Journal of Cell Science & Therapy
ISSN: 2157-7013

Geisbauer et al. J Cell Sci Ther 2011, 2:1
DOI: 10.4172/2157-7013.1000103

Research Article Open Access

Transplantation of Enteric Cells into the Rodent Stomach with Basic Fibroblast Growth Factor

Carrie L. Geisbauer[1], June C. Chapin[3], Benjamin M. Wu[1,2,4] and James C.Y. Dunn[1,2,3]*

[1]*Biomedical Engineering Interdepartmental Program, University of California, Los Angeles, CA*
[2]*Department of Bioengineering, University of California, Los Angeles, CA*
[3]*Department of Surgery, Division of Pediatric Surgery, University of California, Los Angeles, CA*
[4]*Department of Material Science and Engineering, University of California, Los Angeles, CA*

Abstract

Enteric cells, isolated from intestinal smooth muscle strips, were transplanted into the syngeneic rodent stomach in collagen gel with and without basic fibroblast growth factor (bFGF). While growth factor delivery did not affect cell viability after transplantation, bFGF promoted the formation of numerous ganglion-like structures at the injection site and adjacent muscularis. Immunohistochemistry staining revealed the presence of peripherin, S100, and synaptophysin in these ganglion-like structures. A small percentage of transplanted enteric cells expressed peripherin in the injection site after transplantation. Enteric cells transplanted with collagen and bFGF may hold potential as a cellular therapeutic for various motility disorders of the gastrointestinal tract.

Keywords: Cell transplantation; Neural crest stem cells; Enteric nervous system; bFGF

Introduction

The enteric nervous system (ENS) is part of the peripheral nervous system that controls the functions of the gastrointestinal tract [1-7]. The ENS is primarily comprised of the myenteric and submucosal plexi [1,2,4,7,8]. Located between the longitudinal and circular muscle layers of the gastrointestinal tract, the myenteric plexus controls the motility of the gut. The submucosal plexus is located between the circular muscle and the submucosa and is primarily responsible for controlling the secretions of the gut. The development of these plexi originates from the rostrocaudal migration of vagal neural crest cells [5,6,9,10,11]. Enteric neural crest cells require appropriate guiding signals, either neurotropic factors [12-16] or extracellular matrix molecules including collagen or laminins [17,18], to migrate to the appropriate location.

Neural crest stem cells have been investigated via organ cultures and xenotransplantation as a potential cell source for cellular therapeutics. Achalasia, Hirschsprung's diease and other motility disorders may be treated with advances in cell transplantation [7,9]. Brain-derived and gut-derived neural crest stem cells have shown survival, migration and expression of enteric markers when investigated under these conditions [7,9,14,19,20,21]. Neural crest stem cells have also been transplanted into aganglionic gut in organ cultures [22]. These organ culture studies suggest the permissivity of the aganglionic gut to repopulation via stem cell transplantation, however, they do not provide evidence that this effect is also observable *in vivo*.

Basic fibroblast growth factor (bFGF) is a mitogen for a variety of mesodermal and neuroectodermal-derived cells, and its presence has been described in many tissues[23]. bFGF belongs to a large family of related fibroblast growth factors which share angiogenic and growth-promoting properties [23,24]. bFGF exhibits paracrine and autocrine signaling [25] and modulates other neurotropic factors [26]. *In vitro*, bFGF maintains the survival of astroglia cells [27], promotes neurite outgrowth of hippocampal [28] and cortical neurons [29], and simulates division of cortical multipotent stem cells [30,31]. In the ENS, bFGF immunoreactivity has been detected in the majority of neurons and glia in the distal esophagus and colon [25]. Furthermore, bFGF has been shown to stimulate proliferation of neural crest cells *in vitro* [32]. In bFGF knockout mice, enteric ganglia are reduced in diameter, and the number of neurons and glial cells per ganglionic area is decreased [31,33]. bFGF has also been shown to promote the survival of primary intestinal smooth muscle cells in collagen coated scaffolds implanted *in vivo* in the omentum [24].

Type I collagen is the most abundant collagen and is an important extracellular matrix protein for neurons [17,18,34,35]. It assists in the guidance of neural crest stem cells to their appropriate location as well as neurite growth from differentiated neurons [17,18,34,35]. In addition to its importance in the native environment, 3D collagen gels with bFGF have also been shown to promote proliferation, differentiation and network formation of neural stem cells [34,35]. Collagen entrapped neural stem cells have formed networks with active synapses that are stimulated and inhibited by traditional pharmacological methods [34,35]. It is therefore believed that neural stem cells entrapped in type I collagen gels with bFGF may be able to regenerate parts of the nervous system.

In this study, we evaluated the mitogenic and differentiation effects of bFGF on enteric cells when transplanted *in vivo* in the rodent stomach. Peripherin, an intermediate filament in neurons, S100, a glial cell protein, and synaptophysin, the synaptic vesicle protein p38, were all markers used in this evaluation.

