Review

Biomaterial-based interventions for neuronal regeneration and functional recovery in rodent model of spinal cord injury: A systematic review

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Context: There is considerable interest in translating laboratory advances in neuronal regeneration following spinal cord injury (SCI). A multimodality approach has been advocated for successful functional neuronal regeneration. With this goal in mind several biomaterials have been employed as neuronal bridges either to support cellular transplants, to release neurotrophic factors, or to do both. A systematic review of this literature is lacking. Such a review may provide insight to strategies with a high potential for further investigation and potential clinical application.

Objective: To systematically review the design strategies and outcomes after biomaterial-based multimodal interventions for neuronal regeneration in rodent SCI model. To analyse functional outcomes after implantation of biomaterial-based multimodal interventions and to identify predictors of functional outcomes.

Methods: A broad PubMed, CINAHL, and a manual search of relevant literature databases yielded data from 24 publications; 14 of these articles included functional outcome information. Studies reporting behavioral data in rat model of SCI and employing biodegradable polymer-based multimodal intervention were included. For behavioral recovery, studies using severe injury models (transection or severe clip compression (> 16.9 g) or contusion (50 g/cm)) were categorized separately from those investigating partial injury models (hemisection or moderate-to-severe clip compression or contusion).

Results: The cumulative mean improvements in Basso, Beattie, and Bresnahan scores after biomaterial-based interventions are 5.93 (95% CI = 2.41 – 9.45) and 4.44 (95% CI = 2.65 – 6.24) for transection and hemisection models, respectively. Factors associated with improved outcomes include the type of polymer used and a follow-up period greater than 6 weeks.

Conclusion: The functional improvement after implantation of biopolymer-based multimodal implants is modest. The relationship with neuronal regeneration and functional outcome, the effects of inflammation at the site of injury, the prolonged survival of supporting cells, the differentiation of stem cells, the effective delivery of neurotrophic factors, and longer follow-up periods are all topics for future elucidation. Future investigations should strive to further define specific factors associated with improved functional outcomes in clinically relevant models.

Keywords: Spinal cord injuries, Axonal regeneration, Neuronal bridge, Biomaterials, Functional recovery, Translational research

Introduction

The hostile microenvironment at the injury epicenter inhibits neuronal regeneration after spinal cord injury

(SCI).¹ Specifically the myelin breakdown products Nogo and chondroitin sulfate proteoglycans are inhibitory to the advancing growth cone.² Some subsets of macrophages are inhibitory, while others secrete growth factors to facilitate regeneration.^{3–6} Bioengineering strategies have therefore focused on replicating a permissive

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environment for regeneration by designing multimodality interventions that combine biodegradable polymers, neurotrophic factors, and supporting cells for axonal regeneration.^{7,8} A variety of biodegradable polymers, neurotrophic factors, and supporting cells have been investigated in various combinations so far.^{9–32} There is considerable interest in translating appropriate experimental strategies for potential clinical application.³³ In a recent review, the design strategies for two of the most promising interventions (nerve guidance channels and hydrogels) were discussed for enhancing regenerative capacity after SCI.⁸ However, a systematic review of most efficacious axonal regeneration strategies using multimodal biomaterial-based interventions is lacking. Therefore, we systematically reviewed data from existing publications to critically analyse the biomaterial-based strategies and to optimize behavioral recovery after experimental SCI. Such systematic reviews are mainly utilized in clinical medicine to combine results from clinical trials and to draw conclusions regarding treatment effects.³⁴ These have recently been employed in the SCI literature to critically analyse the body of evidence for cell-based therapies, directly applied biologics, and neuroprotective agents.^{8,35–38} The goal of this review is to systematically review the design strategies and the predictors of improved functional outcomes after biomaterial-based multimodal interventions for neuronal regeneration in rodent SCI model.

Methods

The following operational definitions were developed for the purpose of data abstraction and analysis for this systematic review. We defined a biodegradable neuronal bridge as a ‘composite implant fabricated from biodegradable polymer specifically for axonal or cellular guidance’. The neuronal bridge can be either a scaffold or a gel. The consistency of this neuronal bridge was further classified as rigid or soft based on the material used, fabrication process, and scaffold architecture as reported in the publication. Conventionally for neuroprotective strategies the timing of intervention is typically within a few hours after SCI.³⁸ However, since this review explores interventions designed for axonal regeneration, we classified intervention within 72 hours as an acute phase strategy.³⁹ Interventions applied between 3 days and 4 weeks were considered sub-acute and interventions applied after more than 4 weeks were categorized as chronic phase interventions.

To maximize the search sensitivity, a broad search strategy was used in PubMed separately with various search words (e.g. ‘biodegradable scaffold’, ‘neuronal bridge’, ‘neuroregeneration’, ‘hydrogel’, ‘chitosan’,

‘gelatin’, ‘fibrin’, ‘collagen’, ‘polylactic acid’, ‘poly glycolic acid’, ‘poly alpha hydroxy acid’, ‘poly beta hydroxyl butyrate’, ‘methylcellulose’, ‘hyaluronic acid’, ‘polyethylene glycol’) in combination with ‘spinal cord injury’. A similar search was performed in the CINAHL database. Abstract reviews of all articles published in English were then performed.

Inclusion criteria for this study were published investigations that evaluated neuroregeneration using combination strategies with a neuronal bridge fabricated from a biodegradable polymer for axonal or cellular guidance along with either supporting cells or neurotrophic factors in a rodent SCI model. The injury being treated could be induced by contusion, clip compression, transection, or hemisection methods, and the intervention could be administered in acute, sub-acute, or chronic phases. Results from the latest publications were included in situations for which multiple publications reporting results of ongoing research were available. Studies utilizing only the biodegradable polymers alone and those investigating biodegradable polymers for drug delivery after intrathecal or intra-spinal injection were excluded.

