UNIVERSITY OF CALIFORNIA SAN DIEGO

Optimizing Trophic Support of Neural Stem Cell Grafts in Sites of Spinal Cord Injury

A Thesis submitted in partial satisfaction of the requirements for the degree Master of Science

in

Biology

by

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The Thesis of Jacob Newell Robinson is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

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ABSTRACT OF THE THESIS

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Previously we reported that neural stem cells (NSCs) embedded in fibrin matrices containing 9 growth factors and an anti-apoptotic agent, survived and completely filled sites of spinal cord injury. In the current study, we examine whether the number of factors can be reduced while optimizing NSC survival and

filling of the lesion site. NSCs derived from embryonic day 14 F344 rat spinal cord (expressing green fluorescent protein, GFP) were embedded in fibrin matrices containing a defined growth factor cocktail (1 to 4 factors among BDNF, bFGF, VEGF and calpain inhibitor, or original 10 factor combination). Grafts were made into a C5 lateral hemisection of wild-type adult F344 rats two weeks post-injury (N=3-6 per group, total 9 groups). Graft survival was assessed 2 weeks post-grafting. A 4 factor cocktail resulted in graft survival, neuronal differentiation, and filling of lesion site that was equivalent or superior to the 10 factor cocktail. The use of fewer than 4 growth factors, including single growth factors, also frequently but less consistently resulted in NSC survival and fill of lesion site. The effect of fewer growth factors on axon extension from the graft into the host cord is currently under analysis. Collectively, these findings suggest that excellent neural stem cell engraftment and survival can be achieved with a reduced growth factor cocktail, enhancing clinical practicality.

Chapter 1: Introduction

1.1 Spinal Cord Injury

The central nervous system (CNS) is composed of the brain and the spinal cord. The spinal cord is the main pathway that transmits information between the brain and the peripheral nervous system (PNS). It is responsible for motor information descending from the brain, sensory information ascending to the brain, and coordinating various spinal reflexes.

The spinal cord is housed by the vertebral column, 31 individual bone segments grouped into 5 general regions: cervical, thoracic, lumbar, sacral, and coccygeal. Inside the vertebral column encasing the spinal cord are three tissue layers of protection; the dura mater, arachnoid mater, and the pia mater. The spinal cord is divided into 31 segments that correlate to the surrounding segment of vertebrae. At each of these segments, the spinal cord has bundles of spinal nerves that form into spinal roots. Two ventral roots, one on each side, consist of axons from motor neurons that transmit information to the peripheral nervous system from cell bodies within the central nervous system. Similarly, two dorsal roots are made up of sensory axons with one root on either side of the spinal cord.

When this intricate organization of the spinal cord is perturbed, there is immense variability in the symptoms observed depending on the severity and location of the spinal cord injury (SCI). Mild injuries such as a bruise to the spinal cord can result in loss of feeling or function in relatively small regions of the body like a hand or foot. More severe injuries, such as a severing of the spinal cord,

typically result in complete paralysis below the point of injury (McKinley et al., 2007; Sekhon and Fehlings, 2001). Cervical and higher thoracic level injuries can result in quadriplegia while thoracic and lower injuries result in paraplegia. Every SCI can result in a range of outcomes because of the specificity of function each region of the spinal cord is responsible for. Symptoms such as autonomic dysreflexia, bladder/bowel dysfunction, sexual dysfunction, muscle spasms, and chronic pain can occur in various combinations and with a spectrum of severity. (Hulsebosch et al., 2009; Karlsson, 2006; Khastgir et al., 2007; Monga et al., 1999; Rekand et al., 2012; Sipski and Arenas, 2006). In attempt to classify the wide array of injuries, patients are diagnosed using the American Spinal Injury Association (ASIA) Impairment Scale (AIS). This scale ranges A (complete injury and paralysis) through E (normal and intact spinal cord). Neurological testing of sensory and motor function determines very broadly the severity of an injury (Kirshblum et al. 2012). The complexity of the nervous system and the following variability in injuries that can occur illustrates the difficulty in attempting to find therapeutic approaches to curing spinal cord injury.

1.2 CNS versus PNS Regeneration

The nervous system is divided into the central nervous system (CNS) consisting of the brain and spinal cord and the peripheral nervous systems (PNS) composed of nerves that connect the CNS to the rest of the body. Both are made of nervous tissue and composed of neurons, the distinguishing cell of the nervous system. While both systems are strikingly similar on the cellular level,

they are distinctively different in their response to injury. An interesting confound of the mammalian nervous system is the contrast in ability of the PNS to successfully regenerate after injury and the failure in the CNS to spontaneously regenerate. A perfect example of this is seen when CNS axons persist to grow over long distances in peripheral nerve bridges (David and Aguayo, 1981; Houle et al., 2006). Several distinguishing mechanisms have been identified in contributing to the advantageous regenerative feature of the PNS. Firstly, the lesion cavity in the peripheral nerve injury fills with a bridge of permissive physical matrix that promotes axonal attachment and growth (Williams et al., 1983; Chernousov and Carey, 2000; Dubovy, 2004). Secondly, growth factors native to the nervous system are secreted in appropriate spatial and temporal gradients to stimulate axonal regeneration (Terenghi, 1999; Boyd and Gordon, 2003). While similar gradients are present for typical axonal projection in the nervous system during development, they only occur in the PNS promoting recovery during injury state. Thirdly, nuclei of damaged neurons in the PNS undergo activation of a set of genes associated with axonal regeneration, a trigger that does not occur in the CNS (Kury et al., 2001; Navarro et al., 2007). Lastly, the PNS is absent of central myelin and ECM molecules that inhibit axon sprouting and regeneration that have been identified and are present in the CNS (Schwab, 1988; Fawcett, 2006).

Individual investigation of these mechanisms has revealed extremely vital information regarding axonal plasticity and regeneration. However, given the complexity of the natural development of the mammalian nervous system as well

as the vast array of injury that can ensue, a relevant therapeutic approach will likely need to incorporate more than one of these mechanisms. Fundamental challenges for regeneration in the CNS revolve around the magnificently orchestrated events that occur for the naturally developing neuron, undergoing both intrinsic and extrinsic events that result in a prodigiously complex network. It is a great challenge for researchers to overcome the bulwark of the simple inability to replicate the spatial and temporal elements of natural CNS development.

1.3 Previous Use of Growth Factors

The ability of injured adult spinal cord axons to respond to growth factors has been widely studied. It has previously been seen that fibroblasts genetically modified to produce and secrete nerve growth factor (NGF) grafted into collagen matrices of lesioned spinal cords resulted in axonal penetration into the NGF-secreting grafts (Tuszynski et al., 1996). These findings revealed the ability of axons to extend when presented with a permissive environment containing growth factors in an injury state. This and many other studies support the idea that adult axons maintain sensitivity to growth factors after injury and into adulthood (David and Aguayo, 1981).

