Fibrin as a scaffold for delivery of GDNF overexpressing stem cells to the adult rat brain

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ABSTRACT Treatment of neurodegenerative disease is entering a new era where direct intra-cerebral delivery of therapeutic factors aims to restore normality to dysfunctional circuits. Cell-based therapeutic approaches, where virally manipulated mesenchymal stem cells (MSCs) over-expressing glial cell line derived neurotrophic factor (GDNF) are utilised as vehicles to deliver neurotrophic support to the Parkinsonian brain, have shown promising pre-clinical results at preserving dopaminergic neuron integrity. However, poor cell survival following transplantation will hinder clinical progression. One approach to improve MSCs survival following transplantation is to couple the cell engraftment procedure with a scaffold thereby providing a physical substrate upon which to eventually complex pro-survival factors. Evaluation of commercially available, clinically accepted materials with an established safety profile will expedite clinical translation. Therefore, this study sought to determine if a clinically used fibrin scaffold can be utilised as an adjunct to intra-cerebral cell transplantation without evoking an adverse host or stem cell response. Sixteen male Sprague-Dawley rats received bilateral intra-striatal transplants of 30,000 GDNF-transduced MSCs delivered in either control transplantation medium or a fibrin scaffold. Rats were sacrificed 1,

4, 7, and 14 days post-transplantation. Brains were analysed to determine *in situ* polymerisation and biodegradability of the fibrin scaffold, GDNF release from transplanted GDNF-MSCs, survival of the GDNF-MSCs graft and the host's immune response to the transplant. This study found that fibrin scaffold was adaptable to intra-cerebral delivery with successful polymerisation of the fibrin scaffold *in situ*. Inclusion of the fibrin scaffold was not detrimental to cell survival nor did it impede neurotrophin release from entrapped cells. Importantly, the inclusion of the fibrin scaffold was associated with a reduced host astroglial and microglial response compared to cells alone indicative of a favourable biocompatibility profile. Overall, fibrin represents an adaptable scaffold for inclusion in a minimally invasive cell-based therapeutic approach for neurodegenerative diseases.

TEXT

Introduction:

Parkinson's disease is a progressive neurodegenerative movement disorder that affects an estimated 1.2 million people in Europe [1]. Notwithstanding the severe impact that the disease has on the patients' quality of life, Parkinson's disease constitutes a major health economic challenge in terms of direct medical costs as well as in-direct costs from reduced employment[1,2]. As the elderly population grows, the incidence of Parkinson's disease is set to increase substantially in the coming decades [2]. Such projections give a renewed impetus for the need to develop innovative treatment approaches to address the underlying neurodegeneration in an effort to slow disease progression [3].

Therapeutic delivery to the central nervous system (CNS) poses significant challenges for researchers. Unsuccessful delivery approaches for disease modulating interventions continues to hinder the clinical realisation of promising therapeutic candidates. One of the most exciting

novel therapeutic approaches to emerge for the treatment of Parkinson's disease has been the disease modulating neurotrophic factor, glial cell line-derived neurotrophic factor (GDNF)[4]. Extremely promising pre-clinical [5-8] and open-label clinical trials[9-11], confirming the neuroprotective role of GDNF on dopaminergic neurons, renewed enthusiasm that disease modulating approaches were within reach. Unfortunately, issues related to the delivery of GDNF undoubtedly contributed towards the failure to reach satisfactory clinical outcome measures in a double-blind placebo-controlled study where patients received intra-putamenal infusion of recombinant GDNF via implanted catheters [12,13]. Thus, clinical realisation of GDNFs potential appears to be intrinsically related to the successful development of an effective intra-cerebral delivery platform.

In this regard, *ex vivo* gene therapy, where cells are engineered to produce GDNF, may prove a more effective strategy for providing local and sustained delivery of GDNF than simple brain diffusion. Bone marrow-derived mesenchymal stem cells (MSCs) possess numerous desirable characteristics for clinical application given their relatively hypo-immunogenic profile [14-18] as well as their ease of isolation, expansion capacity and amenability to genetic manipulation [19-22]. Importantly, stereotaxic injection of autologous MSCs to Parkinson's disease patients in an open-label trial was not associated with any serious adverse events [23] and highlights the advanced phase that this novel therapeutic concept has reached in a relatively short time frame.

As with all novel therapeutic approaches, systematic and incremental pre-clinical evaluation is essential in order to predict potential caveats to successful clinical translation. While we have shown that virally manipulated, GDNF over-expressing MSCs offer significant potential for the delivery of neurotrophic support to the Parkinsonian rat brain [24,25], successful clinical

translation will undoubtedly be hindered by the poor survival of MSCs following transplantation [16,24]. This concern is further corroborated by a recent study where autologous MSCs genetically engineered to over-express GDNF were unilaterally transplanted into the striatum and substantia nigra of cynomolgus monkeys [26]. While the transplanted GDNF over-expressing MSCs unquestionably provided protection against MPTP-induced neuronal damage (sparing contralateral limb motor function and enhancing striatal dopamine uptake), approximately only 10% of the engrafted cells remained eleven weeks post-transplantation [26]. The limited survival of the GDNF-expressing MSCs may explain why low to non-detectable concentrations of GDNF were recorded in the post-mortem tissue. Thus, strategies aimed at improving the survival of GDNF-expressing MSCs following transplantation to the brain are required in order to ensure the continued advancement with this promising neurotrophin delivery approach.

One such effort to improve MSCs survival following transplantation is to couple the cell engraftment procedure with a biomaterial scaffold thereby providing a physical substrate upon which to complex pro-survival factors. To expedite the development of such a complimentary transplantation scaffold, it would be advantageous to utilise a biomaterial that is commercially available, clinically accepted and has significant pre-clinical data available to substantiate its safety profile. One such material fulfilling these criteria is fibrin, the biologically derived matrix which occurs during the wound healing cascade. Fibrin has favourable architectural and surface properties which make it a desirable material upon which to build a multimodal pro-survival matrix. Indeed, pre-clinical studies have illustrated the capacity of fibrin to facilitate protein [27-33] and gene delivery [34-40] *in vivo*. Moreover, fibrin has been evaluated as an adjunctive material to aid cell transplantation [41-43] further highlighting its potential utility as a complimentary transplantation material.