Publications reporting results of *in vitro* studies evaluating the effect of scaffolds on neuronal cultures, feasibility of experimental strategy in small number of animals focused mainly on biocompatibility, and long-term effects of scaffolds without a control group were also excluded. Publications in languages other than English were not included; although, every attempt was made to acquire similar work published by the authors in English. Experiments conducted on canine or mammalian SCI models were not included because the functional outcome assessment in rodents is standardized.^{40–42} For the purpose of this article, we strictly included studies utilizing biodegradable components; therefore, experiments concomitantly using non-biodegradable scaffolds were excluded. The literature search was carried out in July 2010. The review of the studies was performed in collaboration by two investigators (V.K. and S.K.) and conclusions regarding inclusion or exclusion of studies were done by consensus.

Two investigators (V.K. and S.K.) independently abstracted data in July 2010. Standardized data abstraction sheets were developed, and any discrepancies between the independently abstracted information were resolved with discussion. As per the Quorum guidelines for clinical studies, we reviewed the publications for study design, including the animal numbers, proper experimental and control groups, follow-up period, type of SCI model, and timing of intervention.⁴³ Information regarding various characteristics of neuronal bridges including type of

biodegradable polymer, architecture, and supporting cells and neurotrophic factors were abstracted. The method of fabrication and implantation of the bridge was reviewed in detail. The outcomes assessed by the studies were categorized into behavioral tests, electrophysiology studies, imaging studies, and immunohistochemistry. The information gathered was then scanned for whether the multimodal approach provided contact guidance to regenerating axons, improved functional outcomes, or caused inflammation at the host–tissue interface. Data regarding *in vivo* survival and behavior of implanted cells and the concentration and release of neurotrophic factors were also retrieved. Finally, the implant and the method of implantation were studied for appropriateness for potential clinical application.

Data were entered into an Excel spreadsheet and checked for data entry errors. The meta-analysis was performed with STATA-10®™ (STATA Corp LP, TX, USA). Graphs were additionally constructed with MetaAnalyst Beta 3.13®™ (http://tuftscaes.org/meta_analyst/, Tufts University and Agency for Health Research and Quality (AHRQ), USA) software. For the cumulative meta-analysis of functional scores, the mean Basso, Beattie, and Bresnahan (BBB) scores and standard deviations at the last assessment were included. The BBB scores and respective standard deviations were extrapolated to the nearest decimal point from the published graphs in some studies.^{9,12,30} Studies with no published BBB scores^{10,16,17,19,22,24,29} or with published graphs which did not allow for extraction of values^{20,21,31} were excluded from cumulative meta-analysis. A ‘metan’ command was utilized for calculation of individual and pooled-weighted mean difference between experimental and control groups using the DerSimonian–Laird method. Heterogeneity was calculated with the inverse variance method. The pooled effect estimates were calculated using the random-effects model when the test of heterogeneity was significant. Forrest plots depicting these results were also constructed. A simple regression analysis was performed to investigate the heterogeneity among studies. Mean improvement in BBB score was chosen as the outcome variable whereas covariates analysed were duration of follow-up (fewer than 6 weeks vs. more than 6 weeks), type of biopolymer used (poly (alpha hydroxyacids) vs. others), type of construct (soft vs. rigid), neurotrophic factor binding domain (present vs. absent), type of cells used (stem cells vs. others), and type of multimodal approach used (biopolymer + supporting cells + neurotrophic factors vs. biopolymer with either supporting cells or neurotrophic factors). The cumulative mean improvement in BBB scores was separately calculated for the two groups of studies as categorized by the covariates.

Results

Literature review

A full-text review of 41 articles was performed. As shown in Fig. 1, 17 studies were excluded for reasons such as the reports concerned only cell survival/feasibility studies,^{44–50} preliminary or pilot studies,^{44–60} the study was not specifically designed to study axonal regeneration after implantation of biopolymer based multimodality intervention,^{56,58–60} or a non-biodegradable implant was used in conjunction with the biodegradable scaffold.^{55,57} The results are summarized in Table 1.

Overall characteristics of published studies

Spinal cord transection^{9–11,14,21,23–26,28,31,32} was the most common injury model followed by hemisection.^{12,13,15–19,22,27,29,30} Most studies contained information about the effects of acute intervention after induction of SCI but some studies also contained

Figure 1 Literature search and data abstraction from published studies investigating multimodal approach utilizing biodegradable polymer scaffold, supporting cells, and neurotrophic factors.

information about the effects in the sub-acute or chronic phase.^{13,16,17,20} The number of animals studied ranged from 18 to 89 with follow-up durations ranging from 4 to 40 weeks post intervention.

Implant characteristics

Biopolymer design

Collagen^{9,11-13,16,18} and poly (alpha hydroxyacids)^{14,23,24,26,27,30} were the most common biopolymers

Figure 2 Forrest plot of improvement in mean BBB scores between experimental and control groups among studies investigating biodegradable polymer scaffold, supporting cells, and neurotrophic factors for axonal regeneration in transection model.

Figure 3 Forrest plot of improvement in mean BBB scores between experimental and control groups among studies investigating biodegradable polymer scaffold, supporting cells, and neurotrophic factors for axonal regeneration in hemisection model.

Table 1 Study design and implant characteristics of studies investigating multimodality approach utilizing biodegradable polymer scaffold, supporting cells, and neurotrophic factors