Numerous growth factors have been identified and classified in terms of axonal sensitivity in the CNS. Neuronal populations often have sensitivity to multiple growth factors, resulting in a spectrum of sensitivity indicative of the complexity seen during development. Many of the factors identified are found in

peripheral nerve bridges during injury, suggesting their importance to regeneration. By intuitively evaluating the information known about these growth factors and the sensitivity they incur, candidates for an optimal growth factor cocktail can start to take form.

1.4 Cell Replacement Therapy

A common approach to SCI regeneration is cell replacement therapy (Boyd et al., 2005; Bregman et al., 1993; Faulkner and Keirstead, 2005; Liu et al., 1999; Lu et al., 2005; Lu et al., 2012; Richardson et al., 1980; Xu et al., 1995a; Xu et al., 1995b). One of the main goals of cell transplantation based therapies is to create a lesion site that is a more growth-permissive environment in order to enable axon regeneration across it. Early bridge experiments were done using peripheral nerve grafts showing that CNS axons have the potential to regenerate when lacking the presence of CNS glia and inhibitory environment (David and Aguayo 1981). However, these early PNS grafting studies showed very little differentiation and axon extension.

Cell transplantation studies in SCI have looked to create grafts that use undifferentiated cells that can potentially become the variety of cells needed to support a healthy nervous system. When embryonic stem (ES) cells are grafted into the rat spinal cord, they survive and differentiate into astrocytes, oligodendrocytes, and neurons that can promote some functional recovery (McDonald et al., 1999). However, visualization techniques at the time limited the ability to view the grafts. Later studies showed that grafted neural stem cells

elicit neuroprotective effects, promoting host axonal regeneration and release of neurotropic factors (Lu et al., 2003).

Recently, it was shown that neural stem cells (NSCs) can be grafted into a lesion site embedded in a fibrin matrix with growth factors to produce lesion filling, differentiation, and axon extension long distances from the lesion site (Lu et al., 2012). Because the grafted NSCs ubiquitously expressed green fluorescent protein (GFP), robust axon outgrowth could be visualized in an unprecedented way.

While Dr. Lu's research offers a novel approach and incredible progress in SCI research, the ultimate goal of human translation using his methods remains problematic. One major reason for this is the complexity of the growth factor cocktail he used. Containing 11 growth factors, approval for clinical use of his growth factor cocktail would necessitate an unreasonable amount of control groups, requiring each factor to be individually tested and evaluated for safety. If the amount of growth factors could be reduced while eliciting comparable survival, differentiation, and axon extension, clinical relevance would be significantly more achievable.

Chapter 2: Materials and Methods

2.1 Animals

Adult female Fischer 344 rats weighing 150-200g (n=25) were used for this study. All activity was conducted in strict accordance with the laboratory animal care and safety guidelines set by the NIH. Animals had free access to food and water throughout the study. For all surgeries and perfusions animals were deeply anesthetized using a combination (2 ml/kg) of ketamine (25 mg/ml), xylazine (1.3 g/ml), and acepromazine (0.25 mg/ml).

2.2. Surgery Procedures

Rats (n=25) were deeply anesthetized and fixed in place with stereotaxic equipment. An incision was made through the dorsal epidermis using a #15 blade. The dorsal muscle tissue was cut to expose the vertebral column. In order to expose the spinal cord, a laminectomy on the cervical level 5 vertebrae was performed. A small incision was made in the dura along the midline using a scalpel fitted with a #11 blade. A 2mm block of spinal cord was cut with iridectomy scissors and removed through microaspiration. A visual inspection of the lesion verified that the entire hemi-section was removed. The muscles above the wound were sutured and the skin was secured with wound clips. Animals were given post-operative injections of lactated ringers solution, Banamine, and Ampicillin for 3 days following surgery.

Two weeks after lesion surgeries, animal spinal cords were again exposed. Microinjection needles were filled with either 2.5μL of fibrinogen or 2.5 μL

thrombin cell suspension solutions and fixed into a picospritzer stereotax attachment. The cell suspensions were consecutively and slowly injected. In the lesion the fibrinogen and thrombin solutions mixed in equal parts to form a fibrin matrix suspending the NSCs. Animals received the same post-operative procedures as during lesion surgeries.

2.3 E-14 Dissection and Dissociation

Spinal cords from transgenic Fischer 344 rats constitutively expressing GFP under the ubiquitin C promoter (Baska et al., 2008; Bryda et al., 2006) were dissected and dissociated. Dissected cords were placed in a15 ml conical tube containing 1ml of ice cold Hank's Balanced Salt Solution (HBSS). Cells were dissociated chemically with a 0.125% trypsin (Harris et al., 2007). Dissociated E-14 spinal cord cells were then resuspended in solutions of 25 mg/ml fibrinogen or 25 U/ml thrombin with various combinations of growth factors at a concentration of 200,000 cells/µl. The different combination of growth factor The growth factor cocktail was added to support graft survival and contained BDNF (50 µg/ml), neurotrophin-3 (NT-3; 50 µg/ml), platelet-derived growth factor (PDGF-AA; 10 µg/ml), insulin-like growth factor 1 (IGF-1; 10 µg/ml), epidermal growth factor (EGF; 10 µg/ml), basic fibroblast growth factor (bFGF; 10 µg/ml), acidic fibroblast growth factor (aFGF; 10 µg/ml), glial-cell-line-derived neurotrophic factor (GDNF; 10 µg/ml), hepatocyte growth factor (HGF; 10 µg/ml), and calpain inhibitor (MDL28170, 50 µM)

2.4 Histology and Immunohistochemistry

Four weeks after grafting, animals (n=6) were heavily anesthetized, and transcardially perfused first with ice cold saline, and then 4% paraformaldehyde (PFA) and dissected. Tissue was post fixed in 4% PFA overnight at 4° C and then placed in a 30% sucrose solution for 48 hours at 4°C. Blocks of tissue 2-4 cm around the graft were cut using razor blades, and placed into tissue freezing medium on dry ice. Sections of 30μm were cut using a cryostat.