Materials and Methods

Primary intestinal cell harvest

Animal experiments were performed in accordance with the

***Corresponding author:** James C.Y. Dunn, M.D., Ph.D, UCLA Surgery-Pediatrics, Box 957098, CHS 72-140, Los Angeles, CA 90095-7098. Tel: 310.206.2429; Fax: 310.206.1120; E-mail: jdunn@mednet.ucla.edu

Received November 23, 2010; **Accepted** March 09, 2011; **Published** March 10, 2011

Citation: Geisbauer CL, Chapin JC, Wu BM, Dunn JCY (2011) Transplantation of Enteric Cells into the Rodent Stomach with Basic Fibroblast Growth Factor. J Cell Sci Ther 2:103. doi:10.4172/2157-7013.1000103

regulations of the Animal Research Committee at the University of California, Los Angeles. Intestines of the neonatal Lewis rat pups (1-5 days old) expressing green fluorescent protein (GFP) on the ubiquitin promoter [36] were used for cell isolation. Briefly, the intestines were removed via midline incision and smooth muscle strips containing both longitudinal and circular musculature were gently sectioned from the intestines with fine forceps. The strips were placed on ice in Hank's Balanced Salt Solution (HBSS) without calcium and magnesium (Invitrogen, Carlsbad, CA). The strips were incubated for 35 minutes in a water bath at 37°C with collagenase IV (200 µl of 10 mg ml^{-1} stock dissolved in HBSS with Ca^{2+} and Mg^{2+}) (Sigma-Aldrich, St. Louis, MO) dissolved in 1800 µl of HBSS without Ca^{2+} and Mg^{2+}. Dulbecco's Modified Eagle Medium (DMEM) containing 10% fetal bovine serum (FBS) (Invitrogen) was used to stop the collagenase digestion. The resulting suspension was strained through a 70 µm cell strainer (Becton Dickenson, Franklin Lakes, NJ) prior to use.

Collagen extraction

Type I collagen was extracted from Lewis rat tail tendons. All solutions were maintained at 4°C. Tendons from 4-8 rat tails were pulled by dislocating every other joint in the tail and placed in cold 0.5 M glacial acetic acid (Fisher, approximately 400 ml). The tendons were spun overnight at 4°C with pepsin (Sigma-Aldrich, 0.5 mg/ml) to remove the telopeptides. The solution was centrifuged at 15,000 g at 4°C for 30 minutes. The precipitate was discarded and NaCl (Sigma-Aldrich) was slowly added while stirring for 30 minutes to bring the solution to 2.5 M. The solution was centrifuged at 15,000 g at 4°C for 30 minutes. The supernatant was discarded and the precipitate was resuspended in a 1.0 M NaCl (Sigma-Aldrich), 50 mM Tris (Sigma-Aldrich), pH 7.4 solution. The precipitate was allowed to dissolve into solution overnight. The solution was centrifuged at 15,000 g at 4°C for 30 minutes. The precipitate was discarded and NaCl (Sigma-Aldrich) was added while spinning for 30 minutes to bring the solution to 2.5 M NaCl. The solution was centrifuged at 15,000 g at 4°C for 30 minutes. The supernatant was discarded and the precipitate was resuspended in ~200 ml of 0.5M glacial acetic acid (Fisher). This solution was dialyzed against 0.5 M acetic acid for 8 hours with at least 3 buffer changes. The solution was then dialyzed against 0.001 M hydrochloric acid and spun overnight at 4°C. The collagen concentration was measured to be 4 mg/ml by freeze drying and weight analysis. Type I collagen was aliquotted and stored at 4°C.

Collagen preparation

All solutions were kept at 4°C. Collagen was neutralized prior to use. The collagen solution was mixed with 10x phosphate buffered saline (PBS) (1:10; Invitrogen). Several drops of sterile phenol red were added and the pH was adjusted to 7.4 with 0.01 M NaOH (Fisher). Collagen was mixed 1:1 with HBSS prior to injection.

Surgical procedures

Female Lewis rats (Charles River Laboratories, Wilmington, MA) weighing 200-250 g were used as recipients. The stomach was located through a midline incision under isoflourane anesthesia. 6-0 Prolene sutures (Ethicon, Sommerville, NJ) were used as injection site markers in the gastric wall. Prior to the injection, isolated cells were resuspended in HBSS with India Ink (2% v/v, Becton Dickenson) as an indicator of the injection area. Immediately prior to transplantation, approximately 250,000 cells in HBSS with India ink were mixed with neutralized collagen solution and bFGF (~2.5 ug) depending on the experimental group. The total injection volume was 10 µl. The mixture was injected into the gastric wall via a micro-injector with a ½" long 33G needle (Hamilton, Reno, NV). The abdominal wall was closed using an absorbable 3-0 suture and the skin was closed with a 3-0 non-absorbable suture. After 7 days, the animals were euthanized. The injection site and surrounding gastric wall containing the India ink was removed and divided into equal parts for immunohistochemistry and qRT-PCR for GFP DNA. The number of animals per group was 6.