Fibrin is formed through the polymerisation of the soluble precursor molecule fibrinogen [44], a process that is initiated by the serine protease thrombin. As the dual component fibrin matrix system necessitates the mixing of fibrinogen with thrombin to yield the fibrin scaffold *in situ*, fibrin has traditionally been evaluated in easily accessible compartments such as the spinal cord [45], peripheral nerve [46] or directly to the brain surface [47]. In order to be considered for integration into a neurodegenerative cell-based therapeutic approach, fibrin delivery to the CNS must be minimally invasive and preferably injectable. Therefore, this study sought to determine if a commercially available, clinically accepted fibrin source could be utilised as an adjunct to intra-cerebral cell transplantation without evoking an adverse host or stem cell response.

Experimental Section:

Materials and Methods

All procedures were carried out in accordance with European Union Directive 2010/63/EU and S.I. No. 543 of 2012 and were reviewed and approved by The Animal Care and Research Ethics Committee of the National University of Ireland, Galway. Male Sprague–Dawley rats, sourced from Charles Rivers, UK were used in all experiments.

Experimental Design

Sixteen male Sprague-Dawley rats received bilateral intra-striatal transplants of 30,000 GDNF-transduced GFP-MSCs (hereafter referred to as GDNF-MSCs) delivered in either control transplantation medium (n=8 rats) or a fibrin scaffold (n=8 rats). Rats were sacrificed by anaesthetic overdose and transcardial fixation at 1, 4, 7, and 14 days post-transplantation (n=2 rats per group per time-point yielding n=4 independent transplantation sites per group

per time point). Brains were analysed to determine fibrin scaffold *in situ* polymerisation and biodegradability, GDNF release from transplanted GDNF-MSCs and the host's immune response to the transplant using immunohistochemistry. Survival of the GDNF-MSC graft was studied using fluorescent microscopy.

Mesenchymal Stem Cell Isolation and Expansion

Green fluorescent protein MSCs (GFP-MSCs) were isolated from the bone marrow of 8- to 12-week-old Sprague-Dawley transgenic rats (extracted from the "green rat" SD-Tg (CAG-EGFP) CZ-004Osb [48,49]). Briefly, after euthanasia by CO_2 inhalation, femora and tibiae were dissected and cleaned of musculature. Under sterile conditions, bone ends were removed and marrow was flushed from the femoral and tibial compartments with Alpha MEM-F12 medium. Marrow plugs were pooled, triturated and the cell suspension was centrifuged at 500 x g for 5 min. Cells were counted and plated at a density of 9×10^5 cells cm^{-2} in complete rat MSC medium (44.5% Alpha MEM; 44.5% F12; 10% FBS; 1% penicillin/streptomycin) and incubated at 37^oC in 5% CO_2 at 90% humidity. Non-adherent cells were washed away after 3 days and adherent cells were fed with fresh complete medium. Cells were ready for sub-culture after 16–17 days. MSCs were characterised by differentiation along adipogenic, chondrogenic and osteogenic lineages and FACS analysis demonstrated that cells were negative for CD45 and positive for the transferrin receptor CD71 and the stem cell associated CD172, as previously shown [50].

GDNF-Retroviral Transduction of MSCs

GFP-MSCs were virally transduced with a Moloney murine leukemia virus with GDNF transgene expression driven by the 5' long terminal repeat (LTR) intrinsic promoter as previously described [51].

Surgery

Surgery was performed under gaseous isofluorane anaesthesia (5% in oxygen for induction and 2% in oxygen for maintenance). GDNF-MSCs were transplanted directly into the striatum using a stainless steel cannula at stereotaxic coordinates A.P. 0.0, M.L. ± 3.7 and D.V. -5.0 mm from bregma with the nose bar set to -2.3 mm. Cells were delivered at 10,000 cells/microlitre/minute (using an automated delivery pump) in either control transplantation medium (DMEM/F12, 0.66% glucose, 0.125% $NaHCO_3$, 0.0056M HEPES containing 0.05% DNase) or suspended within the fibrinogen componenet of the fibrin scaffold (1.5 µl of 60 mg ml^{-1} human fibrinogen : 1.5 µl of 4 IU human thrombin; TISSEEL™ [fibrin sealant], Baxter). To avoid polymerisation of the fibrin scaffold within the injection cannula, the fibrinogen and thrombin components were delivered sequentially, physically separated in the cannula with a small volume of sterile saline (0.2 µl) which may dilute the scaffold concentration by a factor of 15.

Tissue Processing

Rats were deeply anaesthetised by pentobarbital injection (100 mg kg^{-1} i.p.) and transcardially perfused with 100 ml of heparinised saline (5000 units L^{-1}) followed by 150 ml of ice cold 4% paraformaldehyde in phosphate buffer. Brains were removed and post-fixed for four hours at room temperature before transferring to 25% sucrose in phosphate buffer. Serial coronal brain sections (30 µm) were cut using a freezing stage sledge microtome (Bright, UK).