Free-floating sections were washed in Tris-Buffered Saline (TBS) then incubated for 1 hour in TBS containing 5% donkey/goat serum, and 0.25% Triton. Sections were then incubated overnight at 4°C in TBS containing 5% donkey/goat serum, 0.25% Triton and primary antibodies against; jellyfish green fluorescent protein indicating graft origin (GFP, rabbit, 1:1,500) adenomatous polyposis coli, a marker of Oligodendroctyes, (APC, mouse monoclonal, 1:400); myelin-associated glycoprotein, indicating myelin (MAG; mouse, 1:200). Sections were washed, and then incubated at room temperature for 2.5 hours with Alexa 488, 594, or 647 conjugated donkey/goat secondary antibodies (1:250) along with nuclear stain DAPI (1:1000).

Chapter 3: Results

3.1 Grafted NSCs with Reduced Growth Factor Cocktail show Survival, Filling, and Differentiation

In order to find a growth factor cocktail with a reduced amount of factors that still maintained the favorable parameters of a healthy graft, 24 adult female Fischer 344 rats received cervical C5 hemisections. Two weeks post-lesion, embryonic day 14 neural stem cells were grafted into a closed lesion site. The lesions were re-exposed without aspirating debris or surgically disturbing the injury in any way. 18 animals received grafts with fibrin matrix and combinations of different growth factors suspended within the fibrin and thrombin during grafting. 6 subjects received only one growth factor of BDNF, 6 received four growth factors of BDNF, VEGF, bFGF, and calpain inhibitor, and the last 6 received the full 11 factor growth factor cocktail (Figure 1). A control of 3 animals received NSC grafts with PBS only and no fibrin matrix (fibrinogen and thrombin). Another control group of 3 animals received NSC grafts with no growth factors and only the fibrinogen and thrombin to create the fibrin matrix. All animals survived two weeks before sacrificing. All groups of grafted NSCs showed the ability to survive, fill the lesion site, and differentiate into mature neurons. Average graft size per every 6^{th} section through the graft measured as a relative pixel area show a significant difference between the Full Cocktail and other groups. The average pixel area per section for the full cocktail was 425,172±85,837, while the four growth factor cocktail and BDNF only averaged

237,339±17,782 and 209,479±26,455, respectively. The control groups of PBS Only and Fibrin Only had an average graft size of 152,974±33,903 and 161,619±49,320 pixels, respectively. It is important to note that a larger graft may not mean an optimal graft.

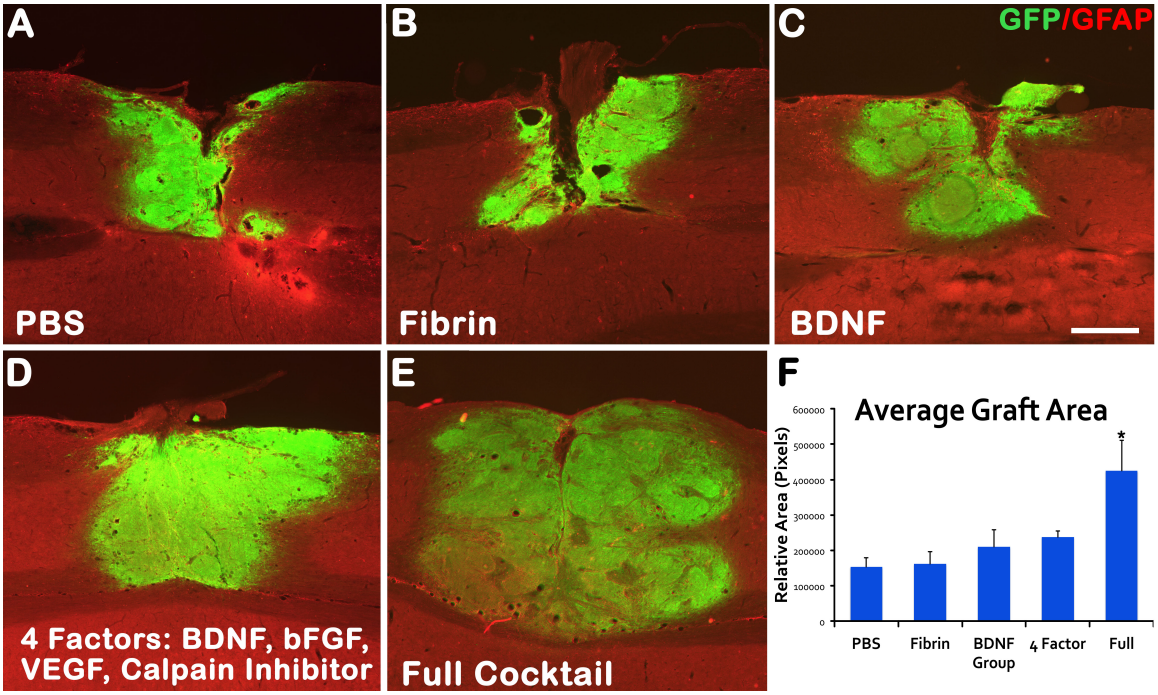

Figure 1. Grafted NSCs with Reduced Growth Factor Cocktail show Survival, Filling, and Differentiation.
Overview of GFP and GFAP fluorescent immunolabeling in horizontal sections demonstrates the ability of the graft to survive and fill the lesion site. Control groups of PBS and Fibrin only (**A,B**) do not show optimal survival or filling of lesion site as seen with BDNF only (**C**), 4 factor (**D**), and Full Cocktail (**E**). (**F**) Average graft sizes as a measurement of average pixels per graft per animal shows only a significant difference between the full cocktail and other groups. The average pixel area per graft for the full cocktail, 4 factor cocktail, and BDNF only measured 425,172 ±85,837, 237,339±17,782 and 209,479±26,455, respectively. The control groups of PBS Only and Fibrin Only had an average graft size of152,974±33,903 and 161,619±49,320 pixels, respectively.
All data represented as mean ± SEM. (*$p<0.05$). Scale Bar: 500μm.

3.2 Four Growth Factor Cocktail Reduces Graft Bulge on Central Canal

Grafts receiving full cocktail, both in this study and previous studies, have repeatedly shown to survive and proliferate abundantly, completely filling the lesion site and bulging against the host tissue and central canal. All 6 animals receiving grafts with a full growth factor cocktail consistently showed this distinguishing characteristic of expanded growth that bulged outward, pressing against the central canal and creating a graft that appears to swell into host tissue (Figure 2). 5 of 6 four factor cocktail grafts instead show a non-perpetrating graft that grows parallel with the central canal and cleanly blending into host tissue, lacking any bowing or bulging effect seen in with the full cocktail (Figure 2). One graft receiving the 4 factor cocktail had lower graft survival relative to the others in the group and the described characteristic is not observed.