qRT-PCR for GFP DNA

Total DNA was isolated from the gastric wall using the Qiagen DNeasy Blood and Tissue Kit (Qiagen) protocol. DNA samples were also treated with RNase (Qiagen). The absorbance of the sample at 260 nm was measured to quantify the total amount of DNA. Real-time PCR was performed using an ABI PRISM 7700 Sequence Detection System (Applied Biosystems) and the PCR master mix from the Quantitect Probe RT-PCR kit (Qiagen). Sequences for the forward and reverse GFP primer were sense, 5-ACTACAACAGCCACAACGTCTATATCA-3 and antisense, 5-GGCGGATCTTGAAGTTCACC-3 [37]. The sequence for the probe was 5-CCGACAAGCAGAAGAACGGCATCA-3 [37]. The probes was labeled with 6-FAM, a fluorescent reporter dye with TAMRA at the 3' end. PCR reactions were carried out in a volume of 20 µl. The following were added to each well of a 96-well RT-PCR plate (Applied Biosystems): 12.5 µl/well of master mix (Qiagen), 1 µl of each primer (forward and reverse: 1800 nM stock), 1 µl of probe (500 nM stock), 3.5 µl of DNase/RNase-free water (Qiagen) and 1 µl (1 ug) of each DNA sample. Each sample was run in duplicate and normalized to a sample containing a known number of GFP+ cells isolated as described above. A standard curve was used to calculate the number of GFP+ cells present in the injection sites.

Immunohistochemistry

After tissue procurement, the rodent stomachs were fixed in 10% formalin for 24 hours at 4°C. Samples were dehydrated and embedded in paraffin wax. Sections, 5 µm thick, were cut and adhered to glass slides. Slides were dewaxed in xylene and rehydrated by washes in 100%, 95%, 70% and 0% ethanol (Fisher Scientific). Antigen retrieval was performed in a citric buffer (Biogenex, San Ramon, CA) for 20 minutes at 95°C and slides were allowed to cool for 30 minutes in a running cool water bath. Slides were incubated in a solution of 4% normal goat serum (Vector labs, Burlingame, CA) and 0.4 % bovine serum albumin (Fisher Scientific) in PBS with 0.5% Tween-20 (PBS/T) for 1 hour to block non-specific secondary binding. Primary antibodies were peripherin (1:200; Millipore), S100 (1:200; Dako) and GFP (1:200; Millipore). Slides were incubated with these antibodies diluted in PBS/T at 4°C for 24 hours. Slides were washed for 30 minutes in PBS/T. Secondary antibodies (goat anti-rabbit Alexa Fluor 488, goat anti-mouse Alexa Fluor 594, Invitrogen) were diluted in PBS/T and slides were incubated for 30 minutes at room temperature in this solution. Slides were again washed for 30 minutes with PBS/T at room temperature. Prolong Gold with Dapi (Invitrogen) was applied to each tissue section and the slides were covered with glass coverslips (Fisher). The mounting media was allowed to cure overnight at room temperature and then stored at 4°C.

Quantification of staining

Histological slides were visualized with an AxioObserver inverted Microscope, Axiocam MRm camera and AxioVision software (Zeiss, Thornwood, NY). Confocal laser scanning microscopy was performed using a Leica SP2 1P-FCS scope and the associated Leica LAS-AF imaging software. Quantification of ganglion-like structures was performed by counting the number of structures per visual field in 3 separate visual fields per section. This process was repeated for 2 additional sections at least 15 µm apart. One-way ANOVA in Microsoft Excel was utilized to determine statistical significance.

Results

Normal rodent stomach

Staining of the normal rodent stomach revealed prominent native ganglia of the myenteric plexus located between the outer longitudinal smooth muscle and inner circular smooth muscle layers (Figure 1). Expression of peripherin, S100 and synaptophysin were predominantly found in the gangla of the myenteric plexus (Figure 1). Expression of these markers was sparse within the longitudinal and circular smooth muscle layers of the normal gastric wall and was not organized as ganglia.

Control experiments

To account for the effect of collagen and bFGF, injections into the gastric wall without cells were performed in control experiments. The histological sections of injections of only HBSS (Figure 2A), neutralized rat tail collagen (Figure 2B) and neutralized rat tail collagen with bFGF (Figure 2C) demonstrated the location of the injection site to be in the submucosal space. Injections with collagen showed a larger and more localized injection site as indicated by the India ink (Figure 2B & 2C). Injections including bFGF showed an increase in the vascularity of the injection site and the surrounding tissue (Figure 2C).