Peroxidase-based Immunohistochemistry

Immunohistochemical staining for fibrin scaffold, GDNF, OX-42 and GFAP was completed on a 1:12 series of free floating sections using a peroxidise-based method as described

previously [24,52]. For each individual antigenic target, all control and treatment groups were processed at the same time to avoid heterogeneity between antibody solutions. In brief, endogenous peroxidase activity was quenched using a solution of 3% hydrogen peroxide/10% methanol in distilled water. Non-specific antibody binding was blocked using 3% normal serum in Tris-buffered saline (TBS) with 0.2% Triton X-100 at room temperature for 1 hour. Sections were incubated overnight at room temperature with the appropriate primary antibody diluted in TBS with 0.2% Triton X-100 (mouse anti-fibrin; goat anti-GDNF 1:200, R&D systems, USA; rabbit anti-GFAP, 1:2000, Dako, UK; mouse anti-OX42, 1:400, Chemicon, Ireland). Sections were incubated with biotinylated secondary antibody for 3 hours (goat anti-rabbit, 1:200, Jackson, UK or horse anti-mouse, 1:200, Vector, UK) followed by 2 hours incubation in streptavidin–biotin–horseradish peroxidase solution (Dako, UK). Sections were developed in 0.5% diaminobenzidine tetrahydrochloride (Sigma, Ireland) or Vector SG (Vector, UK) in Tris buffer containing 0.3 µl ml^{-1} of hydrogen peroxide. Sections were mounted on gelatin-coated microscope slides, dehydrated in alcohols, cleared in xylene, and cover-slipped using DPX mountant (BDH chemicals, UK). For immunofluorescent staining, after blocking non-specific antibody binding, the sections were incubated overnight at room temperature with the appropriate primary antibody (as above). Sections were incubated for three hours with fluorophore-labeled secondary antibody (either goat anti-rabbit Alexa Fluor 546, 1:200, Invitrogen; goat anti-mouse Alexa Fluor 546, 1:200, Invitrogen, UK). Sections were mounted onto gelatin-coated microscope slides and cover-slipped using Fluoromount fluorescence mounting medium (Sigma, Ireland).

Image Analysis

Photomicrographs of peroxidise-based immunostained sections from the striatum were taken using a Nikon WD70 dissecting microscope. Fluorescent photomicrographs were taken with using an Olympus IX81 fluorescent microscope. The presence of the fibrin scaffold, the

survival of the MSC graft [16] and the release of GDNF from the MSC graft were measured by volumetric analyses. The volume of immunostaining was calculated according to Cavalieri's principle, using the cross-sectional areas measured on a series of sections through the striatum with Image J software (ImageJ v1.41o; National Institute of Health, Bethesda, ML, USA). The area and density of microgliosis and astrocytosis was measured from OX-42 and GFAP immunostained coronal sections. For each graft site, a single mid-graft representative section was chosen at the same stereotaxic coordinate of -0.3 to -0.4 mm posterior to bregma, and the area or density of staining was measured using Image J software.

Statistical Analysis

GraphPad Prism 5 statistical software was used to analyse all data. All data are expressed as mean ± standard error of the mean (s.e.m). One-way ANOVA, using Newman-Keuls post-hoc where appropriate, was used to assess the presence of the fibrin scaffold, while two-way ANOVA, with Bonferroni post-hoc test where appropriate, was used to analyse the impact of the fibrin scaffold on all other parameters over time.

Results:

In situ Polymerisation and Degradation Profile of the Fibrin Scaffold in the Rat Striatum

We first sought to determine if the fibrin scaffold was adaptable for intra-cerebral delivery. Individual components of the fibrin system were injected directly into the striatum sequentially, with the fibrinogen component carrying the suspended cells injected initially followed by the thrombin component. Post-mortem histological analyses revealed the successful *in situ* formation of a fibrin scaffold within the striatum (Fig. 1A). Immunohistochemical staining targeting human fibrin revealed that a large fibrin scaffold was evident at the transplantation site day 1 post-transplantation (Fig. 1A&B). By day 4 post-transplantation, the fibrin scaffold was still evident, however, the scaffold had reduced

significantly in volume, indicative of resorption (Fig. 1B: Time, $F_{(3,16)}$=9.50; P<0.01 vs. Day 1 Fibrin by 1-way ANOVA with Newman-Keuls post-hoc test). By day 14 post-transplantation, the fibrin scaffold was difficult to detect and had largely degraded (Fig. 1A&B).

Fibrin Scaffold is Compatible with the Delivery of GDNF-MSCs to the Rat Striatum

We next sought to determine the impact that the fibrin scaffold had on GDNF-MSCs delivery and persistence at the graft site. Rats received intra-striatal injections of GDNF-MSCs delivered in either control medium or with the fibrin scaffold. Qualitatively, when delivered within the fibrin scaffold, the visual appearance of the GDNF-MSCs graft did not differ from cells delivered in control buffer (Fig. 2A). Cells formed a homogeneously dispersed graft at the transplant site (similar to those delivered in control buffer) indicating that the fibrin scaffold did not impede GDNF-MSCs delivery to and dispersal at the graft site (Fig. 2A). Quantitatively, graft volume measurements indicated that GDNF-MSCs delivered within the fibrin scaffold had a similar survival profile as GDNF-MSCs delivered with control buffer. While there was a trend for greater graft volume in the fibrin group, this did not differ significantly from control groups at any time-point (Fig. 2B: Group, $F_{(1,24)}$=2.08; P=0.16, ns). In line with previous published reports from our laboratory [24], the volume of the graft declined significantly over time, with no negative impact from the presence of the fibrin scaffold evident at any time point (Fig. 2B: Time, $F_{(3,24)}$=18.52; P<0.001, by 2-way ANOVA with Newman-Keuls post-hoc test).

Fibrin Scaffold allows GDNF Diffusion from Entrapped Cells following Transplantation

We next sought to determine if the presence of the fibrin scaffold was conducive to neurotrophin diffusion from the entrapped GDNF-MSCs. Following transplantation to the brain, GDNF-MSCs released GDNF into the cerebral tissue with immunohistochemical staining revealing a large neurotrophic milieu surrounding the graft site (Fig. 3A and B). The volume of GDNF released from control and fibrin groups did not differ significantly at any time-point examined (Fig. 3A and C: Group, $F_{(1,23)}$=1.39, P>0.05, ns.), demonstrating that the fibrin scaffold did not impede neurotrophin released from the transplanted GDNF-MSCs. In line with our previously published studies [24], given that the GDNF-MSC grafts did not persist in the brain, the volume of GDNF released into the striatum from the transplanted cells declined significantly over time (Fig. 3A&C: Time, $F_{(3,23)}$=21.07, P<0.001, 2-way ANOVA with post-hoc Newman Keuls).

