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Evaluation of Prototype Cell Delivery Catheters Using Agarose Gel and Cell Culture Experiments

Sagar Panse
Virginia Commonwealth University

Evaluation of Prototype Cell Delivery Catheters Using Agarose Gel and Cell Culture

Experiments

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science at Virginia Commonwealth University.

by

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Abstract

EVALUATION OF PROTOTYPE CELL DELIVERY CATHETERS USING

AGAROSE GEL AND CELL CULTURE EXPERIMENTS

By Sagar Panse, MBBS

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Major Director: Helen Fillmore Ph.D.
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Neurodegenerative diseases and brain tumors affect millions of patients worldwide and are associated with significant morbidity and mortality. The blood brain barrier constitutes a major obstacle to delivery of therapeutic agents administered systemically for treating these disorders. Intracranial drug delivery provides a novel way of bypassing the blood brain barrier and achieving high concentration of therapeutic agents in the brain while avoiding systemic side effects. However damage to tissues during insertion of catheters, release of air in the brain and consequent backtracking of dye are some disadvantages with this mode of treatment. We evaluated prototype cell delivery catheters

(each with outer and inner catheter) developed to minimize these complications. The catheters (1.6 mm small bore and 2.0 mm large bore) were evaluated using agarose gel and cell culture experiments. We initially delivered pheochromocytoma (PC 12) cells through a 25-gauge syringe needle to optimize characteristics for cell growth. We observed by the agarose gel experiments that when the inner catheter was filled and then inserted with the outer catheter, no air bubble or backtracking of dye was seen. PC 12 cells delivered through the prototype catheters showed growth in collagen gel and differentiated into neurons in the presence of neural growth factor. Future studies with animal experiments would be needed to confirm the findings.

I   Introduction

Millions of people worldwide suffer from neurodegenerative disorders and movement disorders, principally Parkinson's disease and Alzheimer's disease. Most of these illnesses manifest themselves later in life. Therefore, correlated to the increase in life expectancy, the number of people affected with these diseases will grow (1). These diseases are a major cause of morbidity. Another major cause of morbidity and mortality are brain tumors (2). The incidence rate of all primary benign and malignant brain tumors is 14.0 cases per 100,000 person-years (5.7 per 100,000 person-years for benign tumors and 7.7 per 100,000 person-years for malignant tumors).

The brain is a very delicate organ. Unfortunately, the same mechanisms that protect it against intrusive chemicals can also frustrate therapeutic interventions. Many existing pharmaceuticals are rendered ineffective in the treatment of cerebral diseases due to our inability to effectively deliver and sustain them within the brain (3). The blood brain barrier (BBB), which is formed by tight junctions of endothelial cells that make up the walls of capillaries within the brain, is almost always a major "obstacle" to the delivery of therapeutic agents administered systemically into the brain. BBB prevents most hydrophilic drugs or drugs of moderate molecular weight from diffusing into the interstitial space of the brain (4). Currently many strategies for the transient alteration of the BBB are under investigation but there is as yet no universally accepted means of

accomplishing that task. As a result many diseases of the CNS including neurodegenerative diseases and brain tumors are left inadequately treated by existing therapies. These diseases may benefit from intracranial drug, growth factor, and growth inhibitor therapy (5). Intracranial therapy is advantageous as it delivers drugs directly into the affected brain region thereby minimizing the systemic side effects seen with traditional administration of drugs. The diseases likely to benefit from intracranial drug therapy are Parkinson's disease, Alzheimer's disease and brain tumors.

1.1 Parkinson's Disease

Parkinson disease (PD) is a progressive neurodegenerative disorder associated with a loss of dopaminergic nigrostriatal neurons. PD is recognized as one of the most common neurological disorders. The major neuropathologic findings in PD are a loss of pigmented dopaminergic neurons in the substantia nigra and the presence of Lewy bodies. The loss of dopaminergic neurons occurs most prominently in the ventral lateral substantia nigra. The three cardinal signs of PD are resting tremor, rigidity, and bradykinesia. Most cases of idiopathic PD are believed to be due to a combination of genetic and environmental factors (reviewed in 6).

The goal of medical management of PD is to provide control of signs and symptoms for as long as possible while minimizing adverse effects. Medications usually provide good symptomatic control for four to five years. After this, disability progresses despite best medical management, and many patients develop long-term motor

complications (7, 8). Additional causes of disability in late disease include postural instability (balance difficulty) and dementia.

Several surgical procedures have been studied in advanced Parkinson's disease (PD), including deep brain stimulation (DBS), thalamotomy, and pallidotomy (9). Thalamotomy and chronic thalamic stimulation are effective in reducing medically refractory tremor (10). Stereotactic surgery has made resurgence in the treatment of PD. Deep brain stimulation of the subthalamic nucleus has been shown to be an effective therapeutic option for well-selected patients with medically intractable symptoms of Parkinson's disease (11, 12, 13). Neural transplantation is a potential treatment for PD because the neuronal degeneration is site and type specific (ie, dopaminergic), the target area is well defined (ie, striatum), postsynaptic receptors are relatively intact, and the neurons provide tonic stimulation of the receptors and appear to serve a modulatory function (14). Multiple sources of dopamine-producing cells, including fetal nigral cells, sympathetic ganglia, carotid body glomus cells, PC-12 cells, and neuroblastoma cells, have been studied. Results have been varied (14,15).