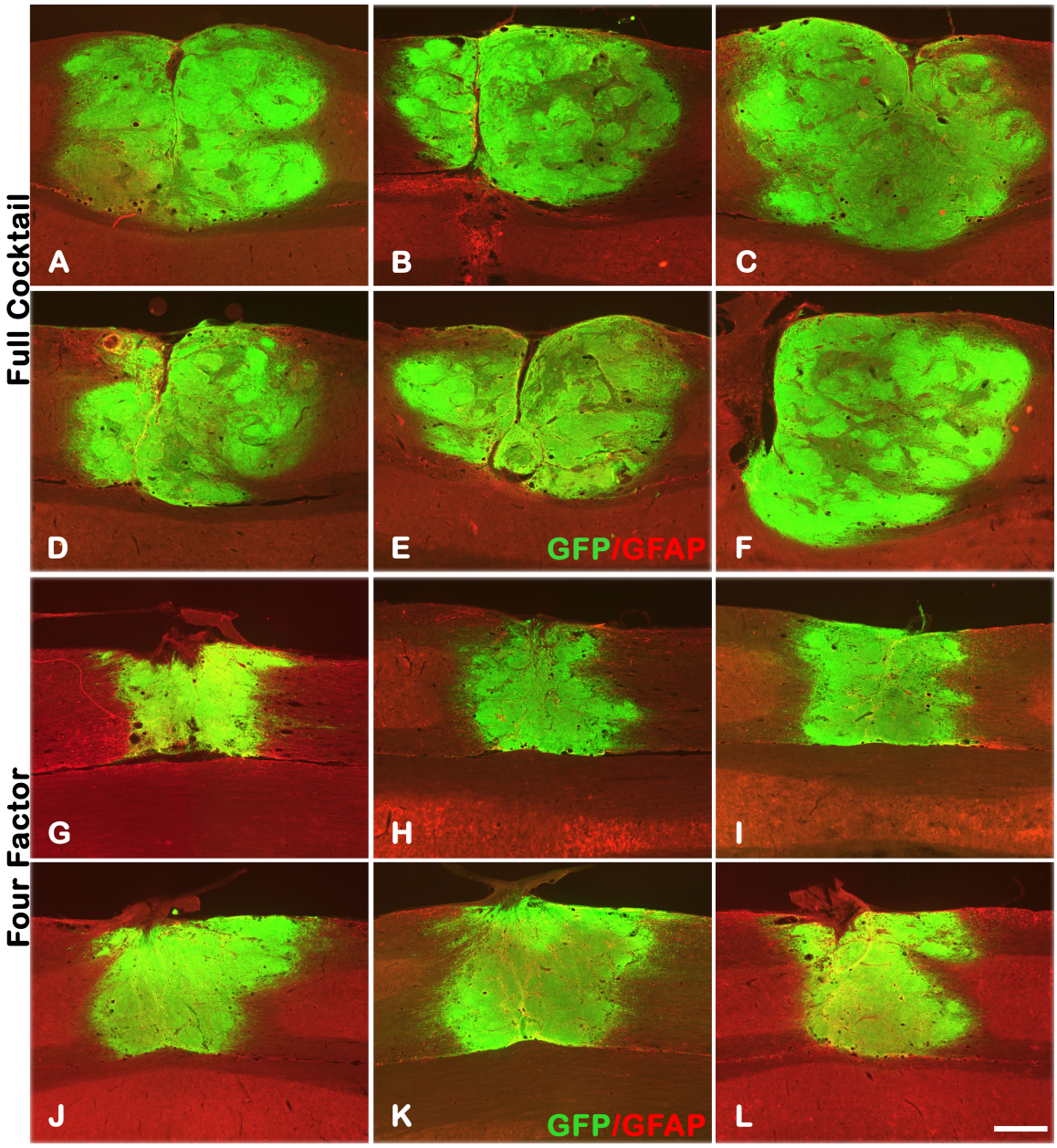

Figure 2. Four Growth Factor Cocktail Reduces Graft Bulge on Central Canal and Host-Graft Interface

(**A-F**) Immunolabeled for GFP and GFAP, horizontal sections show grafts receiving full growth factor cocktail consistently bulging against the central canal and host tissue. A representative image of the graft for all 6 animals has been shown for both groups. (**G-L**) In contrast, grafts receiving the four growth factor cocktail result in a clean parallel border against the central canal and blend in to host tissue in a much less invasive manner at the host-graft interface, completely filling the lesion site while not abundantly proliferating to the extent of the grafts receiving full cocktail.

Scale Bar: 500μm.

3.3 Grafts Receiving Growth Factors Trend Toward Higher Populations of Mature Neurons Compared to Grafts without Factors

Similar to NSCs maturation when grafted with a full growth factor cocktail, grafts receiving four growth factor cocktail also developed into mature neurons. While there was only statistical significance between the number of mature neurons in the full cocktail grafts and the other groups, there was a trend toward increased amount of NeuN+ cell populations, indicating that growth factors contribute to the increased amounts of mature populations within the graft. Control grafts of PBS and Fibrin do show development into mature neurons in the amounts of 1,590±370 and 1,827±270 NeuN+ cells per section per graft, respectively, but not to the extent seen in grafts receiving growth factors. The trend can seen in an increase from a graft with a single growth factor of BDNF having an average of 2,274±305 NeuN+ cells per section per graft and then again with a grafts receiving 4 factors having an average of 3,083±560 cells. Finally, there is a significant increase with grafts receiving all 11 growth factors having an average of 5,741±578 cells.