Host's Microglial Response to the Fibrin Scaffold

This part of the study sought to determine if transplantation of GDNF-MSCs in a fibrin scaffold affected the host's response to the transplanted cells. The area and density of microgliosis was determined using immunohistochemical staining for OX-42, a microglial marker. The area of microgliosis surrounding the cells transplanted in control medium and in a fibrin scaffold differed significantly at day 14 post-transplantation (Fig. 4B; Day 14, $F_{(1,16)}$=2.98; P<0.01). Control and fibrin groups did not differ significantly in density of microgliosis at any time-point examined (Fig. 4B; Group, $F_{(1,16)}$=0.003; P=0.95, ns). Results showed that the area and density of microgliosis surrounding the transplants did not differ significantly for cells transplanted in control medium or in a fibrin scaffold (Fig. 4B: Area: Group, $F_{(1,16)}$=2.98; P=0.10, ns; Density: Group, $F_{(1,16)}$=0.003; P=0.95, ns). Moreover, there was no significant difference in the area or density of microgliosis over the time-points examined (Fig. 4B Area: Time, $F_{(2,16)}$=2.86; P=0.09, ns. Density: Time, $F_{(2,16)}$=0.35; P=0.71,

ns). These results suggest that delivery of GDNF-MSCs within a fibrin scaffold did not negatively affect the host's microglial response to the cells indicating the biocompatibility of the fibrin scaffold. Indeed, inclusion of a fibrin scaffold significantly reduced the host response to the transplanted cells two weeks after transplantation, indicating the biocompatibility of the fibrin scaffold.

Host's Astroglial Response to the Fibrin Scaffold

The area of astrocytosis surrounding the transplants did not differ significantly for cells transplanted in control medium or in a fibrin scaffold (Fig. 5B: Group, $F_{(1,24)}$=2.56; P=0.12, ns.). However, the density of astrogliosis at day 7 post-transplantation differed significantly between control and fibrin groups (Fig. 5B: Day 7, $F_{(1,24)}$= 5.09; P<0.01, 2-way ANOVA with post-hoc Bonferroni). These results suggest that delivery of GDNF-MSCs within a fibrin scaffold did not negatively affect the host's astroglial response to the cells indicating the biocompatibility of the fibrin scaffold. Inclusion of a fibrin scaffold significantly reduced the host response to the transplanted cells 7 days after transplantation, indicating the biocompatibility of the fibrin scaffold.

Discussion:

The present experiment sought to determine the utility of fibrin as an auxiliary biomaterial scaffold for delivery of neurotrophin over-expressing MSCs to the brain. To address this, adult rats received intra-striatal transplants of GDNF over-expressing MSCs delivered in either control transplantation buffer or combined with a fibrin scaffold. This study supports the supposition that the fibrin scaffold can be adapted for intra-cerebral delivery. Sequential injection of the individual components, fibrinogen and thrombin, resulted in the *in situ*

formation of a fibrin scaffold in the striatum. Importantly, the therapeutic molecule, GDNF, was released into the striatal tissue from the stem cells contained within the fibrin scaffold. Inclusion of a fibrin scaffold with the minimally invasive cell transplantation procedure did not induce an aversive host response and stem cell survival was not affected.

In order to be considered for integration in a cell-based therapy for neurodegenerative diseases, fibrin was tested in an intra-cerebral injectable format. Following sequential injection of fibrinogen and thrombin to the rat striatum, a large fibrin scaffold formed in the striatum indicative of successful *in situ* polymerization and confirming a viable delivery approach for the fibrin scaffold. Time-course analyses revealed that the fibrin scaffold had largely undergone resorption one week post-transplant, as expected [53,54]. Proteases such as plasmin and scaffold metalloproteases are principally responsible for the degradation of fibrin scaffold *in vivo* [55]. Our experimental paradigm relied on the *in situ* polymerisation of the fibrin scaffold in order to gain proof-of-concept for the utility of fibrin in a minimally invasive transplantation approach. Therefore, it is likely that endogenous plasminogen from the host was incorporated into the scaffold accelerating the degradation profile compared to that of a pre-polymerised scaffold [53]. While the fibrin scaffold we delivered contained the protease inhibitor aprotinin, this small molecule rapidly diffuses out of matrices and therefore does not provide extended degradation protection. To address this limitation of the relatively rapid resorption of fibrin, Hubbell and colleagues have engineered an aprotinin variant that can be immobilized within fibrin thus significantly extending scaffold longevity [54]. Such an approach could be considered for integration into this system in an effort to prolong scaffold retention. Indeed, exploitation of differential fibrin degradation kinetics offers significant potential to develop a temporal release system to provide various pro-survival factors to promote stem cell survival over time. Our group has previously utilised this property to develop the fibrin-in-fibrin system, where highly cross-linked fibrin microspheres containing

DNA are contained within the fibrin scaffold, with differential degradation processes allowing temporal release of encapsulated molecules [40].

Microarchitecture also affects degradation of the fibrin scaffold by fibrinolysis [44]. Thrombin concentration at the time of gelation has a profound influence on fibrin clot structure. Low thrombin concentrations produce fibrin clots composed of thick, loosely-woven fibrin strands. Higher concentrations are composed of relatively thinner, more-tightly packed strands which are more resistant to fibrinolysis[44]. However, for the current application, the biocompatibility of the microarchitecture with cell viability dominated choice of fibrinogen:thrombin ratios above adapting the microarchitecture to address degradation profile [56]. The concentrations used in this study (60 mg ml^{-1} fibrinogen to 4 IU thrombin) were compatible with cell delivery to the graft site and cell viability as no significant difference in graft persistence was observed when compared to control group. This was assessed using volumetric analysis of the GFP-expressing graft which we have previously shown to be a reliable method for assessing the overall survival of these cells in the rat brain[52]. Our laboratory has shown that the composition of this fibrin scaffold provides an excellent substrate to facilitate viral and non-viral therapeutic gene delivery [34,36-40]. Therefore, it is conceivable that this scaffold may be utilised as a platform to complex pro-survival factors to aid stem cell survival following transplantation.