Recently, Hauser et al reported promising results for 2-year follow-up for 6 patients with PD who underwent bilateral fetal nigral transplantation. Prospective, blinded, randomized studies are being performed to evaluate the long-term safety and efficacy of fetal nigral transplantation in patients with PD (16). Transplantation of fetal porcine cells in patients with advanced PD is now under study. In the laboratory, genetic engineering of cells and the use of stem cells are being investigated (17, 18).

1.2 Alzheimer's disease

Alzheimer's disease (AD) is the most common cause of dementia, which is an acquired cognitive and behavioral impairment of sufficient severity to interfere significantly with social and occupational functioning. At present, the disorder afflicts approximately 5 million people in the United States and more than 30 million people worldwide (19).

The anatomic pathology of AD includes cerebrocortical atrophy (predominantly at the expense of association regions), and neurofibrillary tangles (NFTs) and senile plaques (SPs) at the microscopic level (20). In addition to NFTs and SPs (composed of amyloid protein), many other lesions of AD have been recognized since Alzheimer's original papers. The pathophysiological mechanism underlying the clinical manifestations of AD is corticocortical disconnection due to the loss of medium-sized pyramidal neurons effecting such connections (21). Clinical features are mainly due to cell deterioration in the forebrain cholinergic projection system, particularly, in a structure called nucleus basalis of Meynert (22). The earliest evidence of AD is the onset of chronic, insidious memory loss that is slowly progressive over several years. This can be associated with slowly progressive behavioral changes. Diffuse cortical/cerebral atrophy is expected to occur and can be seen on brain MRI or CT scans. A variety of both behavioral and pharmacologic interventions have been shown to be useful in the management of AD, although their impact is often modest and temporary and does not prevent the eventual relentless deterioration of the patient's condition (23).

Neural stem cell (NSC) grafts present a potential and innovative strategy for the treatment of many disorders of the central nervous system including AD, with the possibility of providing a more permanent remedy than present drug treatments. After grafting, these cells have the capacity to migrate to lesioned regions of the brain and differentiate into the necessary type of cells that are lacking in the diseased brain, supplying it with the cell population needed to promote recovery (24, 25).

1.3 Glioblastoma multiforme (GBM)

GBM is the most common and most aggressive of the primary brain tumors. It is highly malignant, infiltrates the brain extensively, and at times may become massive before turning symptomatic (26).

GBM is an anaplastic, highly cellular tumor with poorly differentiated, round, or pleomorphic cells, occasional multinucleated cells, nuclear atypia, and anaplasia. Presenting features include headaches, seizures and focal neurological deficits (27). Various genetic and molecular abnormalities have been identified. Primary GBM develops in older patients and demonstrates epidermal growth factor receptor over expression, *MMAC1* mutations, *CDKN2A* deletions, and, less frequently, *MDM2* amplifications. Secondary GBM develops in younger patients and contains *TP53* mutations (28, 29).

Available treatment options are surgery, radiotherapy, and chemotherapy (30). Removal and resection of GBM by neurosurgery is mostly incomplete due to the highly infiltrating nature of the tumor (31, 32). Newer modalities of treatment, which are under

investigation, include immunotherapy, antiangiogenesis targeting therapies, biologic therapy, gene therapy and growth factor and second messenger inhibition (33, 34). Many of these modalities would benefit with by utilizing intracranial therapy.

1.4 Intracranial Therapy

Many neural diseases including those mentioned above would benefit from intracranial delivery of drug, chemotherapeutic, growth inhibiting or stimulating factors. The most direct way of circumventing the BBB is to deliver drugs directly to the brain interstitium (35).

Several techniques have been developed for delivering drugs directly to the brain interstitium. Implantable miniature osmotic pumps have been used to provide a continuous supply of drugs or other active biologic factors to the brain and other tissues at a controlled rate. Reservoir limitations as well as drug solubility and stability have, however, restricted the usefulness of this technology. Macroencapsulation, which generally involves loading cells into hollow fibers, then sealing the ends of the fibers and surgically implanting the cell culture device in the brain, has also been used to deliver therapeutic drugs into the central nervous system. However results with this technique have been unreliable (36). Implantable microporous devices that contain hormone releasing cells have been utilized with variable results. Thus, there exists a need for an improved method to deliver cells that can produce biologically active factors to a target region of the brain (36).

Convection-enhanced delivery (CED) via positive pressure infusion through catheters is evolving as a technique for site-specific drug delivery bypassing the BBB. Protocols for CED are designed to deliver clinically useful volumes of therapeutic agents into a specific region of the brain in which adequate concentrations of the drug cannot otherwise be established via systemic delivery. There is data on the delivery of solutions of macromolecules by this technique for the purposes of characterizing the distribution pattern and confirming the safety of the technique (37).

The major advantage of intracranial therapy is site-specific therapy that avoids systemic side effects. Intracranial drug delivery is however not without problems (36). The brain is a very delicate and compact structure. Vital centers are located in close proximity to each other. Inadvertent damage to a center can result in lifelong complications or death. One of the problems with neurosurgical catheters is release of air into the brain (37). When an empty tube is filled with liquid, the air in the tube must go somewhere, but it should not be pushed into the brain where it could cause a backwash of the therapeutic agent along the catheter track. This backtracking of dye can lead to decreased concentration of therapeutic agent at the delivery site and excess concentration at a non-intended area. Both these effects are undesired. The air released in the intracranial cavity itself might cause problems. If air enters in the systemic circulation it can result in local or distant embolism (38). Intracranial air can also exert pressure on tissues resulting in damage. Another problem with intracranial catheters is tissue damage during insertion of catheters. This cannot be avoided but can be minimized by decreasing the frequency of catheter insertion or removal (39, 40).