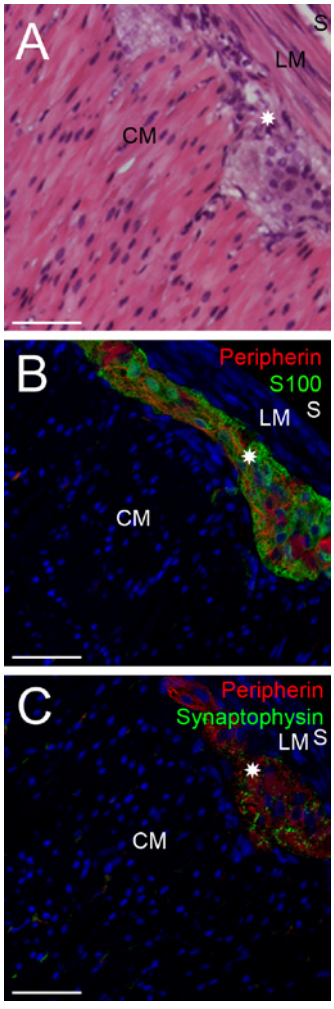

Figure 1: **Normal Rodent Stomach.** Normal rodent stomach sections stained with hematoxylin & eosin (A), peripherin & S100 (B) and peripherin & synaptophysin (C) showed strong staining of the myenteric plexus (starburst) located between the circular (CM) and longitudinal [LM] musculature. Nuclei were stained blue with DAPI. The serosa (S) was labeled and scale bars represent 50 um.

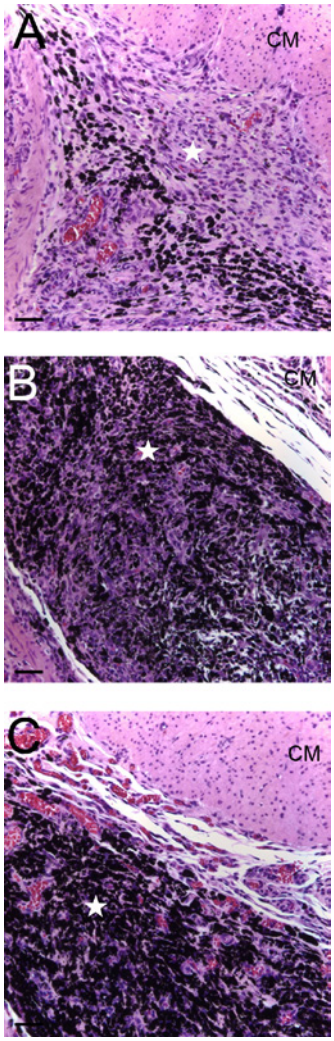

Figure 2: **Injection Sites of Control Experiments.** HBSS (A), collagen (B) and collagen & bFGF (C) were injected without cells into the rodent stomach in control experiments. As expected, injection sites with bFGF showed an increased vascularity in and around the injection site. Staining of serial sections of these histological sections revealed no expression of peripherin or S100. Circular muscle (CM) and injection site (star) were labeled.

Expression of peripherin, S100 and synaptophysin was also investigated in these control experiments. The expression of these markers in the ganglia, in the injection site, and in the adjacent smooth muscle was similar to the expression found in the normal rodent gastric wall (data not shown).

Cell Transplantation with bFGF

Intestinal neuromuscular cells mixed with rat tail collagen were injected into the normal rodent gastric wall. In the presence of bFGF, cells expressing peripherin and S100 were found to be in close proximity to each other in the center of the injection site (Figure 3A & 3C). The neuron-like cells expressing peripherin were enveloped by glial-like cells expressing S100 and formed ganglion-like structures in the presence of bFGF. The number of such ganglion-like structures per high

power field was 2±1, or approximately 6 per mm^2. In the cell injection sites without the simultaneous delivery of bFGF, these ganglion-like structures were never observed. In both groups, cells expressing peripherin were primarily located near the center of the injection site, whereas cells expressing S100 were distributed throughout the injection site with a slight increase toward the outer edge of the site.

There was also an increase in the co-localization of peripherin with synaptophysin in the cell injection sites when bFGF was simultaneously delivered (Figure 3D) as compared to the cell injection sites without bFGF delivery (Figure 3B). Synaptophysin, a synaptic vesicle glycoprotein, marks synaptic vesicles that would be present in active synapses. Therefore, active synapses appeared to be present in the injection site of the enteric cells transplanted with bFGF (Figure 3D). The number of ganglion-like structures expressing peripherin and synaptophysin per high power field in the injection site was 6±3 (approximately 18 per mm^2) in the group with bFGF and 1±1 (approximately 3 per mm^2) in the group without bFGF (p=0.0002).

Cells expressing peripherin and S100 were also found in the smooth muscle adjacent to the injection site of transplanted cells (Figure 4). Peripherin and S100 expressing cells were located in close proximity to each other and resembled ganglion-like structures in the smooth muscle adjacent to the injection site when bFGF was simultaneously delivered with the cells (Figure 4C). Peripherin and S100 expressing cells were also identified in the smooth muscle of the experimental group without bFGF; however, these cells did not localize in close proximity to each other nor formed ganglion-like structures. In the smooth muscle near the injection site, the number of ganglion-like structures per high power field that expressed peripherin and S100 was 3±2 (approximately 9 per mm^2) in the group with bFGF and 0 in the group without bFGF. Co-localization of peripherin and synaptophysin was also found in the smooth muscle adjacent to the injection site when enteric cells were transplanted with bFGF (Figure 4D). The number of active synapses was higher than that found in the normal smooth muscle (Figure 1C). The number of ganglion-like structures expressing peripherin and synaptophysin per high power field was 5±3 (approximately 15 per