Treatment of neurodegenerative disease is entering a new era where invasive direct intra-cerebral delivery of therapeutic reagents (growth factors, gene therapy, deep brain stimulation) aims to restore normality to dysfunctional circuits. Astrocytes constitute the predominant glial cell type that functions to control the adult CNS environment and have a dominant role in tissue reaction. In this study, inclusion of the fibrin scaffold did not aggravate an exacerbated host astroglial response compared to the delivery of cells alone. Indeed inclusion of the scaffold reduced the astroglial response of the host to the grafted cells

at seven days post-transplant. To our knowledge, there are no other published studies where direct intracerebral delivery and *in situ* polymerization of fibrin has been investigated making direct comparisons difficult. However, De Faveri and colleagues used the same fibrin scaffold (TISSEEL™) loaded with primary hippocampal cells to coat a microelectrode and examined the host response following implantation to the rat brain [57]. The authors reported that inclusion of the fibrin coating resulted in a weaker astroglial response as compared to a bare electrode at day seven post-implantation, highlighting fibrins biocompatibility with intra-cerebral applications. Investigation of the host microglial response to fibrin also highlighted the biocompatibility of this biomaterial with no exacerbation of a host response in response to this material.

Conclusions:

Overall, this study found that inclusion of fibrin as a complimentary biomaterial scaffold for cell transplantation is not associated with any detrimental effects on cell survival or host response. Therefore, fibrin offers significant potential as a suitable injectable material upon which to build a pro-survival platform for cell transplantation to deep brain parenchymal structures. Further studies are warranted to complex various pro-survival factors in the scaffold to address the multitude of insults that contribute to poor cell survival following transplantation. Such approaches offer significant potential to further the era of direct intra-cerebral cell delivery approaches for neurodegenerative diseases.