1.5 Prototype cell delivery catheters

We used experimental models to evaluate prototype cell delivery catheters designed to minimize the above-mentioned complications. The new catheters are made of two catheters, one inside the other. The inner catheter has an end pore while the outer catheter has 4 spiral pores. The inner pore can be aligned with one of the spiral pores on the outer catheter. When any agent is to be delivered using the catheters, first the inner catheter is filled with the agent. When sufficient pressure has been generated by the weight of the column in the inner catheter, the agent starts flowing through the end pore of the inner catheter into the outer catheter and then through the spiral pores of the outer catheter to the outside. Once the outer catheter is guided into place, the inner catheter can be primed with a therapeutic agent, such as a gene therapy or cellular mixture, and inserted into the outer catheter. Multiple combinations of priming the catheters and insertion are possible and are mentioned in detail under material and methods.

As the smaller catheter goes in, air is displaced through the narrow space between the two catheters. This air can escape to the outside between the inner and outer catheters or pass through one of the spiral pores into the brain. The size of the spiral pores is 1 mm allowing only a small quantity of air to pass through. A porthole at the end of the inner tube is aligned with one in the outer tube, allowing the therapeutic agent to flow into the brain and minimizing air leak. The inner catheter can be withdrawn and reloaded while the outer catheter remains in place. This eliminates the potential for tissue damage from reinsertion. The catheters were intended to be used for intracranial delivery of different

agents. We evaluated the catheters using two separate 3-dimensional gel models, a dye model and a cell culture model.

The dye experiments were used to characterize leakage of air and backtracking of dye using the catheters. Agarose gel at 0.6% concentration in TBE (Tris-Borate-EDTA) buffer closely resembles in vivo brain during infusion studies, with respect to several critical physical characteristics (41). Broaddus et al has shown that agarose gel (0.6%) serves as a workable surrogate for *in vivo* bulk mammalian brain tissue in exploratory studies of the intraparenchymal positive pressure infusion of therapeutic agents (42). We used different combinations of catheters to inject bromophenol blue dye into the agarose gel. The dye as it passed into the gel was visualized. The presence and maximum two dimensions of the air bubble produced, if any, were measured in mm. Backtracking of the dye was also noted.

In the second set of experiments we delivered cells through prototype cell delivery catheters into collagen gel using a stereotactic assembly under aseptic conditions. We used pheochromocytoma (PC12) cells for infusion. We first infused the cells through a 25-gauge needle syringe to study cell growth and distribution as well as NGF induced differentiation. After determining a favorable microenvironment for cell growth, we infused cells through the catheters into 4ml collagen gel and into a sandwich gel of 2 ml collagen and 2 ml agarose on the top. Differentiation of cells after adding neural growth factor was observed and studied.

The 0.6% agarose gel and collagen gel experiments revealed that the catheters could be used for experimental purposes for the delivery of dye and cells. When the inner

catheter was primed with therapeutic agent, inserted into the outer catheter and then into gel, minimal air leak and backtracking of dye were noted. Further studies including animal infusion studies are needed. If these studies reveal a favorable outcome, the catheters have a good potential in delivering intracranial agents. This will have a significant impact in the treatment of neurodegenerative diseases and brain tumors.

1.6   **Specific Aims**

Experiments were conducted to study the properties of prototype cell delivery catheters and their effectiveness for delivering different agents. This was done with 2 groups of experiments with specific aims:

1. Agarose gel model: To determine optimal combination of inner and outer catheters to eliminate air leak and backtracking of dye.
2. Cell Culture model: To investigate the effect of delivery of cells into a 3-dimensional gel using catheter combination found to be optimal in specific aim 1 on cell growth and differentiation.