Author-year	Type of biodegradable polymer and implant architecture	Cell encapsulation and concentration	Neurotrophic factor and concentration	Spinal cord injury model	Number of animals studied	Follow-up (weeks)
Oudega-2001 ²⁴	PLA50 and PLA100/10 6 mm long × 2.7 mm diameter tubes	Schwann cells (140 × 10 ⁶ cells/ml)	Not studied	T8-T9 transection	45	16
Novikov-2002 ²²	PHB fibers, 2-3 mm long and coated with alginate hydrogel and fibronectin	Schwann cells (80 × 10 ⁶ cells/ml)	Intrathecal BDNF-NT-3 used in comparison group	C3 hemisection	48	8
Teng-2002 ³⁰	PLGA (50-50) with polylysine Bilayered 3 × 4 × 1.5 mm scaffold	Neural stem cells (5 × 10 ⁵ cells/ml)	Not studied	T9-T10 hemisection	48	14
King-2003 ¹⁹	Fibronectin Unidirectional mats (10-100 μm)	Not studied	NT-3, BDNF, NGF	Thoracic hemisection	26	4
Joosten-2004 ¹⁸	Collagen gel 2 mm block	Neonatal astroglial cells (1.5 × 10 ⁵ cells/ml)	No	Thoracic hemisection	20	4
Patist- 2004 ²⁶	Freeze-dried PLA Tubular scaffold 4 mm long × 2.6 mm diameter with longitudinally oriented pores (<10 and 75-200 μm)	Not studied	BDNF	T9-T10 transection	49	8
Hurtado-2006 ¹⁴	Freeze-dried Poly D, L lactate. Tubular scaffold 4 mm long × 2.6 mm diameter with longitudinally oriented pores (<10 and 75-200 μm)	Modified Schwann cells (1 × 10 ⁶ cells/ml)	NT-3, BDNF	T9-T10 transection	37	6
Iwata-2006 ¹⁶	80% collagen-based hydrogel cylinders with uniaxial orientation of DRG constructs	Elongated DRG constructs	NGF	T9-11 hemisection. Sub-acute	25	4
Piantino-2006 ²⁷	PLA-PEG-PLA hydrogel <i>In situ</i> photo-polymerization of the hydrogel	Not studied	NT-3	T8 hemisection	18	6
Rochkind-2006 ²⁸	Dextran-gelatin tube with nanofibers filled with NVR-N gel. Tube diameter 2 mm and 0.4 mm wall thickness filled with 50-100 μm bundle of nanofibers	Nasal olfactory mucosal cells (1 - 2 × 10 ⁶ cells/ml) and human embryonic spinal cord cells (1 - 2 × 10 ⁶ cells/ml)	NVR-N gel containing NGF, BDNF	T7-T8 transection	20	8-40
Stokols- 2006 ²⁹	Templated agarose scaffold Multicomponent fiber bundles 1.5 mm long and channel diameter of 200 μm, wall thickness 100 μm	Bone marrow stem cells (1 × 10 ⁵ cells/ml)	BDNF	C4 aspiration lesions	37	4
Yargin-2006 ³¹	Spherogel surrounded with collagen film	Embryonic nerve cells (250 000 cells/animal)	Not studied	T10 transection	28	8-12

Continued

Table 1 Continued

Author-year	Type of biodegradable polymer and implant architecture	Cell encapsulation and concentration	Neurotrophic factor and concentration	Spinal cord injury model	Number of animals studied	Follow-up (weeks)
Guo-2007 ¹¹	Type 1 collagen scaffold 2 × 2 × 2 mm ³	Schwann cells (1 × 10 ⁴ cells/ml) Neuronal stem cells (1 × 10 ⁵ cells/ml)	NT-3	T8-T12 transection	60	8
Zhang-2007 ³²	Gelatin foam 2 × 2 × 2 mm ³	Schwann cells (1 × 10 ⁴ cells/ml) Neuronal stem cells (1 × 10 ⁵ cells/ml)	NT-3	T-9 transection	37	8
Nomura-2008 ²⁰	Chitosan channels 8 mm long × 1.8 mm diameter with 0.2 mm wall thickness. Fibrin sealant used	Intercostal nerve grafts	Not studied	T8 clip compression, sub-acute and chronic ®	89	SA 15 and C 18
Nomura-2008 ²¹	Laminin-coated chitosan channels 10 mm long × 4.1 mm diameter with 0.2 mm wall thickness	Neuronal stem cell-derived progenitor cells (3 × 10 ⁷ cells/ml)	Not studied	T8 transection	46	14
Pan-2008 ²⁵	Fibrin glue	Neural stem cells (5 × 10 ⁵ cells/ml)	Granulocyte- colony stimulating factor (50 µg/kg)	T8-9 transection	40	12
Han- 2009 ¹²	Linear ordered collagen scaffold Multiple 6 mm long × 2 mm diameter fibers	Not studied	CBD-BDNF	T8-T10 hemisection	40	15
Hatami-2009 ¹³	3D Type 1 collagen scaffold	Human embryonic stem cells-derived neuronal progenitor cells (unknown concentration)	Not studied	T-10 hemisection. Sub-acute	45	5
Itosaka-2009 ¹⁵	Thin fibrin fibers forming uniform mesh with pore size 2-5 mm	Bone marrow stem cells (3 × 10 ⁵ cells/ml)	Not studied	T8 hemisection	19	4
Johnson-2009 ¹⁷	Fibrin-based scaffold created <i>in situ</i> from 10 µl fibrin spheres	Not studied	NT-3	T9 hemisection. Sub-acute	28	2
Olson-2009 ²³	85:15 PLGA. 2 mm × 3 mm tube with seven 660 µm channels	Schwann cells (4.76 × 10 ⁵ cells/ml) Neuronal stem cells (4.76 × 10 ⁵ cells/ml)	Not studied	T8-9 transection	42	4
Fan-2010 ⁹	Linear collagen scaffold 2 mm long × 2 mm diameter	Not studied	CBD-NT-3	T8-T10 transection	72	16
Gros-2010 ¹⁰	Templated agarose scaffold. Uniform channels of diameter 200 µm with wall thickness of 100 µm	Bone marrow stem cells (7.5 × 10 ⁴ cells/ml)	NT-3 added within the scaffold as well as distal to the site of injury	C4 transection	30	4

used, followed by fibrin.^{15,17,25} Most of the studies described various scaffold designs whereas some utilized gel preparations of biopolymers.^{18,25,27,31} Linearly-oriented scaffolds were investigated in 12 studies with a pore size varying from 10 to 660 µm.^{9,10,12,14,16,19,22,23,26,28-30} Scaffold-binding domains for neurotrophic factors were employed in two studies^{9,12} whereas one study described the effect of controlled release of neurotrophic factors from a heparin-based delivery system.¹⁷ In some early

publications, the architecture of the biopolymer implant deteriorated over time.^{14,24,31} In more recent literature, the neuronal bridges maintained their architecture up to several weeks after implantation.^{10,13,20,21} Among the interventions studied, only a few are amenable for implantation using minimal access microinjection into the spinal cord.^{15,25,27} Most bridges, however, would require extensive procedures to create an implantation cavity (Table 2).