FIGURES

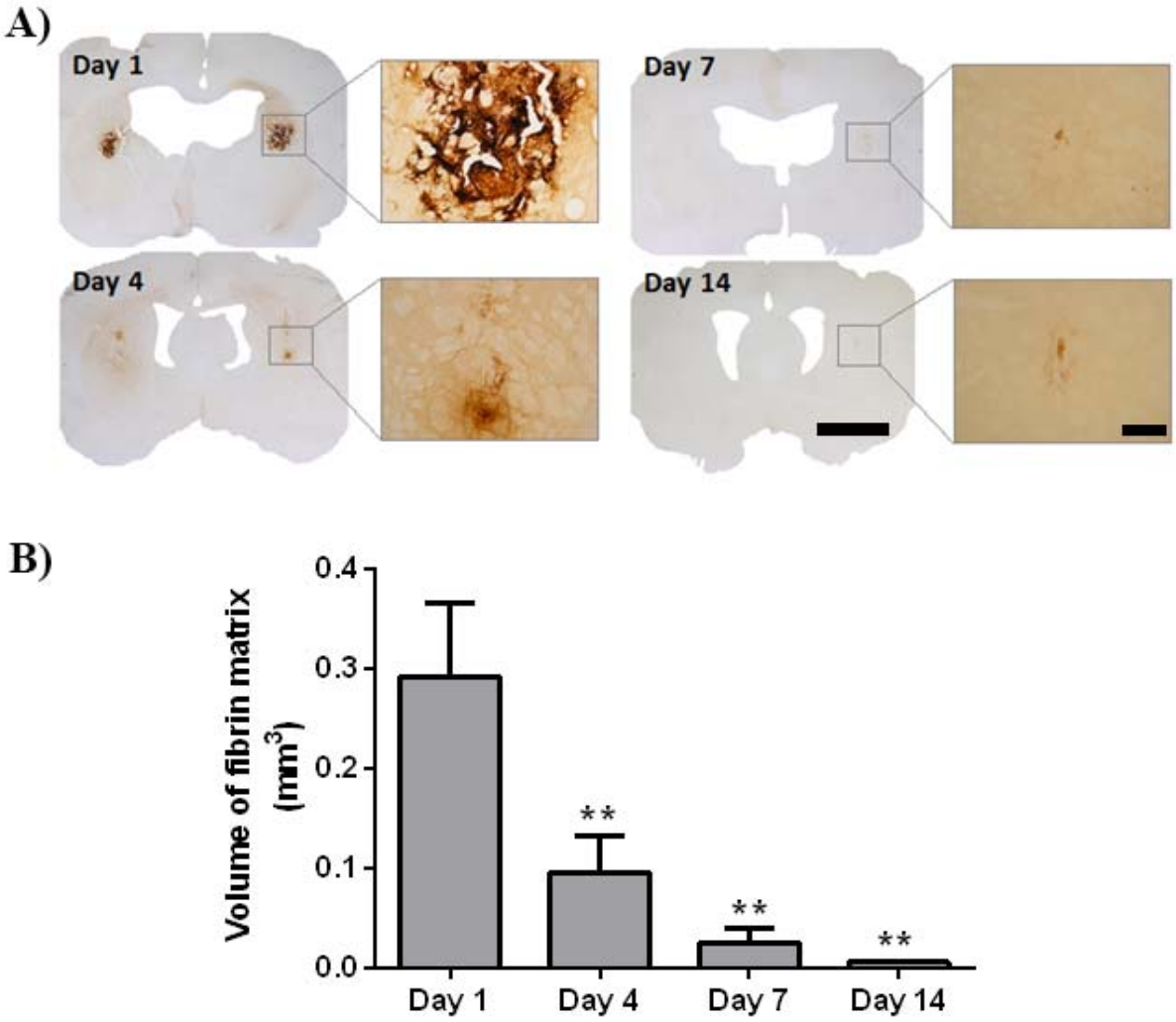

Figure 1. *In situ* polymerization of the fibrin scaffold in the intact rat brain. Following sequential delivery of fibrinogen and thrombin to the rat brain, the *in situ* polymerization and biodegradability of the scaffold was assessed using immunohistochemistry targeting human fibrin. A) Representative photomicrographs showing successful formation of the fibrin scaffold following injection of human fibrinogen and human thrombin into intact rat striatum. B) Although the fibrin scaffold was visible at all time-points, the volume of the scaffold decreased significantly over time (**P<0.01 vs. Day 1 Fibrin by 1-way ANOVA with Newman-Keuls post-hoc test). All data expressed as mean ± s.e.m. Left scale bar = 3mm, right scale bar = 500 µm.

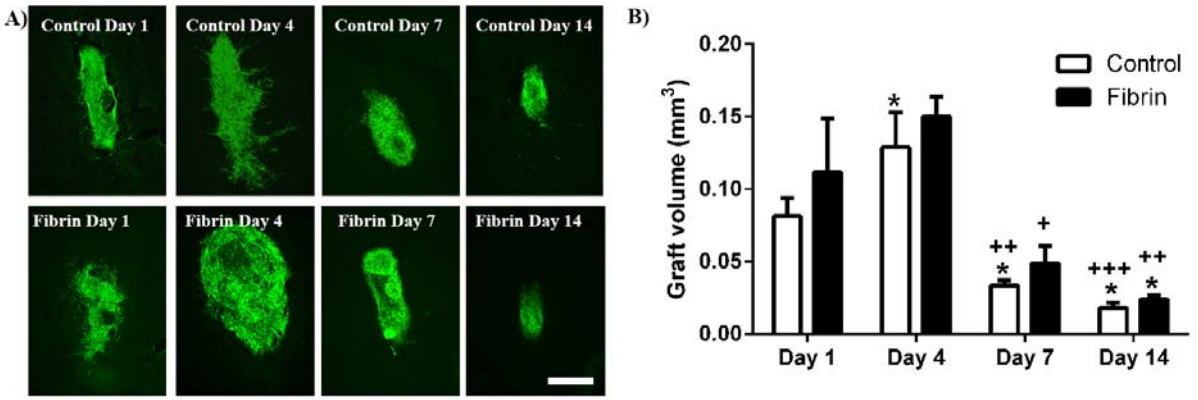

Figure 2. Impact of the fibrin scaffold on survival of GDNF-MSCs in the rat striatum. Rats were intra-striatally transplanted with GDNF-MSCs delivered in either control medium or with a fibrin scaffold. A) Representative fluorescent photomicrographs illustrate the presence of the GDNF-MSCs (identified by their strong GFP expression) at each time point. Qualitatively, inclusion of the fibrin scaffold did not inhibit cell delivery to and retention at the graft site compared with control. B) Delivery of cells within a fibrin scaffold had no significant effect on GDNF-MSCs survival when compared with cells delivered in control medium. Additionally, the volume of the GDNF-MSCs transplant declined significantly with time (*P<0.05 vs. relevant Day 1 group, +P<0.05, ++P<0.01, +++P<0.001 vs. relevant Day 4 group by 2-way ANOVA with Newman-Keuls post-hoc test). All data expressed as mean±sem, scale bar = 500 µm.