II   Material and Methods

2.1 Catheters

Prototype cell delivery catheters were evaluated. The catheters were obtained from Image Guided Neurologics, Melbourne, Florida. Each catheter has an inner and outer catheter (fig.1). The inner catheter has a terminal pore, while the outer catheter has 4 spiral pores on the side. The terminal pore of the inner catheter could be aligned with one of the spiral pores of the outer catheter. For all experiments unless specified otherwise, the terminal pore was aligned with one of the spiral pores and the other three spiral pores were covered with tape. The maximum length of the outer catheter was 6 inches and that of the inner catheter was 6.75 inches. Two separate catheters that differed in the diameters of their outer and inner catheters were evaluated (fig. 2). The diameters of the outer catheters were 1.6 mm and 2.0 mm for the small bore and large bore catheters respectively. The diameter of the inner catheter of the small bore catheter was 0.8 mm and of the large bore catheter was 1.0 mm. When the inner catheter was inserted into the outer catheter, an air column was present between the 2 catheters. This air could exit the system through the open spiral pore or through the gap between the inner and outer catheters.
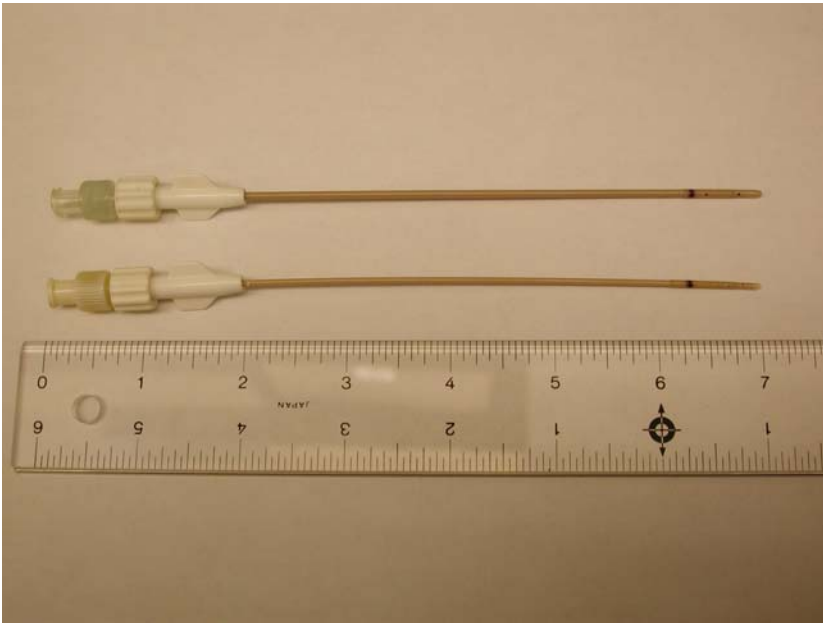
Figure1. 2.0 mm large bore catheter (top) and 1.6 mm small
bore catheter (bottom). Each catheter has inner and outer
catheters.
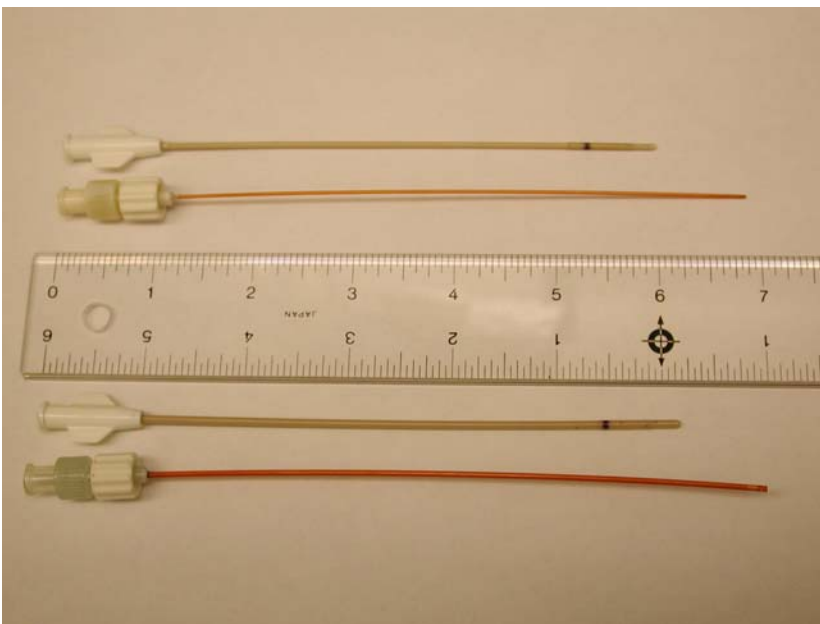
Figure 2. Inner and outer tubes of the catheters. The 1.6 mm
small bore catheter is on the top and the 2.0 mm large bore
catheter is at the bottom.

2.2 Agarose gel preparation

Agarose gel was prepared by mixing agarose powder and 1X TBE (89mM Tris, pH 8.4; 2mM EDTA) solution. The mixture was heated until all the powder had dissolved and boiled for 5 min. When the resulting solution had cooled to about 50°C, approximately 500 ml was poured into a rectangular container made of thin-walled transparent Plexiglas™ of dimensions 10 cm x 10 cm x 7 cm, to grossly simulate a large fraction of the volume of human brain (41). The agarose gel solidified as it cooled to room temperature. This apparatus containing agarose gel is referred to as the brain box.

2.3 Brain box

Chen et al. have shown that 0.6% agarose gel in the brain box grossly simulate the distribution characteristics of the human brain (41). Navigus trajectory guide was firmly attached to the detachable lid of the brain box. The transparent material of the brain box allowed good visualization of dye.

2.4 Navigus

Navigus is a disposable magnetic resonance (MR)-compatible trajectory guide made from plastic. It was obtained from Image-Guided Neurologics, Melbourne, FL. The Navigus trajectory guide was mounted in the rectangular lid of the container and served as the insertion and guidance path for the catheter that would deliver the infusate into the gel (42, 43, 44).

2.5 Collagen gel preparation

The collagen gel was made by adding 8 ml of type I collagen solution (obtained from Vitrogen, Inc) and 1 ml of Minimal Essential Medium (MEM). 1ml of 0.1 N Sodium chloride was added drop by drop till the color changed to pink. The mixture was then spun at 4000 rpm for 6 minutes to remove any air bubbles. It was then poured into 4-6 cell culture inserts that were placed in a 6 or 12 well cell culture plate. The collagen gel solidified in approximately 1-2 hours. The entire process was carried out under aseptic conditions. Collagen was used as it has been shown to support the growth of pheochromocytoma cells in cell cultures (45).