Table 2 Assessment of outcomes by published studies investigating multimodality approach utilizing biodegradable polymer scaffold, supporting cells, and neurotrophic factors

Author– year	Experimental and control groups/random allocation of animals in groups	Contact guidance (axons regenerated in scaffold)	Improvement in functional outcomes /blinded assessment	Inflammation in surrounding tissue	Survival and behavior of implanted cells <i>in vivo</i>	Concentration and release of neurotrophic factor <i>in vivo</i>	Comments
Oudega–2001 ²⁴	PLA50 with SC, PLA100/10 with SC No	More regeneration in PLA100/10, + myelinated fibers. Possible dieback after 2 months	Not studied Not applicable	Reactive astrocytes present at interface but no microglia or macrophages	SC survived for 16 weeks	N/A	Collapse of tubes over time
Novikov–2002 ²²	PHB coated with fibronectin and alginate, PHB coated with fibronectin and alginate + SC, alginate + fibronectin, alginate, fibronectin, gelfoam, i.t. BDNF, i.t. NT-3, no treatment No	PHB fibers with alginate/fibronectin and Schwann cells have synergistic effect. Growth across the distal end	Not studied Not applicable	Not studied	Not studied	N/A	Implant forms a bridge and resulted in survival of 75% of rubrospinal neurons
Teng–2002 ³⁰	Scaffold + NSC*, scaffold, NSC, no treatment* Yes	Corticospinal fibers regenerating across the implant	11 ± 1.5 vs. 6.5 ± 1.25 Yes	Scaffold may impede scarring and cyst formation at the injury site	More cells survived in the presence of scaffold	N/A	Scaffold may help in tissue preservation. No differentiation of stem cells
King–2003 ¹⁹	Fibronectin mats with either NT-3 or BDNF or NGF or saline No	Regeneration of both central and peripheral fibers in longitudinal and fascicular pattern	Not studied Not applicable	Not studied	N/A	Not studied	Good integration of mats with but not good approximation with edges in some. Decreased cavitation in spinal cord
Joosten–2004 ¹⁸	Collagen + neonatal astrocytes*, collagen* No	Yes, 102 ± 25 axons in experimental group. Fibers did not grow across the implant	13.4 ± 0.3 vs. 12.6 ± 0.2 No	Small rim of reactive astroglia at the interface, microglia in the matrix	Uniform distribution within matrix at 4 weeks	N/A	Collagen implant inhibits inflammation and scar formation
Patist– 2004 ²⁶	Scaffold+ BDNF + fibrin glue containing FGF*, scaffold with fibrin glue and FGF*, Fibrin+ FGF No	Yes, but no regeneration across the implant. No. of myelinated axons higher in fibrin only group (204 ± 46). Earlier appearance of myelination in BDNF group	6.5 ± 0.2 vs. 5.7 ± 0.6 Yes	GFAP-positive cells at the interface +microglia in the matrix	N/A	Not studied	No effect on tissue loss in both rostral and caudal spinal cord but BDNF implants promoted cell survival in proximal stump + vascularization

Hurtado–2006 ¹⁴	Scaffold +GFP-SC*, scaffold +D15A–GFP - SC*, fibrin only No	Lesser dieback of cut neurons in D15A–GFP-SCs group. No regeneration across the implant. More myelinated axons outside the implant	6.79 ± 0.31 vs. 6.33 ± 0.49 Yes	Modest GFAP-positive cells at the periphery along with a GFAP-free region at the interface as well	SCs mainly at the interface. Decreasing number over time	Not studied	Parallel orientation less after 1 week + vascularization
Iwata–2006 ¹⁶	Elongated DRG constructs with scaffold, DRG constructs without scaffold, scaffold No	Elongated DRG axons extended rostrally and caudally from implant to host tissue	No functional improvement Yes	Not studied	Survival of DRG neurons up to 1 month	N/A	Cyclosporine used post operatively
Piantino–2006 ²⁷	Hydrogel +NT-3*, hydrogel +PBS* No	Significant regeneration across the lesion from multiple dorsal tracts. Collateral contribution from surviving fibers	16.43 ± 0.86 vs. 13.75 ± 0.72 Yes	GFAP-positive cells at the periphery of the hydrogel	N/A	NT-3 concentration averaged over the sample was 50 ng/ml/g	Burst release of NT-3 over the first 24 hours, sustained release over 2 weeks
Rochkind–2006 ²⁸	Scaffold +NOM cells, scaffold + human embryonic spinal cord cells*, no treatment* No	Nerve fibers observed crossing the reparative tissue	8 ± 4 vs. 0.125 ± 0.331	Glial scar surrounding the section cavity	Not studied	N/A	Implant sutured
Stokols– 2006 ²⁹	Scaffold, scaffold + BDNF–GFP BMSC in matrigel, scaffold + BDNF–GFP BMSC in fibrin, scaffold + GFP BMSC in matrigel, Scaffold + GFP BMSC in fibrin, no treatment No	Yes Linear growth of axons with myelination. More in BDNF group. No regeneration across the distal end	Not studied	No fibrous tissue surrounding scaffold, reactive astroglia and macrophages surrounding implants	Longitudinal orientation of cells within the scaffolds	Not studied	Excellent integration of scaffold in host tissue with 97% channels containing cells
Yargin– 2006 ³¹	Spherogel + embryonic nerve cells, spherogel, only collagen film No	More thin myelinated fibers across the implanted site in the spherogel + cell group	Yes BBB 7–8 in spherogel + embryonic nerve cell group No	Significant scar tissue at the interface	Not studied	N/A	Immunofan used post operatively Spherogel slowly resorbed and replaced by connective tissue