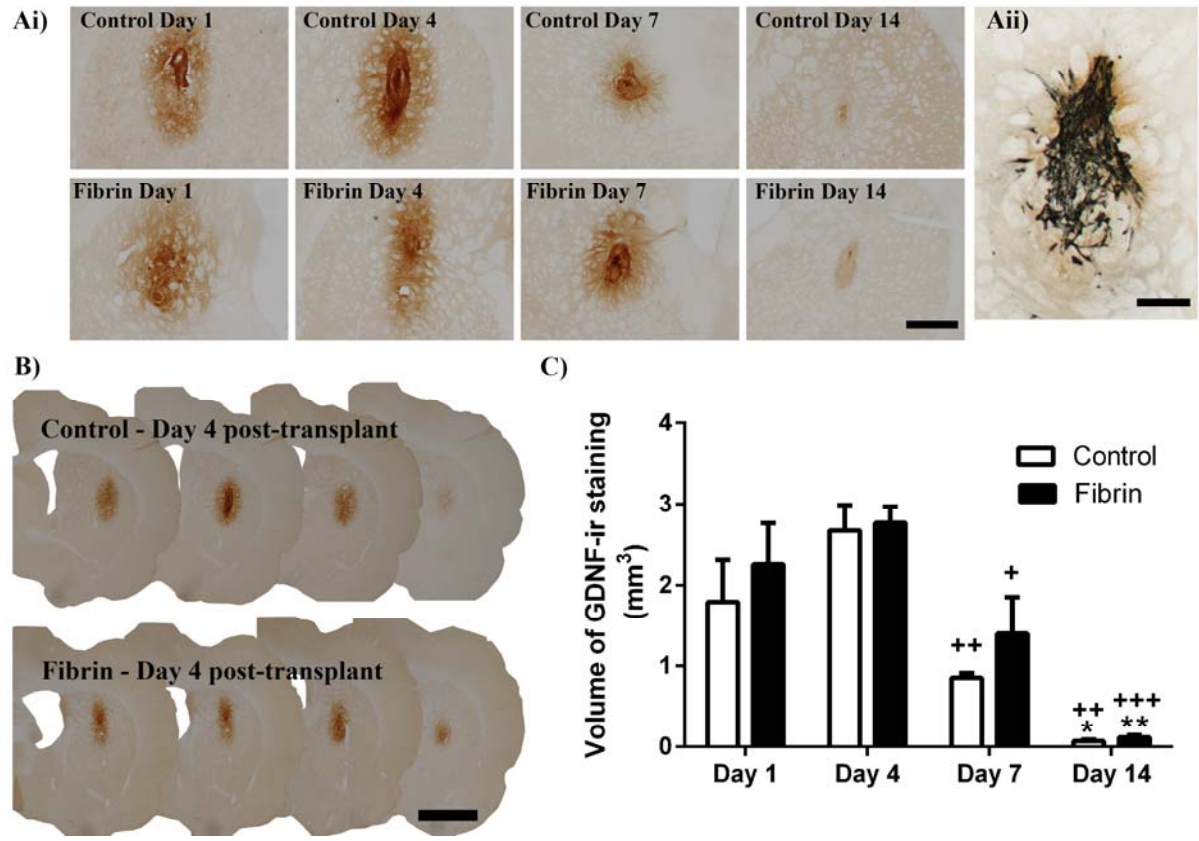

Figure 3. Impact of the fibrin scaffold on GDNF release into the striatum from GDNF-MSCs. Ai) Representative photomicrographs of GDNF release over time from GDNF-MSCs delivered in control medium or fibrin scaffold. Aii) Dual GFP (navy blue) and GDNF (brown) immunohistochemistry indicated that GDNF was secreted into the striatum from the transplanted cells. B) Staining on a series of coronal sections through the brain indicate that the presence of the fibrin scaffold (lower panel) does not impede global release of GDNF to the surrounding tissue. C) Although the level of GDNF secretion did not differ between Control and Fibrin groups, the volume decreased in both groups significantly over time (*P<0.05, **P<0.01 vs. relevant Day 1 group, +P<0.05, ++P<0.01, +++P<0.001 vs. relevant Day 4 group by 2-way ANOVA with post-hoc Newman Keuls). These results suggest that while delivery of GDNF-MSCs within a fibrin scaffold did not improve GDNF secretion, it also did not impede release. All data shown as mean ± s.e.m. Scale bar 500 µm, 100 µm and 3mm

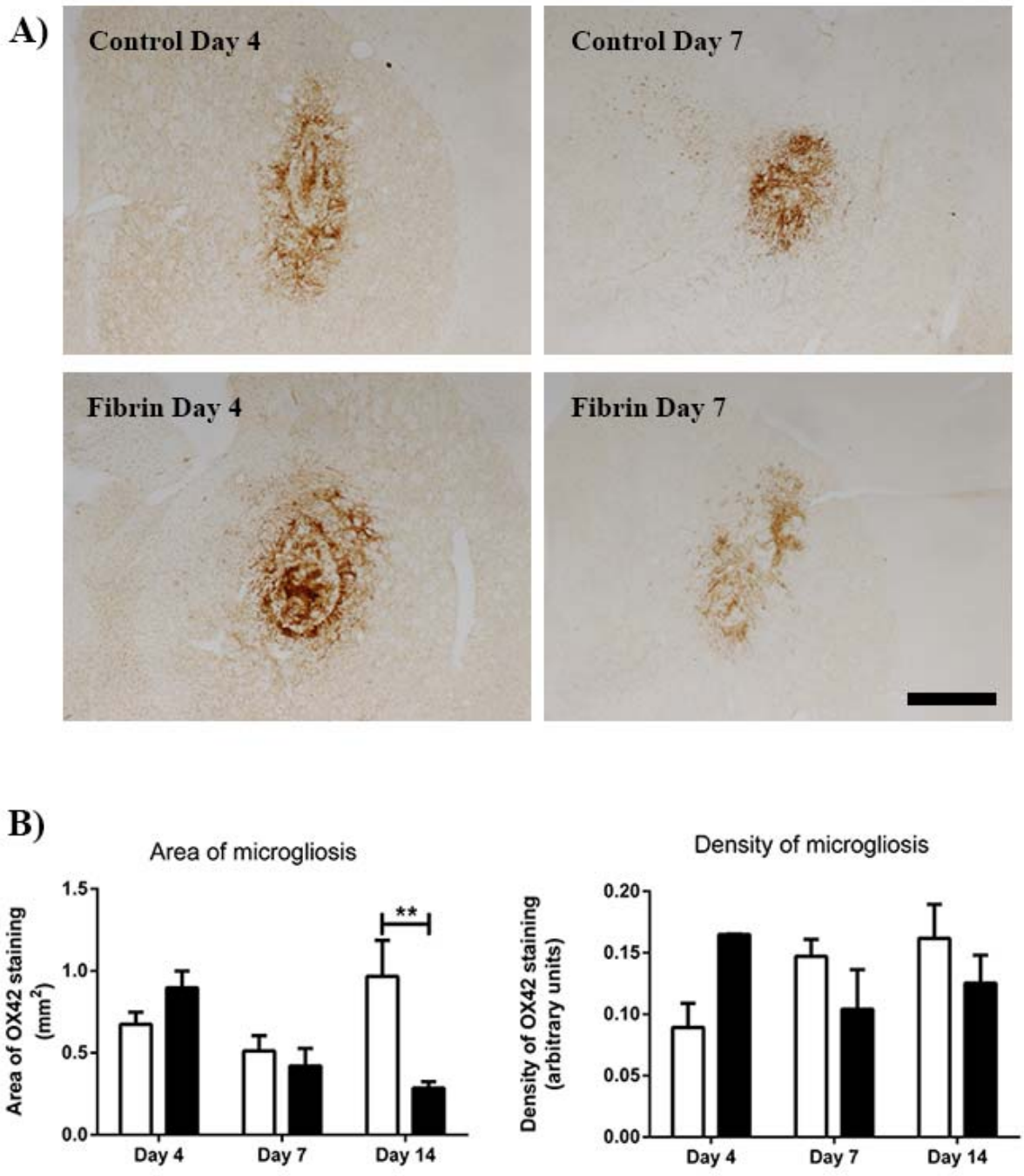

Figure 4. Impact of the fibrin scaffold on the host microglia response to the transplant. Immunohistochemical staining for OX42 was used to assess the microglial response to the fibrin scaffold. A) Representative photomicrographs of OX42 immunostaining in the striatum of control and fibrin groups at day 4 and 7 post-transplant. B) Quantification of staining revealed that the density of microglial cells did not differ when Control and Fibrin groups were compared at any time-point. Area of microgliosis surrounding Control and Fibrin transplant groups significantly differed at day 14 post-transplantation (**P<0.01 by 2-way ANOVA with post-hoc Bonferroni). All data shown as mean ± s.e.m., scale bar = 500 μm.