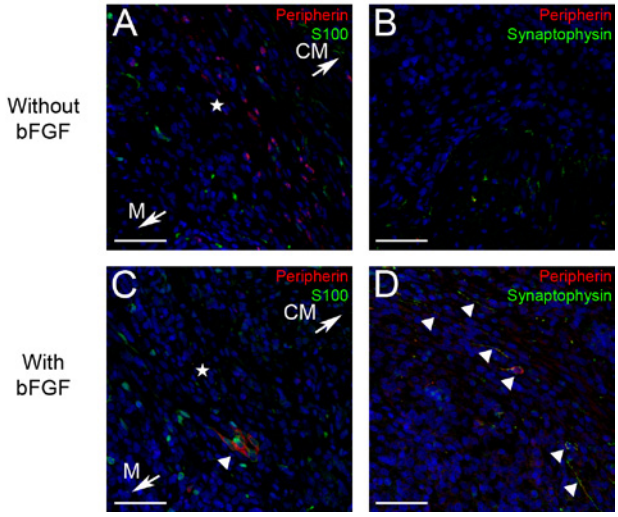

Figure 3: **Transplantation of Enteric Cells with and without bFGF.** Approximately 250,000 enteric cells were injected in the rodent stomach with and without bFGF. Peripherin (red) and S100 (green) expression in the injection site without bFGF (A) and with bFGF (C) show the formation of ganglion-like structures in the injection site (star) in the presence of bFGF(triangle, C). Peripherin (red) and synpatophysin (green) expression in the injection site without bFGF (B) and with bFGF (D) show synaptic vesicles are present in these ganglion-like structures (triangle, D) that are formed in the presence of bFGF. The direction of the mucosa (M) and circular muscle (CM) are noted with arrows on A & C and this orientation is consistent throughout the figure. Nuclei are stained with DAPI (blue) and scale bars represent 50 um.

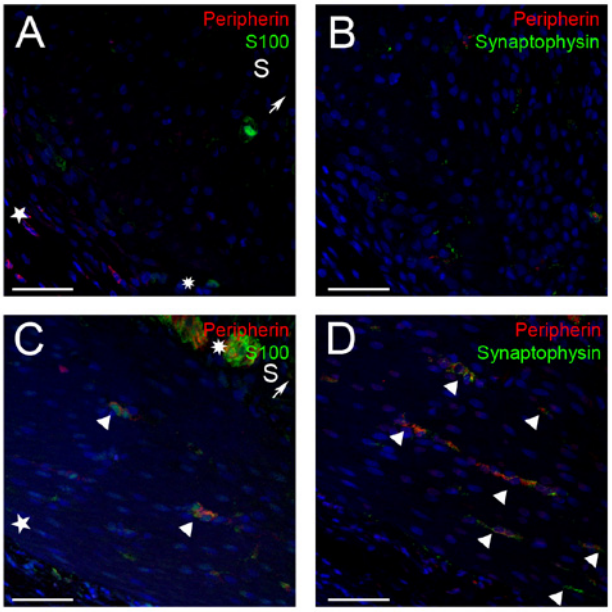

Figure 4: **Peripherin, S100 & Synaptophysin Expression in the Musculature Adjacent to Injection Site.** Approximately 250,000 enteric cells were injected (star) in the rodent stomach with and without bFGF. Peripherin (red) and S100 (green) expression in the muscle adjacent to the injection site without bFGF (A) and with bFGF (C) revealed the presence of ganglion-like structures (triangle) not present in the native stomach musculature. Peripherin (red) and synaptophysin (green) expression in the muscle adjacent to the injection site without bFGF (B) and with bFGF (D) revealed the presence of synaptic vesicles at the newly formed ganglion-like structures. Native ganglia (starburst) and the direction of the serosa (arrow) were labeled. Nuclei were stained blue with DAPI and scale bars represent 50 um.

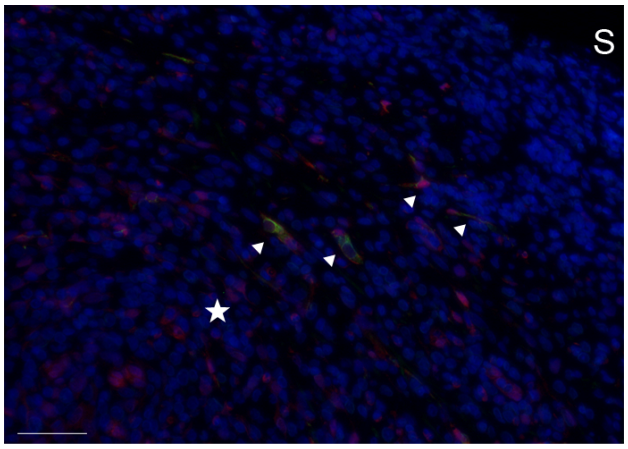

Figure 5: **GFP Expression in the Injection Site.** GFP (red) and peripherin (green) expression in the injection site of approximately 250,000 GFP+ enteric cells injected into the rodent gastric wall with collagen and bFGF. While GFP expression was found throughout the injection site, very few cells had co-localization of peripherin and GFP expression (triangle). The serosa (S) and the injection site (star) were labeled. Nuclei were stained blue with DAPI and scale bars represent 50 um.

mm^2) in the group with bFGF and 1±1 (approximately 3 per mm^2) in the group without bFGF (p=0.0003). Additionally, the size of the ganglion-like structures was qualitatively noted to be larger in all of the animals that received cell transplantation with bFGF.