Continued

Table 2 Continued

Author– year	Experimental and control groups/random allocation of animals in groups	Contact guidance (axons regenerated in scaffold)	Improvement in functional outcomes /blinded assessment	Inflammation in surrounding tissue	Survival and behavior of implanted cells <i>in vivo</i>	Concentration and release of neurotrophic factor <i>in vivo</i>	Comments
Guo– 2007 ¹¹	Scaffold with NSC, scaffold with SC + NSC, scaffold + Lac-Z NSC, scaffold + NT-3 SC NSC*, no treatment*, normal controls	Regeneration of both sensory and motor fibers across the transection site	10.76 ± 3.43 vs. 0.54 ± 0.32	Not studied	More NSC differentiated into neurons in NT-3 SC group	Not studied	Integration of scaffold in tissue
Zhang– 2007 ³²	No Scaffold + pre-differentiated NSC pretreated with NT-3 SC*, scaffold + pre-differentiated NSC pretreated with Lac-Z SC, scaffold + undifferentiated NSC + SC, scaffold + culture media only*, normal controls	Regenerating neurons with myelination. 5HT neurons also regenerated across the distal interface. 611.13 ± 37.4 axons in pre-differentiated NSC group	No 6 ± 1.41 vs. 0.8 ± 0.84	Decreased chondroitin sulfate proteoglycan surrounding the scaffold	More NSC differentiated into neurons in NT-3 SC group	NT-3 expression persisted for the entire duration	Tissue bridge across transection cavity
Nomura–2008 ²⁰	Yes Chitosan + peripheral nerve grafts, chitosan, no intervention No	Yes, 35 260 ± 10 590 axons after sub-acute implantation with chitosan channels and nerve grafts	No No functional improvement Yes	Reactive astrocytes present at interface along with microglia and macrophages	Nerve grafts degenerated after 2 weeks	N/A	No visible degradation of channels after 14 weeks
Nomura–2008 ²¹	Scaffold + brain-derived NSPC, scaffold + spinal cord-derived NSPC, scaffold, no treatment No	Both myelinated and unmyelinated axons in scaffold but no growth across the cavity.	No functional improvement Yes	Not studied	More NSPC survived in brain group and differentiated into adult glial cells	N/A	Thick tissue bridge in scaffold group. Cyclosporine used post operatively
Pan–2008 ²⁵	Fibrin+ NSC+ G-CSF*, fibrin + NSC, fibrin + G-CSF, fibrin* No	Significantly more neurons in NSC + G-CSF group	11.67 ± 0.17 vs. 3.86 ± 0.26 Yes	Not studied	Not studied	Not studied	Regenerating bridges at the interface. Spine stabilization performed
Han– 2009 ¹²	Scaffold + collagen-binding domain +BDNF*, scaffold + BDNF, scaffold + saline* No	Fascicular linear ordered growth of nerve fibers	18 ± 1.75 vs. 4.75 ± 3.75 Yes	Not studied	N/A	Gradual release of BDNF from CBD–BDNF over 14 days	

Hatami– 2009 ¹³	Scaffold + embryonic stem cell-derived NPC*, scaffold, no treatment*	Not studied	19 ± 0.5 vs. 17.3 ± 0.8	Not studied	Majority of cells undifferentiated after 5 weeks	N/A	Scaffold promotes more cells differentiating to neurons
Itosaka–2009 ¹⁵	Yes Scaffold + BMSC*, BMSC, saline* No	Not studied	Yes 14.8 ± 4.7 vs. 6.6 ± 5.2 No	No significant changes in surrounding inflammation	BMSC survived, migrated, and differentiated into neurons and glia	N/A	Cyclosporine used post operatively
Johnson–2009 ¹⁷	Scaffold + delivery system + 500 ng/ml NT-3, scaffold + delivery system + 1000 ng/ml NT-3, scaffold+ 1000 ng/ml NT-3, scaffold, no treatment	20 ± 6% more axons in 500 ng/ml HBDS as compared to 1000 ng/ml suggesting dose-controlled release is important for neuronal growth	Not studied Not applicable	Significantly decreased reactive astroglia surrounding the implant No difference in concentration of chondroitin sulfate or macrophages	N/A	Not studied	—
Olson–2009 ²³	No Scaffold + SC, scaffold +NSC*, scaffold + matrigel*	More axonal regeneration in SC group (<3000 axons)	1.92 ± 0.43 vs. 0.96 ± 0.04	Not applicable Not studied	Both NSC and SC survived up to 4 weeks	N/A	Scaffold channels demonstrated tissue bridge
Fan–2010 ⁹	No Scaffold +binding domain +NT-3*, scaffold +NT-3, scaffold +PBS, binding domain +NT-3, no treatment*, sham	Regeneration of both corticospinal and 5HT fibers across the lesion	Yes 6.6 ± 2 vs. 0.5 ± 1	Yes Dense scar at the interface	N/A	Not studied	
Gros–2010 ¹⁰	No Scaffold +MSC + NT-3 injection + conditioning, scaffold +MSC + NT-3 injection, scaffold +MSC, scaffold, no scaffold Yes	Oriented regeneration but limited reentry to distal stump. 340 + 25 axon/channel (8500 axons/scaffold) +myelination	Yes Not studied Not applicable	Yes Thick fibrous tissue at distal end of scaffold, no astrocytes +microglia at the interface Not applicable	Not studied	Not studied	Scaffolds maintained honeycomb appearance after 4 weeks

*Comparison groups for assessment of improvement in BBB scores.
BMSC, Bone marrow derived stem cell.