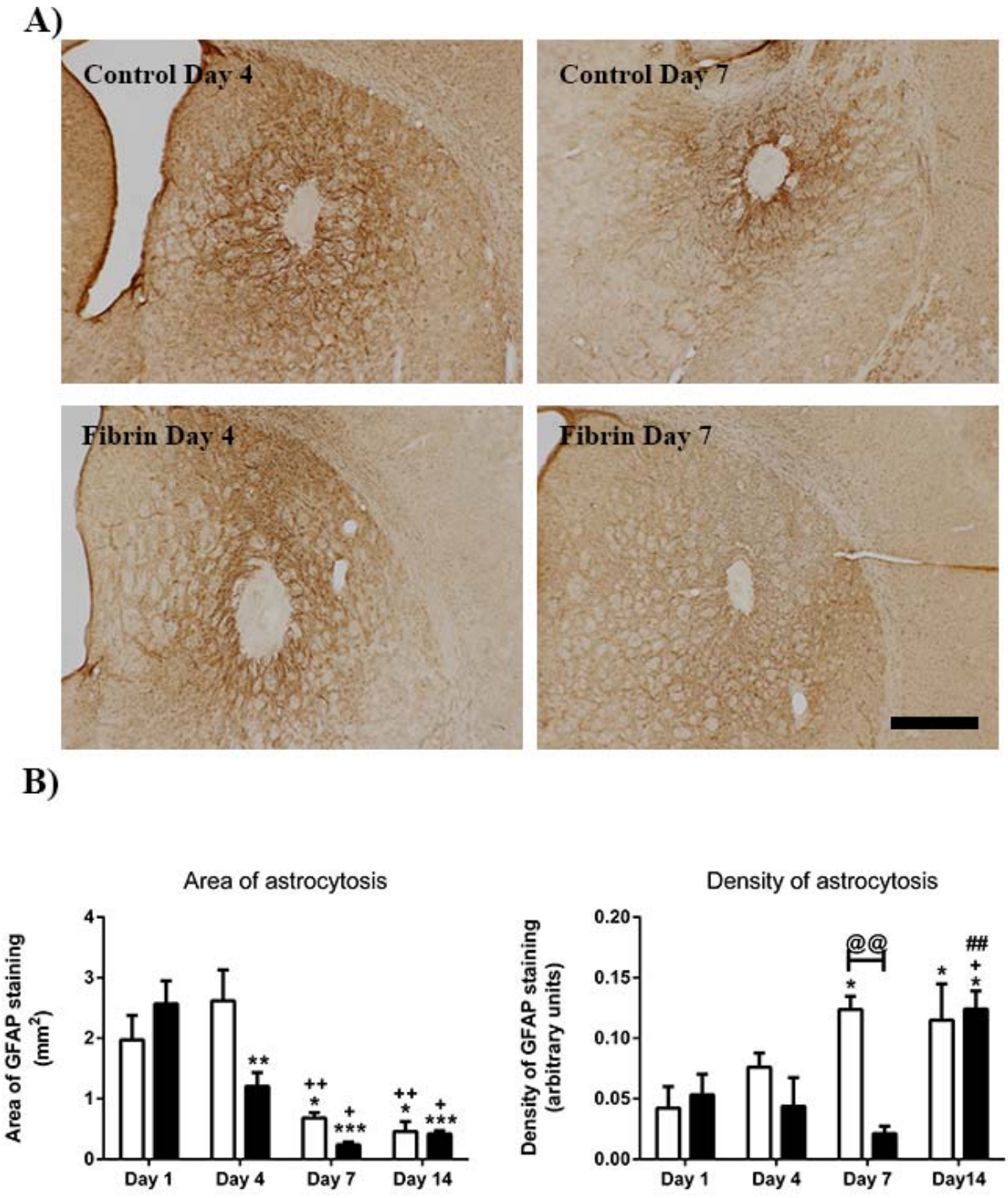

Figure 5. Impact of the fibrin scaffold on the host astroglial response to the transplant. Immunohistochemical staining for GFAP was used to assess the microglial response to the fibrin scaffold. A) Representative photomicrographs of GFAP immunostaining in the striatum of control and fibrin groups at day 4 and 7 post-transplant. B) Area of astrogliosis induced by Control and Fibrin groups did not differ, but declined in both groups over time (*P<0.05, **P<0.01, ***P<0.001 vs. relevant Day 1 group, +P<0.05, ++P<0.01 vs. relevant Day 4 group by 2-way ANOVA with post-hoc Newman-Keuls). This suggests the fibrin scaffold did not produce a prominent effect on area of astrogliosis. Density of astrogliosis

was significantly reduced in the Fibrin group at day 7 post-transplantation (@@P<0.01 by 2-way ANOVA with post-hoc Bonferroni), and the density of astrocytosis surrounding the transplant increased significantly over time (*P<0.05 vs. relevant Day 1 group, +P<0.05 vs. relevant Day 4 group, ##P<0.01 vs. relevant Day 7 group by 2-way ANOVA with post-hoc Newman-Keuls). All data expressed as mean ± s.e.m, scale bar = 500 μm.

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The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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