GFP was used as a method of tracking the fate of the transplanted enteric cells. While cells expressing GFP were found in the injection site and in the smooth muscle adjacent to the injection site (Figure 5), very few of the cells also expressed peripherin. qRT-PCR for GFP DNA was used to quantify the number of GFP cells present in the retrieved tissue. Approximately 22,000 ± 3,300 GFP cells were present in the injection site of the group without bFGF, as compared to approximately 44,000 ± 32,000 GFP cells in the group with bFGF (p=0.55).

Discussion

Enteric neural progenitor cells have shown great promise as a potential cellular therapeutic for achalasia, Hirschsprung's disease and other motility disorders of the gastrointestinal tract. Enteric cells, investigated in this study, are a population of cells believed to contain the enteric neural progenitor cells along with glial, smooth muscle cells, fibroblasts and interstitial cells of Cajal. In our previous studies, enrichment of the population for low affinity nerve growth factor receptor, a known neural crest stem cell marker, did not significantly change the results *in vivo* [38].

In this study, a carrier, collagen, was utilized to assist in the delivery of the cells and bFGF to the injection site. Type I collagen was chosen due to its prominence in the extracellular matrix and its role in supporting neurite adhesion and growth [8,10,39]. Rat tail collagen was extracted and selected for its limited immunogenicity and ability to mimic the native physiological setting of neurons [40,41]. Transplantation of enteric cells with collagen localized the injection better than enteric cells transplanted in HBSS. In pilot studies, similar results were seen when human fibrinogen was used as the cell carrier. It is also probable that commercially available extracelluar matrix products such as PureCol and Matrigel would perform similarly to the type I collagen used in this study.

bFGF was utilized because of its known mitogenic and angiogenic properties. It is possible that bFGF acts to promote cell survival and differentiation by increasing the vasculature in and around the injection site. An increase in angiogenesis would support the survival and growth of cells in the center of the injection site over injections without bFGF. The dose of bFGF was selected based on the *in vitro* effect on this cell population [14,39,42-44]. Pilot studies involving higher doses of bFGF (up to 7.5 μg) produced no significant improvement over the dose used in this study.

Endothelin-3, a growth factor required for the normal development of the enteric nervous system, was also investigated in our preliminary studies [45]. Endothelin-3 supported the expression of p75, a known neural crest stem cell marker, and differentiation by enteric cells *in vitro*. This effect, however, was not seen when endothelin-3 was used in our *in vivo* experiments. It is possible that the dosage and/or availability of endothelin-3 were not optimized for proliferation and differentiation of enteric cells *in vivo*. More investigation of the *in vivo* response of this growth factor is required for more successful outcomes with these cells.

The number of GFP cells in the injection site and surrounding gastric tissue likely underestimates the total number of GFP cells in the recipient animal. The entire stomach and surrounding organs of the rodent would need to be assessed to achieve a more accurate account of the injected cells that survived transplantation. Our previous studies have shown that the number of cells present in the injection site is similar, regardless of whether collagen or HBSS are used as the carrier [38]. The relatively low number of transplanted cells that express peripherin suggests that additional expression of this marker may be derived from native cells induced by the transplantation of enteric cells into the gastric wall. Additionally, it has been reported that neural stem cells lose expression of GFP after differentiation [46]. Regardless of the mechanism, the expression of neural and glial markers not normally seen in the rodent gastric wall was consistently observed in our experimental groups when enteric cells were transplanted.

Our results confirm previous works that have shown the survivability of transplanted cells in the gut [14,22]. This study further shows that the addition of bFGF may support cell survival and induce the formation of ganglion-like structures in the injection site and adjacent musculature in the gastrointestinal tract. The ability of these enteric cells to survive *in vivo* transplantation and induce ganglion-like expression of peripherin, S100 and synaptophysin in a diseased state will need to be documented in the future. This work provides motivation to perform similar experiments in the diseased state as well as future studies to determine the contractility and synaptic transmission in the diseased gastrointestinal tract. To the authors' knowledge, this is the first *in vivo* validation that enteric cells may be a viable cell source for a cellular therapeutic for various motility disorders of the gastrointestinal tract.

Acknowledgements

Financial support provided by the Fubon Foundation and Stein-Oppenheimer Foundation.

The authors would like to thank Dr. Matthias Stelzner for the breeder pairs of GFP+ Lewis rats, Dr. Patricia Zuk for her guidance with immunohistochemical and qRT-PCR techniques, Dr. Andrea Kasko for the use of her microscope and imaging software, Dr. Matthew Schibler for his expertise and assistance with confocal microscopy. Histology related support was provided by the UCLA Translational Pathology Core Laboratory. bFGF supplied by the NIH Preclinical Repository. Confocal laser scanning microscopy was performed at the CNSI Advanced Light Microscopy/Spectroscopy Shared Resource Facility at UCLA, supported with funding from NIH-NCRR shared resources grant (CJX1-443835-WS-29646) and NSF Major Research Instrumentation grant (CHE-0722519).