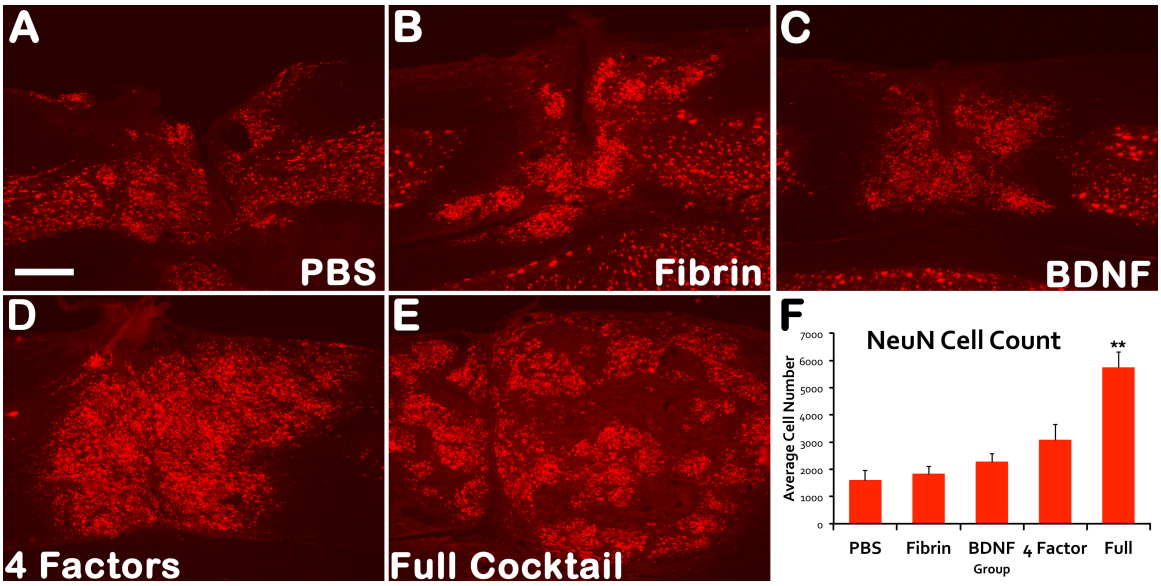

Figure 3. Grafts Receiving Growth Factors Trend Toward Higher Populations of Mature Neurons Compared to Grafts without Factors
(**A-B**) Control grafts of PBS and Fibrin stained for NeuN show populations of mature neurons that increase in amount as growth factors are added. (**C-E**) When growth factors are added, there is a trend toward an increased amount of mature neurons.
(**F**) Control grafts receiving PBS and Fibrin only had an average of 1,590±370 and 1,827±270 NeuN+ cells per section per graft, respectively, while grafts with a single factor of BDNF and 4 Factor Cocktail resulted in of 2,274±305 NeuN+ and 3,083±560 cells per section per graft. There was significant increase when grafted with full growth factor cocktail at 5,741±578 cells per section per graft. All data represented as mean ± SEM. (**$p<0.01$). Scale Bar: 500μm.

3.4 Four Growth Factor Cocktail Results in More Homogenous Distribution of Neurons Within Graft

Grafts receiving full cocktail when stained with NeuN, a mature neuronal marker, show a distinct tendency of GFP+ cells clustering into pockets (Figure 4). Co-labeling with NeuN in the graft shows a distribution of differentiated mature neurons that is very heterogeneous, with clear pockets of cells aggregating together, leaving large spaces empty of any neuronal cell bodies. This starkly contrasts the homogenous distribution of cells in grafts receiving four factors. These grafts have an even and uniform distribution of cells across the entirety of the graft, lacking any major clusters of dense populations of cells. This is likely a beneficial characteristic that could lead to other favorable characteristics.

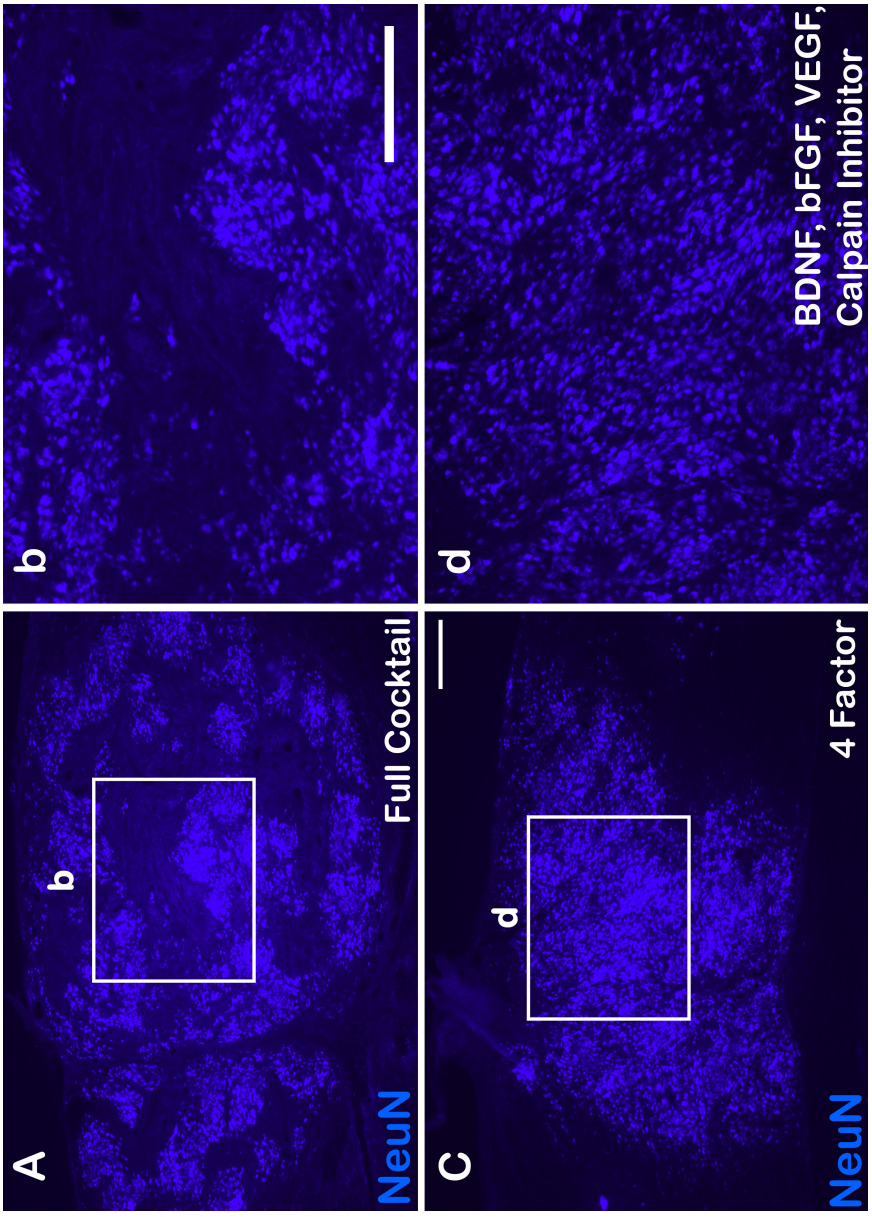

Figure 4. Homogenous Distribution of NeuN+ Cells within Grafts Receiving 4 Factor Cocktail

(**A**) 4x image of NeuN+ graft with full growth factor cocktail at higher magnification (**b**) reveals large pockets of emptiness, showing a tendency to cluster into pockets of very dense regions of mature neurons. (**C**) This is not seen in the grafts receiving 4 growth factor cocktail, where instead an (**d**) even and homogeneous distribution of cells is observed.