Supporting cells

Many studies (18 of the 24) incorporated various supporting cells in the multimodal approach. Stem cells were the most commonly studied supporting cells^{10,13,15,21,25,29,30} followed by Schwann cells.^{11,14,22–24,32} Although the concentration of stem cells^{10,25,30} ranged from 7.5×10^4 to 5×10^5 cells/ml, the concentration of Schwann cells^{11,24} varied greatly from 1×10^4 to 140×10^6 cells/ml. Most of the studies did not comment on the degree of purity of various cell lines. Recently, Schwann cells modified to release various neurotrophic factors have been studied as well.¹⁴ Other cell types studied are neonatal astroglial cells,¹⁸ nasal olfactory mucosal cells,²⁸ human embryonic spinal cord cells,²⁸ embryonic nerve cells,³¹ neural stem cell-derived progenitor cells,²¹ and human embryonic stem cells-derived neuronal progenitor cells.¹³ Most investigators documented prolonged survival of implanted supporting cells in the presence of biopolymers, but five studies did not comment on implanted cell survival.^{10,22,25,28,31} In most studies, stem cells remained undifferentiated. Some studies did include information about increased differentiation of stem cells into neuronal cell lines in the presence of 3D collagen^{11,13} and fibrin²⁵ scaffolds.

Neurotrophic factors

Neurotrophic factor 3 (NT-3)^{9–11,14,17,19,27,32} was the most commonly studied neurotrophic factor followed by brain-derived neurotrophic factor (BDNF),^{12,14,19,26,29} nerve growth factor,^{16,19} and granulocyte-colony stimulating factor (G-CSF)²⁵. Hurtado *et al.*¹⁴ investigated the composite molecule with both NT-3 and BDNF properties as well. As mentioned previously, various factor delivery methods have been employed including scaffold-binding domains^{9,12} and a heparin-based delivery system.¹⁷ Piantino *et al.*²⁷ elegantly demonstrated the pharmacokinetics of the NT-3 *in vivo* with a concentration of 50 ng/ml/g average over the samples. The majority of studies ignored this aspect, and only two other studies documented the duration of release of neurotrophic factors *in vivo*.^{12,32} Johnson *et al.*¹⁷ also reported a dose–response curve for axonal regeneration with more growth at a target delivery of 500 ng/ml of NT-3 compared to 1000 ng/ml.

Effect on the pathogenesis of SCI

Several studies described the presence of vascularization^{14,26} and the cellular bridge after implantation of a neuronal bridge.^{19,21–23,25,29,32} Although most studies described continued inflammation at the graft–host tissue interface, Joosten *et al.*¹⁸ also documented a

decrease in inflammation after implantation of a collagen-based gel. Similarly, others have observed less inflammation with subcutaneous administration of G-CSF up to 5 days after intervention.²⁵

Axonal regeneration

Axonal regeneration was reported in the majority of publications except two reports.^{13,15} Although most of these investigations revealed improved regeneration, growth across the distal stump was not observed in several studies.^{10,18,21,26,29} However, regeneration across the injury site was also described in several studies with reports of growth of both central and peripheral fibers including both corticospinal and rubrospinal pathways.^{9,11,22,28,32} Less dieback of the injured neurons was reported by some^{14,19,22,30} along with collateral contribution from existing neurons.²⁷ Several investigators even described a linear and fascicular pattern of growth in injured tracts.^{10,12,19,29} Overall myelination in the regenerating neurons was also observed.^{24,26,29,32} Only a few studies reported the number of regenerating axons^{10,17,18,20,23,26,32} with a range from a few hundred^{18,26,32} to a several thousand.^{10,20,23}

Assessment of functional outcomes

Functional outcomes were assessed by 18 studies using either the BBB or Tarlov scales (Table 2).^{9,11–16,18,20,21,23,25–28,30–32} Results from four studies could not be utilized for cumulative meta-analysis due to lack of published means and standard deviations in comparison groups.^{16,20,21,31} Data, in three of the publications, were extrapolated from published graphs, which reported mean scores and standard deviations for behavioral outcome and were used in the cumulative meta-analysis.^{9,12,30} The functional outcomes were separately analysed for the severe SCI (transaction, severe clip compression (≥ 16.9 g), and contusion (≥ 50 g/cm)), partial SCI (hemisection, mild clip compression (< 16.9 g), and contusion (< 50 g/cm)) groups.

Function outcomes in severe SCI model

Among the eight studies evaluating biomaterials for neuronal regeneration with transection SCI, the model showed improvement in BBB scores in five.^{9,11,14,23,26,28,32} Pan *et al.*²⁵ reported a mean BBB of 3.86 after performing a transection of 2 mm implying an incomplete injury. Similarly the mean BBB scores for studies by Patist *et al.*²⁶ and Hurtado *et al.*¹⁴ are 5.7 and 6.33, respectively. For studies reporting functional improvement in transection model, the mean difference in BBB score improvement between experimental and control groups was 5.93 (95% CI = 2.41–9.45) (Figure 2).

Table 3 Simple linear logistic regression analysis of effect of various study and implant characteristics on functional outcomes

Study characteristics		Number of studies	Cumulative improvement in BBB
Follow-up period*	>6 weeks	10	5.81 (3.16–8.46)
	<6 weeks	4	1.17 (0.69–1.64)
Scaffold containing binding domain	Yes	2	9.84 (2.34–17.33)
	No	12	4.01 (2–6.01)
Biopolymer used*	Poly (alpha-hydroxyacids)	5	1.73 (0.89–2.57)
	Others	9	6.72 (3.70–9.73)
Scaffold direction	Linear	7	4.09 (2.69–5.5)
	Others	7	5.07 (1.79–8.35)
Scaffold construct	Soft	4	4.64 (–0.11–9.39)
	Rigid	10	4.54 (3.29–5.80)
Type of cells used	Stem cells	6	6.02 (2.23–9.81)
	Others	8	3.34 (2.19–4.48)
Multimodality approach	Scaffold + cells + NTF	5	6.23 (1.96–10.5)
	Scaffold + cells or Scaffold +NTF	9	3.29 (2.36–4.22)