2.6 Sandwich gel preparation

Collagen gel was prepared using the method mentioned in the above paragraph. Collagen gel (2 ml.) was added to 4-6 cell culture inserts that were placed in a 6 or 12 well cell culture plate and allowed to solidify. Agarose gel was prepared as mentioned above. The gel was allowed to cool partially and before it could solidify it was added to the solidified collagen gel. The entire process was carried out under aseptic conditions.

2.7 Bromophenol blue dye

Bromophenol blue dye was used for infusion during the agarose gel experiments. It has been shown that the blue color of the dye allows its easy visualization in the agarose gel (47). Chen et al have shown that the diffusion characteristics of bromophenol blue dye in agarose gel are similar to those in the brain (41).

2.8 Pheocromocytoma cells (PC12)

Pheochromocytoma cells obtained from rodents were used for cell culture experiments. PC12 cells were available as a cell culture suspension. These cells have the potential to differentiate into neurons in the presence of neural growth factor (47, 48, 49). The growth and differentiation of PC12 cells infused by the cell delivery catheters were studied. The cells were harvested each week to maintain viability. Before infusion the cells were harvested using the following protocol. The cell suspension was centrifuged at 1000 rpm for 5 min. The supernatant was discarded and the cells were washed with Hanks Balanced Salt Solution to free the cells. The cell suspension was again centrifuged at 1000 rpm for 5 min. The supernatant was discarded and the cells were washed with RPMI (Roswell Park Memorial Institute) medium (50). The cells were then counted using a Neubar's chamber™. For cell infusion experiments a final cell concentration of 1 million / cm³ was obtained by diluting with RPMI medium.

2.9 Neural growth factor (NGF)

NGF is a neurotropic protein that plays a critical role in the development of sympathetic and some sensory neurons in the nervous system (51, 52). Recombinant NGF was obtained from R&D systems; Inc. NGF has been shown to favor differentiation of pheochromocytoma cells in cell cultures (53, 54).

2.10 Statistics

Size of air leak during dye experiments was compared using ANOVA analysis.
Paired t test was also used for comparison. SPSS software was used to compute the
statistics. A p value of less than 0.05 was considered significant.

2.11 Dye Experiments

The 0.6 % agarose gel was prepared in the brain box. With the help of a Navigus
trajectory guide, the catheters were inserted in the gel. Three combinations were possible
for infusion of dye into agarose gel through the catheters:

1. Inner catheter filled with dye and inserted into outer catheter and then into the gel

2. Outer catheter inserted in gel and then dye filled inner catheter inserted in the outer
catheter

3. Both inner and outer catheters inserted in gel and then the inner catheter filled with
dye.

 The above sets of experiments were conducted with both the 1.6 mm small bore and 2.0
mm large bore catheters (see table1). Each experiment with each catheter was repeated
three times. For combination 2 and 3, with the 2.0 mm large bore catheter additional
modifications were tested. The inner catheter fitted inside the outer catheter with 2
possibilities. The inner catheter could be inserted into the outer catheter and then locked.
Theoretically the locking mechanism will not allow air in the column between the inner
and outer catheters to escape to the outside. The other possibility was to insert the inner
catheter into the outer catheter and keep it unlocked. With the unlocked position of the

inner catheter, air in the column between the catheters will have the potential to escape outside and would not be forced to enter the gel (see table 2). We wanted to test the hypothesis that the unlocked position of the inner and outer catheters would result in less significant air bubble formation in the gel and backtracking.

Using a three-way connector, the catheter was connected to a dye-filled syringe, which was mounted on a flow rate monitor. The flow rate monitor allowed infusion through the catheters at a fixed rate. The flow rate was kept constant at 1 μl / min for 1 hour. Chen et al. reported that infusing bromophenol dye at a rate of 1 μl / min for 1 hour into agarose gel allows good visualization of dye as it passes through the catheter into the gel. The infusion was started and bromophenol blue dye as it passed into the gel was visualized. The presence and maximum dimensions of the air bubble produced, if any, were measured in mm. The visible surface area of the air bubble was calculated as the product of the maximum dimensions. Backtracking of the dye was also noted. The whole experiment was video recorded and digital pictures were taken at every 5-minute interval.

Table 1. Schematic representation of dye experiments performed using 1.6 mm small bore catheter.

Dye experiments with 1.6 mm small bore catheter
Inner catheter filled with dye and inserted into outer catheter and then into the gel
Outer catheter inserted in gel and then dye filled inner catheter inserted in the outer catheter
Both inner and outer catheters inserted in gel and then the inner catheter filled with dye
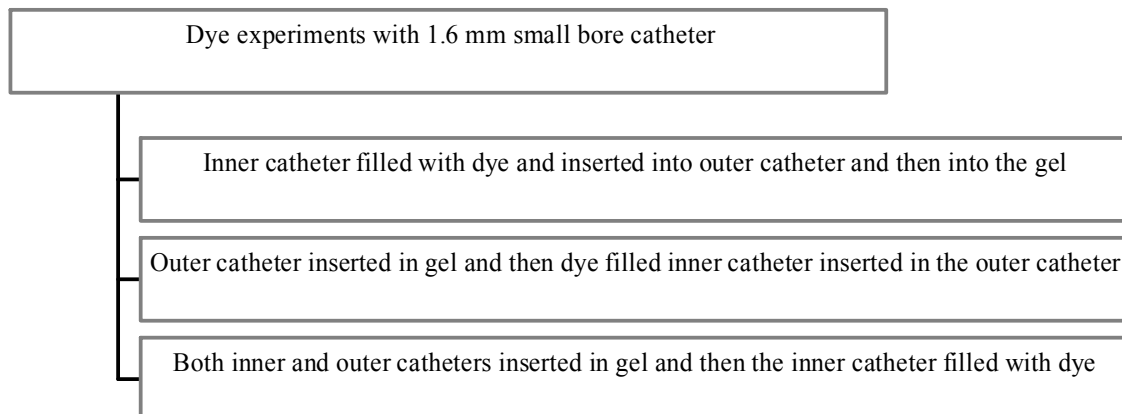
Table 2. Schematic representation of dye experiments performed using 2.0 mm large bore catheter.

