can be fabricated in the form of a solid foam, nanofibrous matrix, microsphere, or hydrogel; scaffolds can be surface engineered to provide an extracellular matrix mimicking environment for better cell adhesion and tissue in-growth and can be designed to release bioactive molecules, such as growth factors, DNA, or drugs, in a sustained manner	46
14.	To develop electrospun nanofibers as useful nanobiomaterials, surfaces of electrospun nanofibers can be chemically functionalized by plasma treatment, wet chemical method, surface graft polymerization, and co-electrospinning of surface active agents and polymers for achieving sustained delivery through physical adsorption of diverse bioactive molecules such as anti-cancer drugs, enzymes, cytokines, and polysaccharides; surfaces can be chemically modified with immobilizing cell specific bioactive ligands to enhance cell adhesion, proliferation, and differentiation by mimicking morphology and biological functions of ECM.	47
15.	Biomaterials can enable and augment the targeted delivery of drugs or therapeutic proteins to the brain, allow cell or tissue transplants to be effectively delivered to the brain; help to rebuild damaged circuits; promote regeneration; repair damaged neuronal pathways in combination with stem cell therapies; nanotechnology allows greater control over material–cell interactions that induce specific developmental processes and cellular responses including differentiation, migration and outgrowth	48
16.	Application of biomaterials in (i) shunting systems for hydrocephalus, (ii) cortical neural prosthetics, (iii) drug delivery in the CNS, (iv) hydrogel scaffolds for CNS repair, and (v) neural stem cell encapsulation for neurotrauma	49
17.	Recent in vivo studies for the regeneration of injured spinal cord, including stem-cell transplantation, application of neurotrophic factors and suppressor of inhibiting factors, development of biomaterial scaffolds and delivery systems, rehabilitation, and the combinations of these therapies	50
18.	Applications of polymer-based delivery of small molecule drugs, proteins, and DNA specifically relevant to neuroscience research; the fabrication procedures for the polymeric systems and their utility in various experimental models; unique experimental tools with downstream clinical application for the study and treatment of neurologic disease offered by biomaterials	51
19.	Approaches adopted for management of SCI including pharmacologic and gene therapy, cell therapy, and use of different cell-free or cell-seeded bioscaffolds; developments for therapeutic delivery of stem and nonstem cells to the site of injury; application of cell-free/cell-seeded natural and synthetic scaffolds	52

S. No.	View point discussed in the review	Reference
20.	bioengineered strategies for spinal cord repair using tissue engineered scaffolds and drug delivery systems; degradable and non-degradable biomaterials; device design; combination strategies with scaffolds.	53
21.	The merger between the two powerful disciplines—biomaterials engineering and stem cell biology	54
22.	Inclusive survey of biopolymers seeded with Schwann cells (SCs) to be used for axonal regeneration in the nervous system	55
23.	Recent studies that utilize electrospun nanofibers to manipulate biological processes relevant to nervous tissue regeneration, including stem cell differentiation, guidance of neurite extension, and peripheral nerve injury treatments	56
24.	Smart biomaterials are capable to carry and deliver cells and/or drugs in the damaged spinal cord; an overview of a wide range of natural, synthetic, and composite hydrogels	57
25.	Self-assembled materials provide the ability to tailor specific bulk material properties, such as release profiles, at the molecular level via monomer design; an overview of self-assembling molecules, their resultant structures, and their use in therapeutic delivery; current progress in the design of polymer- and peptide-based self-assembled materials.	58

Table 5. Leading review reports incorporating the inherent mechanistic and fabrication details of various neural architectures employed for neural tissue engineering and drug delivery

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