* $P < 0.05$.**Functional outcomes in mild or moderate SCI model**

The cumulative mean improvement in BBB among six studies employing a hemisection model was 4.44 (95% CI = 2.65–6.24). These results are not statistically significant from the transection model of injury ($P = 0.94$). Combining all the studies investigating incomplete SCI models the cumulative mean improvement in BBB is 4.23 (95% CI = 1.66–6.91) (Figure 3). There is a trend towards greater improvement in scores over time.^{12,13,15,25}

Predictors of functional outcomes

All the studies were incorporated in a meta-regression analysis to study the predictors of functional outcomes (Table 3). The covariates chosen were year of publication, model of injury, length of follow-up period, type of polymer used, architecture of the polymer, and type of cells used. The mean improvement in BBB scores among studies published before 2006 was significantly lower than for those published after 2006 (1.98 (1.08–2.88) vs. 6.6 (3.48–9.73)). A step-wise regression analysis was performed to study the effect of various factors on the functional improvement after intervention. The cumulative mean improvement in BBB scores among studies with follow-up greater than 6 weeks was statistically different from the others (5.81 (3.16–8.46) vs. 1.17 (0.69–1.64)). Similarly, the outcome appears to be adversely affected by the presence of poly (alpha hydroxyacids) biopolymer from the others (1.73 (0.89–2.57) vs. 6.72 (3.70–9.73)). Although the presence of a binding domain for neurotrophic factors resulted in more than two-fold increases in BBB scores, this improvement was not statistically significant (9.84 (2.34–17.33) vs. 4.01 (2–6.01)). Other factors including scaffold direction (linear vs. others), type of construct (soft vs. rigid), type of cells

used (stem cells vs. others), and type of multimodal approach used (biopolymer + supporting cells + neurotrophic factors vs. biopolymer with either supporting cells or neurotrophic factors) did not seem to influence the outcome in a statistically significant way.

Discussion

This systematic review analyses the results from published studies investigating axonal regeneration utilizing a neuronal bridge fabricated from biodegradable polymer, supporting cells, and neurotrophic factors. Functional outcomes and their predictors were also analysed.

Biopolymer scaffolds**Design strategies and future challenges**

The most common design for the biopolymer scaffolds was the unidirectional linear orientation. Most scaffolds were 2–4 mm in length; however, some publications reported implants as large as 10 mm.^{20,21} Among the publications reviewed, only three polymer designs are suitable for a minimally-invasive microinjection in the immediate post-injury phase.^{15,25,27} Despite the concerns of worsened injury from the pressure wave associated with microinjection technique it remains one of the main options for direct delivery of therapeutics after SCI.⁶¹ Since most of the preclinical studies were carried out in transection or hemisection models, the implantation of the scaffold in the lesion cavity was feasible. On the contrary, the majority of human SCI is a consequence of contusion mechanisms.⁶² The proposition of the placement of a scaffold by creating a cavity within the injury epicenter would not be particularly attractive for clinical application. With the recent FDA approval for clinical trials for human embryonic stem cell implantation in polymer scaffold, the

fabrication of neuronal bridges in the future should be done with a potential human application in mind.⁶³ A recently developed grading scheme to predict the translational potential of proposed interventions may be a useful guide for the future design of implants.⁶⁴

Future studies investigating novel SCI treatment should also incorporate methodological and design considerations to increase the external validity, specifically, the replicability of the experiment. Recent NIH funding for replication studies in SCI reinforces the fact that there are interventions that have led to contradictory results in different labs.⁶⁵ Ethical conduct of experiments with accurate reporting of outcomes is crucial. A detailed description of surgical procedure, parameters of SCI creation, and details of novel intervention administered in publications are also important. Other methodological considerations to increase validity include randomization of animals to receive either treatment or no treatment and blinded assessment of functional outcomes. Randomization of animals to experimental groups should ideally be carried out after creation of SCI. Among the studies reviewed only three publications reported randomization of animals to treatment groups.^{13,30,32} But a majority of the studies carried out blinded assessments of functional outcomes.^{9,12–14,16,20,21,23,25–30}

Neuronal regeneration and SCI pathogenesis

The relationship between extent of neuronal regeneration and functional improvement remains to be explored. Some studies reported a number of regenerating axons within the neuronal bridge but the data were not sufficient for a valid conclusion.^{10,17,18,20,23,26,32} A uniform approach involving quantification of the number, type, direction, and extent of regeneration in future studies can provide evidence for correlations between these two parameters. The detrimental effect of various inflammatory markers on regeneration in the central nervous system is well established.¹ Although some authors have reported less inflammation with particular scaffolds¹⁸ or neurotrophic factors,²⁵ the relative extent of inflammation with various neuronal bridges cannot be assessed with the current qualitative reporting (for example, ED-1 or GFAP staining). Therefore, more precise reporting of the number and distribution of astrocytes, macrophages, and microglia – especially at the implant–host interface – is desirable for future studies.

Functional outcomes and its predictors

Functional testing was not performed by one-fourth of the publications reviewed.^{10,17,19,22,24,29} In future investigations, every effort should be made to study functional

outcomes beyond 6 weeks to establish the utility of the intervention. As the functional outcomes continue to improve, kinematic gait analysis is recommended for the study of subtle differences between the groups.⁶⁶

The BBB scores in various published studies have steadily improved over the years and several factors may be responsible for this effect, including long-lasting degradation profiles of neuronal bridges, higher purity of supporting cells used for implantation, and better delivery of neurotrophic factors. Some initial studies reported degradation of biopolymer quite early after implantation and proposed that this could be detrimental to the neuronal regeneration and subsequent functional outcomes.^{14,24,31} Oudega *et al.*²⁴ reported breakage in both the PLA50 and PLA100/10 scaffold tubes with a resultant increase in dieback after 2 months. Similarly, Hurtado *et al.*¹⁴ reported less longitudinal orientation in the implanted tube after 1 week of implantation. This could be an explanation for the significantly poor gain in BBB scores among studies investigating poly (alpha hydroxyacids) (1.73 (0.89–2.57) vs. other polymers 6.72 (3.70–9.73)). Yargin *et al.*³¹ reported resorption and replacement of spherogel with connective tissue over time. Early degradation of biopolymers does not provide axonal or cellular guidance for regeneration. In more recent studies, however, the degradation profiles of the polymers were more durable with reports of architecture being maintained over several weeks after implantation.^{10,13,20,21}