Dye experiments with 2.0 mm large bore catheter
Inner catheter filled with dye and inserted into outer catheter and then into the gel
Outer catheter inserted in gel and then dye filled inner catheter inserted in the outer catheter

Inner catheter locked	Inner catheter unlocked

Both inner and outer catheters inserted in gel and then the inner catheter filled with dye

Inner catheter locked	Inner catheter unlocked

2.12 Cell Culture experiments

2.12.1 Cell culture experiments using 25 gauge syringe needle

Infusion experiments with a 25-gauge syringe needle were conducted to study the baseline characteristics of cell infusion and growth in gel. The goal of these experiments was to optimize the conditions favorable for cell growth and visualization of cells in gel.

Collagen gel was prepared as mentioned above. Collagen (4 ml) was added to cell culture inserts of 6-12 well plates and allowed to solidify. The entire procedure was done in the hood with thorough aseptic measures. PC 12 cells were harvested as per the above-mentioned protocols. Cell count was made and diluted to achieve a cell count of approximately 1 million / cm³. The cell suspension was then filled in the 25 μl sterilized syringe needle. The syringe needle was attached to a flow rate monitor. The tip of the 25-gauge syringe needle was inserted into the collagen gel in the cell culture insert, which was placed on a lab jack. The tip of the syringe needle was kept around 1.5 cm below the gel surface. The infusion was started and the flow rate was kept constant at 20 μl / min for a period of 3 minutes and then stopped. The tip of the needle was removed from the gel. The insert with the collagen gel was placed in the 6 or 12 well plate and incubated for 7 days. Cell growth in the gel was followed over 7 days under microscope and digital pictures were taken.

Another set of infusion experiments with the needle was performed to identify cell growth characteristics after vertical and horizontal implantation in collagen gel. To ensure precise delivery of cells in the gel, a guide cannula was used. The tip of the needle passed through the guide cannula into the gel. In one set of experiments to achieve

vertical implantation of cells, the guide cannula was firmly attached to top cover of 6 well plates. A hole was drilled in the cover of the culture plate. The cannula was placed in the hole and glued firmly to the side. The same cover was used for all experiments. Each time the cover with the guide cannulae was sterilized by dipping in alcohol for 12 hours and then exposing to UV light for 1 hour. In another set of experiments the cannula was firmly attached to the side of the 6 well plate. This assembly allowed cells to be delivered laterally into the gel. Fig. 3 shows a 12 well plate with vertically implanted guide cannulas. Fig. 4 shows the infusion assembly used for delivering cells by a needle passed through the vertical guide cannula into the gel. Fig. 5 shows a cell culture insert with cannula implanted in its lateral aspect.

Figure 3. 12 well plate with vertically placed guide cannulae. Collagen gel was made in cell culture inserts, which were then placed in the plate. Tip of the 25-gauge syringe needle passed through one of the guide cannula into collagen gel.
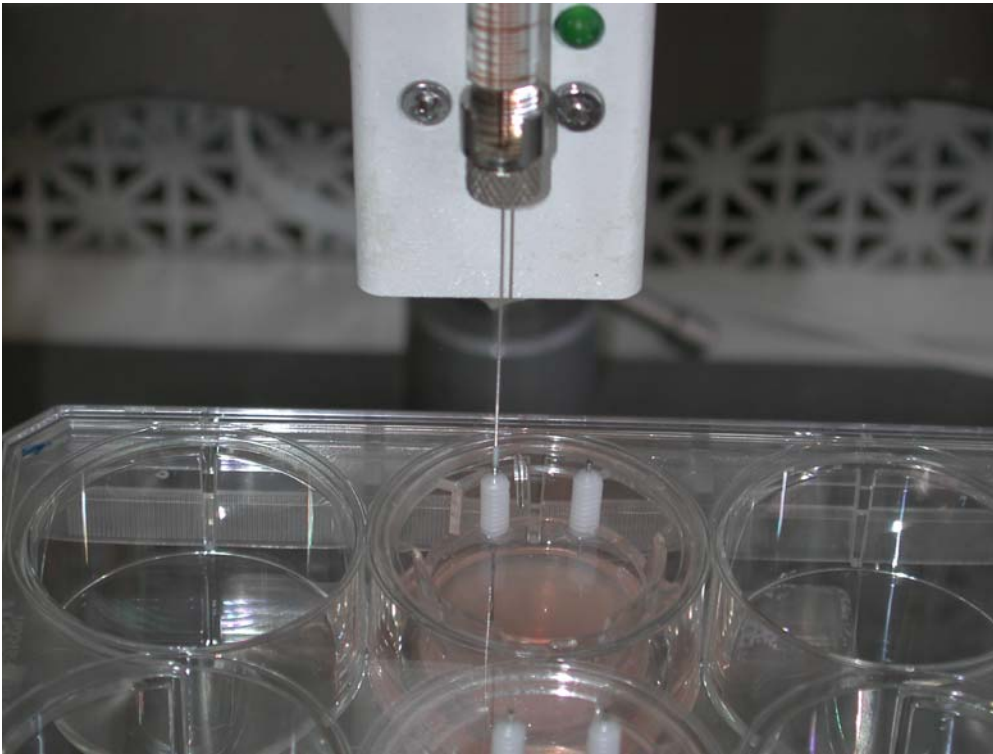
Figure 4. Closer view of the 25-gauge syringe needle infusion through vertically implanted guide cannula. Cells were infused by the 25-gauge syringe needle passing through the guide cannula into collagen gel inserts. This assembly allowed identification of baseline characteristics for cell growth in gel.