All data represented as mean ± SEM. (**p<0.01). Scale Bar: **A** and **C**:500μm; **b** and **d**: 400μm

3.5 Assessment of Four Factor Cocktail to Further Reduction Possibilities

To further assess the effect and optimization of the four factor cocktail, the four factor growth factor cocktail was further reduced. 31 adult female Fischer 344 rats received cervical C5 hemisections. Two weeks post-lesion, embryonic day 14 neural stem cells were grafted into a closed lesion site. The lesions were re-exposed without aspirating debris or surgically disturbing the injury in any way. 15 animals received grafts with fibrin matrix and combinations of the four growth factors suspended within the fibrin and thrombin during grafting. 4 subjects received only one growth factor of VEGF (Figure 5), 6 received two growth factors of BDNF, VEGF (Figure 6), two groups of 6 subjects received three growth factors of BDNF, VEGF, and Calpain inhibitor and the other BDNF, VEGF, and bFGF (Figure 7). An additional control of 3 animals received NSC grafts with PBS only and no fibrin matrix (fibrinogen and thrombin). All animals survived two weeks before sacrificing. All groups of grafted NSCs showed the ability to survive, fill the lesion site, and differentiate into mature neurons. Some groups showed similar morphological characteristics seen in the four factor cocktail. To further assess differences amongst the groups quantitatively, average graft size throughout the graph was measured (Figure 8). Average graft size per every 6^{th} section through the graft measured as a relative pixel area. The average pixel area per section for the single factor of VEGF was 253,658±34,551, while a two growth factor cocktail of BDNF and VEGF averaged 153,910±22,100. The three growth factor groups of BDNF, VEGF, and Calpain inhibitor and BDNF, VEGF, and bFGF had an average graft size of 161,843±21,442 and 111,864±18,203

pixels, respectively. The second group of animals receiving four factor cocktails averaged a graft area of 173,124±22,158. A Control of PBS alone was repeated and had an average graft area of 122,307±24,697. Compiled average graft size between all groups in the both portions of the experiment was compiled (Figure 8). While comparatively these groups show lots of qualitative information, further assessment of axons outgrowth is needed to detect further differences between the groups. We plan to use stereological methods to estimate the total number of axons using non-bias methods in the future.

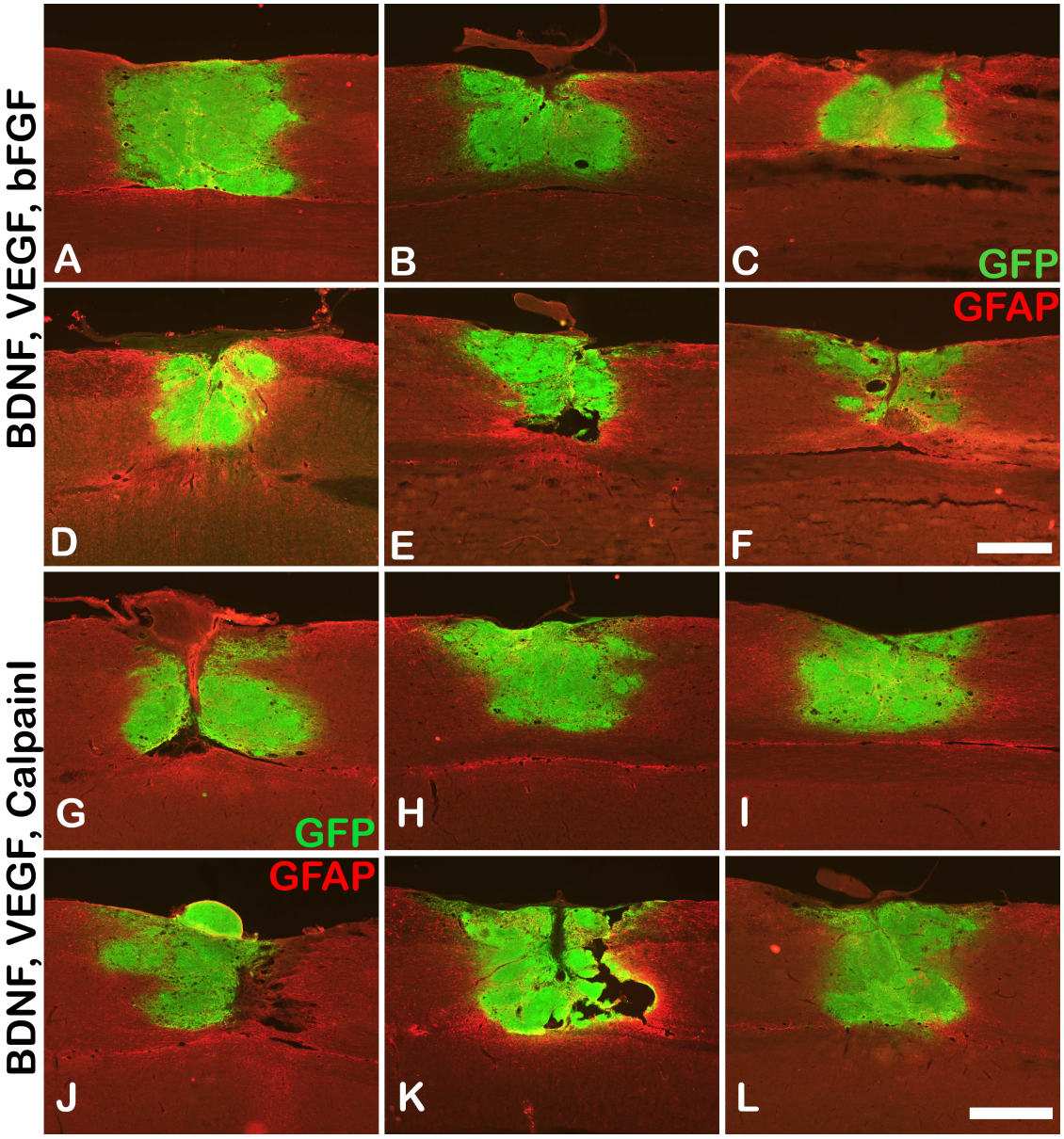

Figure 5. Grafting of NSCs with 3 Growth Factors to Further Assess Four Factor Cocktail.
(**A-F**) Immunolabeled for GFP and GFAP, one representative horizontal section from each of 6 animals show grafts receiving BDNF, VEGF, and bFGF growth factor cocktail. (**G-L**) Similarly, 6 grafts receiving the growth factor cocktail combination of BDNF, VEGF, and Calpain Inhibitor result in similar morphological characteristics of the four factor cocktail, completely filling the lesion site while not abundantly proliferating to the extent of the grafts receiving full cocktail. Scale Bar in **A** and **L**: 500μm.