Disclosures

The authors have no conflicts of interests to disclose.

References

1. Furness JB (2000) Types of neurons in the enteric nervous system. J Auton Nerv Syst 81: 87-96.
2. Furness JB (2006) The enteric nervous system. Malden, Massachusetts, Blackwell Publishing, USA.
3. Furness JB (2006) The organisation of the autonomic nervous system: peripheral connections. Auton Neurosci 130: 1-5.
4. Furness, JB, Costa M (1987) The enteric nervous system. Edinburgh, New York: Churchill Livingstone.
5. Le Douarin N (1982) The neural crest. Cambridge, UK, Cambridge University Press.
6. Le Douarin N, Kalcheim C (1999) The neural crest, 2nd ed. Cambridge, UK: Cambridge University Press.
7. Gershon MD (2007) Transplanting the enteric nervous system: a step closer to treatment for aganglionosis. Gut 56: 459-461.
8. Hansen MB (2003) The enteric nervous system I: organisation and classification. Pharmacol Toxicol 92: 105-113.
9. Burns AJ, Pasricha PJ, Young HM (2004) Enteric neural crest-derived cells and neural stem cells: biology and therapeutic potential. Neurogastroenterol Motil 16 Suppl 1: 3-7.
10. Le Douarin NM, Dupin E (2003) Multipotentiality of the neural crest. Curr Opin Genet Dev 13: 529-536.

11. Young HM, Ciampoli D, Hsuan J, Canty AJ (1999) Expression of Ret-, p75(NTR)-, Phox2a-, Phox2b-, and tyrosine hydroxylase-immunoreactivity by undifferentiated neural crest-derived cells and different classes of enteric neurons in the embryonic mouse gut. Dev Dyn 216: 137-152.

12. Golden JP, DeMaro JA, Osborne PA, Milbrandt J, Johnson EM Jr (1999) Expression of neurturin, GDNF, and GDNF family-receptor mRNA in the developing and mature mouse. Exp Neurol 158: 504-528.

13. Heuckeroth RO, Enomoto H, Grider JR, Golden JP, Hanke JA et al. (1999) Gene targeting reveals a critical role for neurturin in the development and maintenance of enteric, sensory, and parasympathetic neurons. Neuron 22: 253-263.

14. Mosher JT, Yeager KJ, Kruger GM, Joseph NM, Hutchin ME et al. (2007) Intrinsic differences among spatially distinct neural crest stem cells in terms of migratory properties, fate determination, and ability to colonize the enteric nervous system. Dev Biol 303: 1-15.

15. Newgreen D, Young HM (2002) Enteric nervous system: development and developmental disturbances--part 2. Pediatr Dev Pathol 5: 329-349.

16. Newgreen D, Young HM (2002) Enteric nervous system: development and developmental disturbances--part 1. Pediatr Dev Pathol 5: 224-247.

17. Bannerman PG, Mirsky R, Jessen KR, Timpl R, Duance VC (1986) Light microscopic immunolocalization of laminin, type IV collagen, nidogen, heparan sulphate proteoglycan and fibronectin in the enteric nervous system of rat and guinea pig. J Neurocytol 15: 733-743.

18. Jacobs-Cohen RJ, Payette RF, Gershon MD, Rothman TP (1987) Inability of neural crest cells to colonize the presumptive aganglionic bowel of ls/ls mutant mice: requirement for a permissive microenvironment. J Comp Neurol 255: 425-438.

19. Micci MA, Kahrig KM, Simmons RS, Sarna SK, Espejo-Navarro MR et al. (2005) Neural stem cell transplantation in the stomach rescues gastric function in neuronal nitric oxide synthase-deficient mice. Gastroenterology 129: 1817-1824.

20. Micci MA, Learish RD, Li H, Abraham BP, Pasricha PJ (2001) Neural stem cells express RET, produce nitric oxide, and survive transplantation in the gastrointestinal tract. Gastroenterology 121: 757-766.

21. Micci MA, Pasricha PJ (2007) Neural stem cells for the treatment of disorders of the enteric nervous system: strategies and challenges. Dev Dyn 236: 33-43.

22. Almond S, Lindley RM, Kenny SE, Connell MG, Edgar DH (2007) Characterisation and transplantation of enteric nervous system progenitor cells. Gut 56:489-496.

23. Gospodarowicz D, Neufeld G, Schweigerer L (1986) Molecular and biological characterization of fibroblast growth factor, an angiogenic factor which also controls the proliferation and differentiation of mesoderm and neuroectoderm derived cells. Cell Differ 19: 1-17.

24. Lee M, Wu BM, Stelzner M, Reichardt HM, Dunn JC (2008) Intestinal smooth muscle cell maintenance by basic fibroblast growth factor. Tissue Eng Part A 14: 1395-1402.