Figure 5. Cell culture insert with laterally implanted guide cannula. The cell culture insert with the cannula was placed in a 6 well plate. Cell suspension was drawn in a needle and tip of the 25-gauge syringe needle passed through the cannula into collagen gel. This assembly however resulted in poor cell delivery and growth and was not used for prototype catheter experiments.

2.12.2 Cell culture experiments using prototype cell delivery catheters

By performing the 25 gauge syringe needle cell culture experiments we found that type I collagen gel provided a suitable microenvironment for growth of PC12 cells. In addition the vertical infusion of cells was found to allow better cell visualization than horizontal implantation.

Collagen gel was prepared as mentioned above. Collagen was added to cell culture inserts of 6 or12 well plates unless specified otherwise and allowed to solidify. 4 ml of Collagen gel was added to each insert. However 4 ml collagen gel was too dark and did not allow good visualization of cells. To overcome this problem we used a sandwich gel with 2 ml of collagen gel on the bottom with 2 ml of agarose gel on top. The sandwich gel was prepared in 6 well plate inserts. The whole procedure was done in the hood with thorough aseptic precautions. PC 12 cells were harvested as per the above-mentioned protocols. Cell count was made and diluted to achieve cell count around 1 million similar to the needle experiments. Both 1.6 mm small bore and 2.0 mm large bore catheters were evaluated. Sterilized catheters were primed in the following way. First the inner catheter was filled with the cell suspension, and then inserted into the gel along with the outer catheter. We used this combination of inner and outer catheters as our agarose gel experiments proved that this combination was least likely to produce air bubble and backtracking of agent in the gel. The catheter was attached to a 10 ml syringe mounted on a flow rate monitor. The entire assembly was mounted on a stereo tactic frame to ensure precise delivery of cells in the gel. The cell culture insert with solidified sandwich gel was placed on a lab jack. The tip of the cell suspension primed catheter was

inserted in the gel. The infusion was started and continued at a rate of 20 μl / min to achieve a uniform cell count delivered to the gel. At the end of three minutes, the infusion was stopped and the tip of the catheter was removed from the gel. Similar to the 25 gauge syringe needle experiments, the cell culture inserts were kept in an incubator and cell growth was followed daily and picture recorded.   The infusion assembly with catheter mounted on a frame is shown below in fig. 6 . Figure 7 provides a closer view of the catheter with its tip inserted in collagen gel. Table 3 shows a schematic representation of cell culture experiments using 25 gauge syringe needle and prototype cell delivery catheters.

Figure 6. Infusion assembly with 2.0 mm large bore catheter and cell culture insert. Collagen gel was prepared in 6 well plate cell culture insert and then placed on a lab jack. The catheter was connected to a syringe mounted on a flow rate monitor.
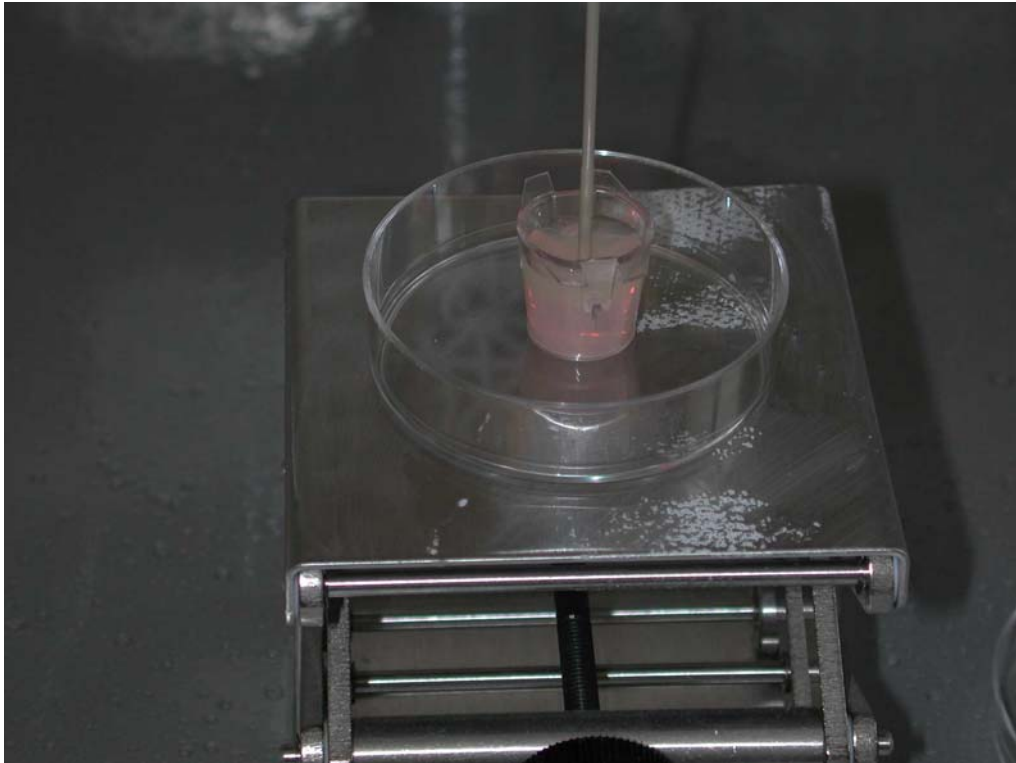
Figure 7. Closer view of the 2.0 mm large bore catheter tip in cell culture insert. Collagen gel was added to the cell culture insert. The tip of the catheter was maintained around 1.5 cm below the collagen gel surface.