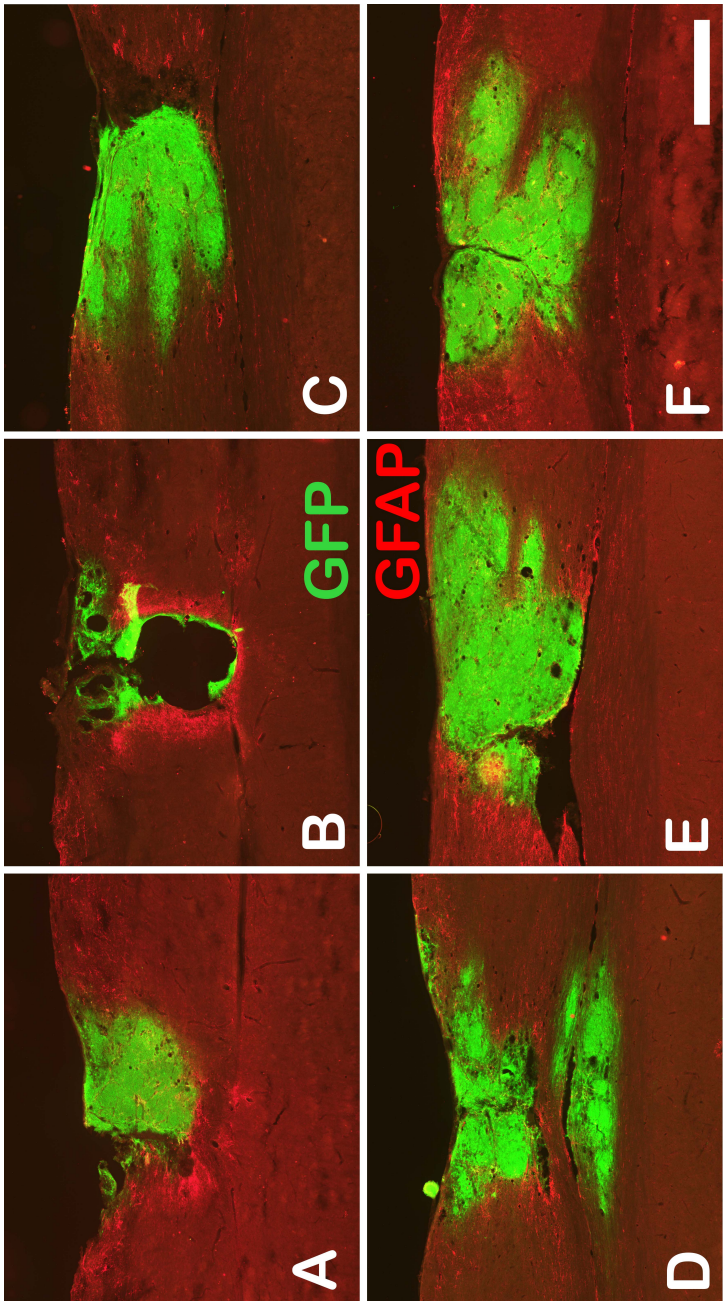

Figure 6. Grafting of NSCs with 2 Growth Factors of BDNF and VEGF to Further Assess Four Factor Cocktail.
(**A**-**F**) Grafts receiving only two growth factors of BDNF and VEGF horizontally sectioned and stained for GFP and GFAP. One representative section from each animal is shown.
Scale Bar: 500μm.

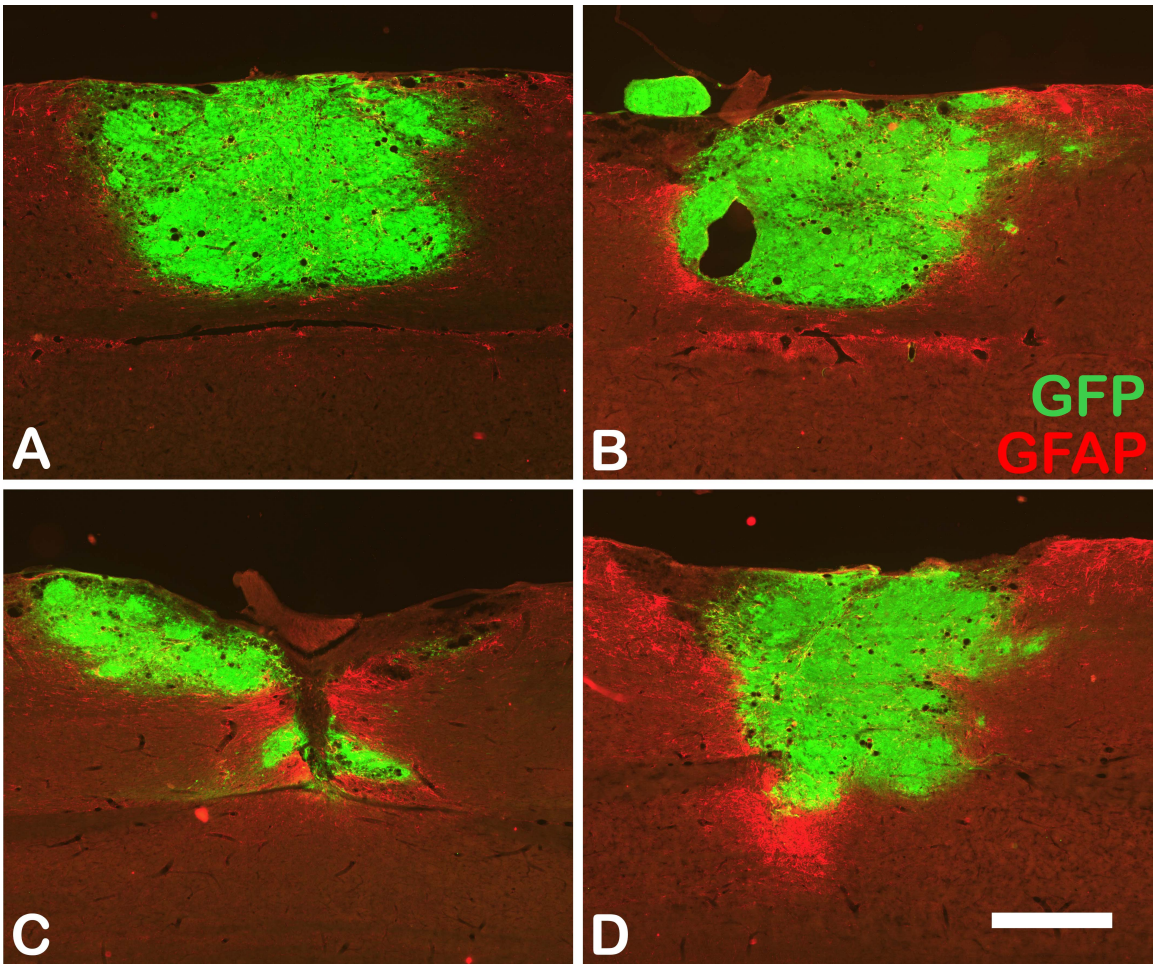

Figure 7. Grafting of NSCs with Single Growth Factor of VEGF to Further Assess Four Factor Cocktail

(**A**-**D**) Grafts receiving only one growth factor of VEGF horizontally sectioned and stained for GFP and GFAP. One representative section from each animal is shown. While this group had a much larger average graft size than the four factor cocktail itself (see Figure 8), further non-biased assessment of graft characteristics, including axon outgrowth, will be done in the future.
Scale Bar: 500μm.