25. Chadi G, Gomide VC, Rodrigues de Souza R, Scabello RT, Mauricio da Silva C (2004) Basic fibroblast growth factor, neurofilament, and glial fibrillary acidic protein immunoreactivities in the myenteric plexus of the rat esophagus and colon. J Morphol 261: 323-333.

26. Hagl CI, Klotz M, Wink E, Kranzle K, Holland-Cunz S et al. (2008) Temporal and regional morphological differences as a consequence of FGF-2 deficiency are mirrored in the myenteric proteome. Pediatr Surg Int 24: 49-60.

27. Bikfalvi A, Klein S, Pintucci G, Rifkin DB (1997) Biological roles of fibroblast growth factor-2. Endocr Rev 18: 26-45.

28. Ray J, Peterson DA, Schinstine M, Gage FH (1993) Proliferation, differentiation, and long-term culture of primary hippocampal neurons. Proc Natl Acad Sci USA 90: 3602-3606.

29. Matsuda S, Saito H, Nishiyama N (1990) Effect of basic fibroblast growth factor on neurons cultured from various regions of postnatal rat brain. Brain Res 520: 310-316.

30. Eckenstein FP (1994) Fibroblast growth factors in the nervous system. J Neurobiol 25: 1467-1480.

31. Kniss DA, Burry RW (1988) Serum and fibroblast growth factor stimulate quiescent astrocytes to re-enter the cell cycle. Brain Res 439: 281-288.

32. Murphy M, Reid K, Ford M, Furness JB, Bartlett PF (1994) FGF2 regulates proliferation of neural crest cells, with subsequent neuronal differentiation regulated by LIF or related factors. Development 120: 3519-3528.

33. Hagl CI, Holland-Cunz S, Schafer KH (2003) What do knockout models teach us about the enteric nervous system? Eur J Pediatr Surg 13: 170-175.

34. Lin HJ, O'Shaughnessy TJ, Kelly J, Ma W (2004) Neural stem cell differentiation in a cell-collagen-bioreactor culture system. Brain Res Dev Brain Res 153: 163-173.

35. Ma W, Fitzgerald W, Liu QY, O'Shaughnessy TJ, Maric D et al. (2004) CNS stem and progenitor cell differentiation into functional neuronal circuits in three-dimensional collagen gels. Exp Neurol 190: 276-288.

36. van den Brandt J, Wang D, Kwon SH, Heinkelein M, Reichardt HM (2004) Lentivirally generated eGFP-transgenic rats allow efficient cell tracking in vivo. Genesis 39: 94-99.

37. Pan D, Gunther R, Duan W, Wendell S, Kaemmerer W et al. (2002) Biodistribution and toxicity studies of VSVG-pseudotyped lentiviral vector after intravenous administration in mice with the observation of in vivo transduction of bone marrow. Mol Ther 6:19-29.

38. Geisbauer CL, Chapin JC, Wu BM, Dunn JC (2011) Transplantation of Enteric Cells Expressing p75 in the Rodent Stomach. J Surg Res [Epub ahead of print].

39. Kruger GM, Mosher JT, Tsai YH, Yeager KJ, Iwashita T et al. (2003) Temporally distinct requirements for endothelin receptor B in the generation and migration of gut neural crest stem cells. Neuron 40: 917-929.

40. Bergsteinsdottir K, Hashimoto Y, Brennan A, Mirsky R, Jessen KR (1993) The effect of three dimensional collagen type I preparation on the structural organization of guinea pig enteric ganglia in culture. Exp Cell Res 209: 64-75.

41. Houweling DA, Lankhorst AJ, Gispen WH, Bar PR, Joosten EA (1998) Collagen containing neurotrophin-3 (NT-3) attracts regrowing injured corticospinal axons in the adult rat spinal cord and promotes partial functional recovery. Exp Neurol 153: 49-59.

42. Bixby S, Kruger GM, Mosher JT, Joseph NM, Morrison SJ (2002) Cell-intrinsic differences between stem cells from different regions of the peripheral nervous system regulate the generation of neural diversity. Neuron 35: 643-656.

43. Kruger GM, Mosher JT, Bixby S, Joseph N, Iwashita T et al. (2002) Neural crest stem cells persist in the adult gut but undergo changes in self-renewal, neuronal subtype potential, and factor responsiveness. Neuron 35: 657-669.

44. Morrison SJ, White PM, Zock C, Anderson DJ (1999) Prospective identification, isolation by flow cytometry, and in vivo self-renewal of multipotent mammalian neural crest stem cells. Cell 96: 737-749.

45. Bondurand N, Natarajan D, Barlow A, Thapar N, Pachnis V (2006) Maintenance of mammalian enteric nervous system progenitors by SOX10 and endothelin 3 signalling. Development 133: 2075-2086.

46. Vroemen M, Weidner N, Blesch A (2005) Loss of gene expression in lentivirus- and retrovirus-transduced neural progenitor cells is correlated to migration and differentiation in the adult spinal cord. Exp Neurol 195: 127-139.