The improvement in isolation techniques of various supporting cells can be another factor accounting for improved functional outcomes overtime. A majority of studies did not report the purity of their cell lines; therefore, this variable could not be studied in the regression analysis. Survival of the cells in the biopolymer microenvironment is another factor that could influence outcomes in several studies. Although the majority of the studies documented the presence of implanted cells several weeks after intervention, currently a quantitative determination of cell survival is not feasible. Greater stem cell differentiation into neuronal cell lines is another recent major advancement. Guo *et al.*¹¹ observed significantly more implanted stem cells differentiating into neurons in the presence of a Type 1 collagen scaffold and NT-3 secreting Schwann cells (20.34 ± 4.16% vs. 12.07 ± 2.35%). Similar results were reported by Pan *et al.*²⁵ who used a fibrin scaffold and subcutaneous administration of G-CSF. About one-third of implanted bone marrow stem cells in the gray matter expressed markers for mature neurons in the presence of fibrin as reported in another publication.¹⁵ Overall, after considering the plasticity of implanted

scaffolds, as mentioned previously, the adjunct of prolonged survival and differentiation into neurons results in effective cellular replacement after injury in the spinal cord and presumably improves outcomes as well.

A significant development that appears to improve functional outcomes is effective delivery of neurotrophic factors. Although this variable did not reach statistical significance, mean improvement in studies utilizing binding domains was 9.84 (95% CI = 2.34 – 17.33) compared to 4.01 (95% CI = 2 – 6.01) for other studies. Studies investigating this strategy incorporated collagen-binding domains for prolonged delivery of NT-3 and BDNF.^{9,12} Utilizing transection and hemisection models, these investigators reported robust regeneration across the interface into the distal stump along with significant improvement in functional outcomes. The *in vivo* release and concentration of neurotrophic factors remains largely absent from the current literature, but this information is needed for future studies to study the effects on functional regeneration.

Finally, it should be emphasized that the duration of follow-up significantly affected functional outcomes. The mean improvement in BBB scores among studies with a follow-up of more than 6 weeks was 5.81 (3.16 – 8.46) compared to 1.17 (0.69 – 1.64) for studies with a follow-up less than 6 weeks. Continued regeneration across the injured tissue could explain this finding as demonstrated by electrophysiological data from some studies.^{11,25,28} Pan *et al.*²⁵ reported significantly increased amplitudes and decreased latencies 12 weeks after implantation of neuronal bridges consisting of fibrin glue and neuronal stem cells along with subcutaneous G-CSF administration. Similarly, Guo *et al.*¹¹ reported recovery of cortical sensory and motor-evoked potential amplitude and latencies in the group transplanted with a collagen scaffold with NT-3 secreting Schwann cells and neuronal stem cells 60 days after implantation. Rockkind *et al.*²⁸ also reported similar trends in their study. Although the effects of training and compensation are difficult to dissect from this observed improvement, current evidence favors follow-up periods more than 6 weeks.

Limitations

Meta-analysis is mainly utilized in clinical medicine to combine the results of multiple randomized controlled trials.³⁴ Because the publications included in this systematic review randomly allocated animals to treatment and control groups, the combination of their results using meta-analytic technique is valid. Meta-analysis offers the advantages of simultaneously studying a

variety of variables, which would otherwise require significant time, material, and personnel resources.

This meta-analysis allowed us to investigate functional outcomes after implantation of neuronal bridges composed of biodegradable polymers, supporting cells, and neurotrophic factors. Functional outcomes have been progressively improving with more recent strategies. Factors associated with this improvement include the type of polymer used and a follow-up of more than 6 weeks. Incorporation of binding domains for neurotrophic factors also improves functional outcomes.

It is however, essential to note that the implications of functional outcomes represented by improved BBB scores in this paper are not to express supraspinal vs. spinally mediated mechanisms or, even more, to describe voluntary vs. involuntary function, but to express an absolute change in function via BBB scores post injury vs. post intervention. A statistically significant increase in BBB score was used as a representation of improved functional outcome. This, however, does not imply a clinically meaningful (supraspinal input-mediated, BBB score 12 and above) recovery in functional scores and therefore is a limitation of this review.⁶⁷ Supraspinal input vs. spinally mediated mechanisms of functional recovery are important topics for further research and discussion with a focus beyond the scope of this article.

Conclusion

A significant progress has been made in design of multi-modality interventions for application in SCI. However, the translational potential of these interventions must be weighed in the context of human SCI. Contrary to the more commonly investigated transection/hemisection models, the majority of human SCI is a consequence of contusion mechanisms.⁶² Whereas implantation of scaffolds may be feasible in the former, a more practical implantation method for contusive SCI must be kept in mind for clinical application.

A standardized and quantitative approach to SCI research is critical. Our study shows that a follow-up of more than 6 weeks is essential to adequately assess the effects of a study intervention on functional outcomes. As discussed in this study, the functional outcomes were studied by a majority of studies reviewed. However, the determinants of improved functional scores still remain elusive. For example, the correlations between the extent of neuronal regeneration and the degree of myelination and functional outcomes are not entirely clear. Qualitative descriptions of relationships between the effects of inflammation and other inhibitory

factors on regeneration are amply assessed in the literature, but a quantitative assessment using a measurement of biomarkers is desirable in future. Similarly, the relationship between survival and differentiation of supporting cells and effective *in vivo* delivery of neurotrophic factors require rigorous investigations. In conclusion, future studies investigating the issues related to the interaction between functional improvement and extent of inflammation, axonal regeneration, scaffold architecture, supporting cell concentration, and survival will require more careful analysis.

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