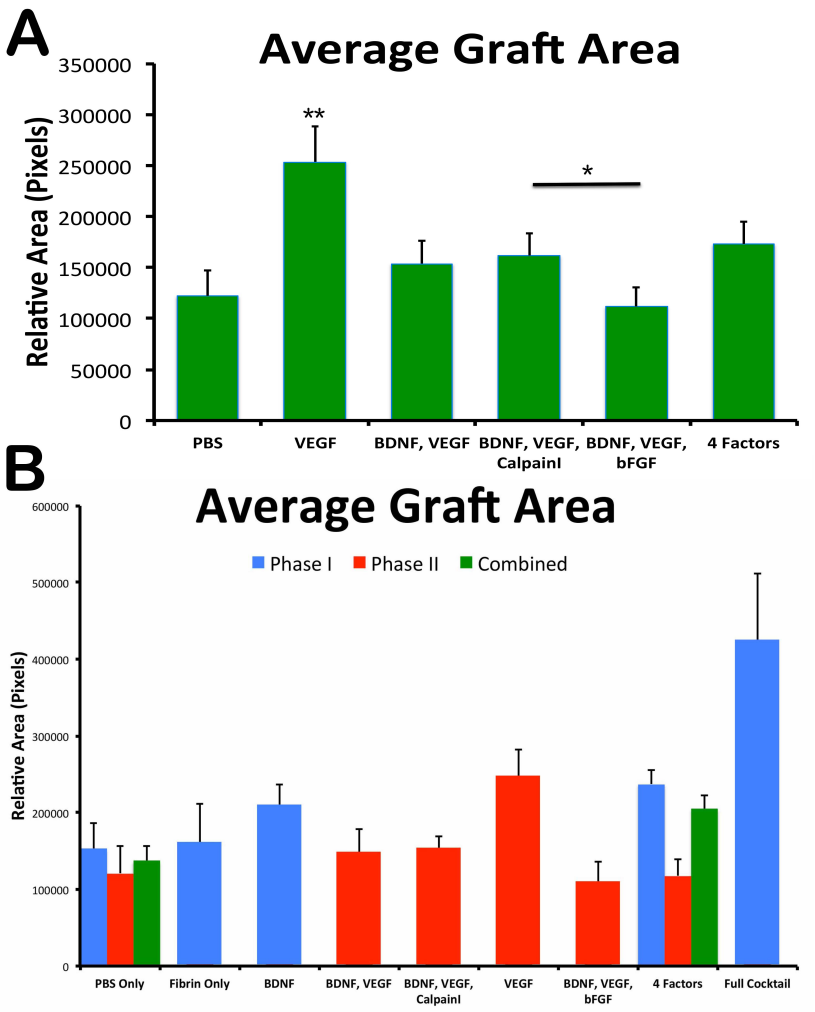

Figure 8. Grafted NSCs with Reduced Four Growth Factor Cocktail show Survival and Fill with Comparable Average Graft Size.
(**A**) Average graft sizes as a measurement of average pixels per graft per animal for the single factor of VEGF was 253,658±34,551, while a two growth factor cocktail of BDNF and VEGF averaged 153,910±22,100. The three growth factor groups of BDNF, VEGF, and Calpain inhibitor and BDNF, VEGF, and bFGF had an average graft size of 161,843±21,442 and 111,864±18,203 pixels, respectively. The second group of animals receiving four factor cocktails averaged a graft area of 173,124±22,158. A Control of PBS alone was repeated and had an average graft area of 122,307±24,697. There was a significant difference observed between the two groups receiving three factors as well as between VEGF alone and all other groups. (**B**) Compilation of all average graft size data amongst all groups.
All data represented as mean ± SEM. (*$p<0.05$, **$p<0.01$).

Chapter 4: Discussion

The goal of everyone working in SCI research has the ultimate goal of human translation. Every piece of information, small and large, contributes towards learning bits of information that will hopefully one day end of helping humans that suffer from CNS system injury and disease. While the work of Dr. Paul in has made tremendous progress towards better studying and understand regeneration in the spinal cord, there is no question that his methods could be improved to generate as impressive of results with a more practical means of achievement (Lu et al., 2012). The growth factor cocktail used in Dr. Lu's work is simply impractical with 11 growth factors because of the need to know the individual function of each of these factors in humans. The data presented in this study shows that superior morphological characteristics can be elicited with a one to four growth factor cocktail. The implications of these findings suggests a much more feasible method for human translation.

One astonishing feature of grafting methods used in Dr. Lu's work is the complete lesion filling observed. While the survival and differentiation within these grafts is remarkable, they arguably achieve too much growth. It is possible these grafts grow at such significant rates that they protrude and disturb host tissue. This can be seen by the bulging of grafts into host tissue rostral and caudal to the graft as well as the central canal. As these studies suggest, elimination of certain factors can restrict growth to fill the lesion site alone, with subsidiary disruption of intact tissue. What is achieved with the given four factor

cocktail is a cellular replacement of lesioned tissue without excess and unnecessary growth.

Another characteristic that may heavily contribute to a more desirable graft survival is the distribution of cells throughout the graft. The clustering of cells within grafts using 11 growth factors is very apparent. This distribution likely disrupts any potential for axon extension, only allowing the most accessible neuronal cell bodies to extend axons out from the graft. While we did observe qualitatively comparable amounts of axon density rostral and caudal to the lesion in a reduced growth factor cocktail, we plan to use stereological methods to estimate the total number of axons using non-bias methods in the future.

The potential has been proposed that less than four factors are needed for graft survival. This issue was address by further analyzing the four growth factor cocktail and grafts with combinations of three or less factors. The data presented shows that various combinations can achieve certain favorable morphological characteristics observed in the four factor cocktail. While no combination seems to achieve every quality, it is likely that the factors work in combination, either together or in competition, resulting in a unique harmony of biological activity. Further assessment of these groups, including axonal outgrowth, will provide more insight as to these individual harmonies within combinations of growth factors.

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