Table 3. Schematic representation of cell culture experiments using 25 gauge syringe needle and prototype cell delivery catheters.

Cell culture experiments

Using 25 gauge syringe needle

Vertical infusion into gel	Horizontal infusion into gel

Using prototype cell delivery catheters

Neural growth factor added	No neural growth factor added
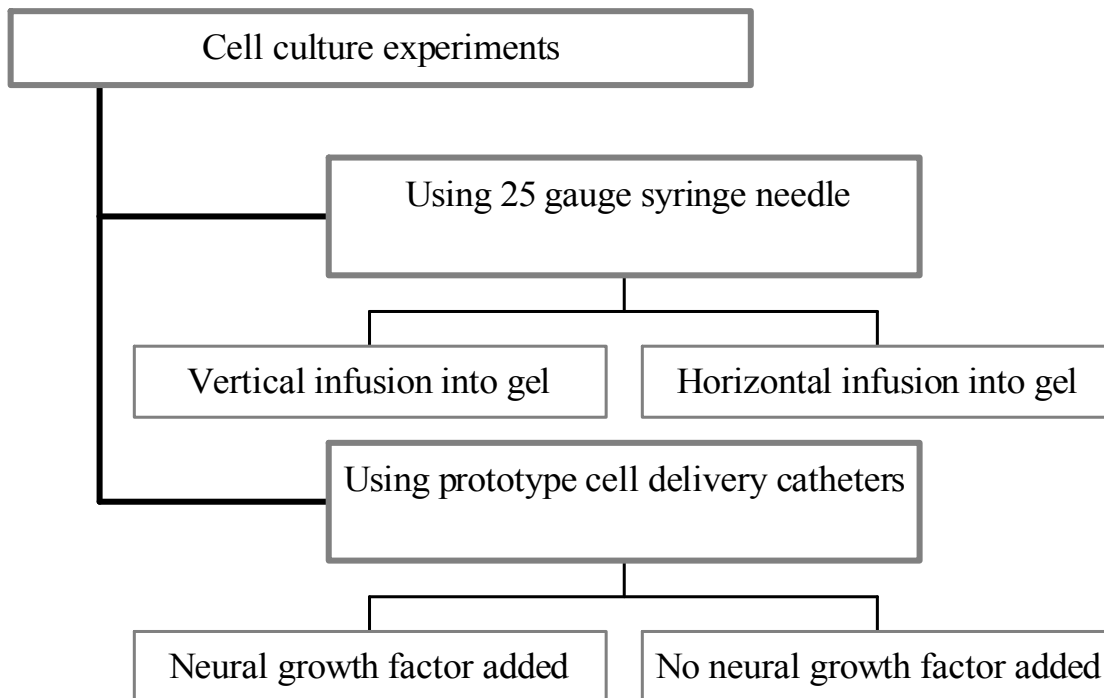
III Results

3.1 Dye Experiments

  Dye experiments were carried out as per the procedure mentioned above in methods section. Both 1.6 mm small bore and 2.0 mm large bore catheters were evaluated using the following 3 combinations of insertion in gel. In the first combination, the inner catheter was filled with dye and inserted into the outer catheter and then into the gel. Another set of experiments was conducted with outer catheter inserted and then dye filled inner catheter. And finally both inner and outer catheters were inserted in the gel and then filled with dye. In addition combinations 2 and 3 were performed with the large bore catheter with the inner catheter in locked and unlocked positions. Each type of experiment was repeated three times to ensure reproducibility and accuracy. Bromophenol blue dye was infused through the catheter for 1 hour to measure air bubble production and presence or absence of backtracking of dye. At the end of the infusion 2 dimensions of air bubbles produced and presence or absence of backtracking of dye were noted.

3.1.1 Small bore catheter:

  In the first set of experiments, the inner catheter was first filled with dye and inserted into the outer catheter and then into the gel, no air bubble and backtracking of

dye were seen. The same results were obtained each time the experiment was repeated.

Since there was no visible air bubble observed, the mean and SD of air bubble produced

were estimated as zero. A representative picture of results obtained with this combination

of insertion of inner and outer catheters is shown in figure 8. Table 4 lists the size of the

air bubbles in square cm. and the presence or absence of backtracking of dye.
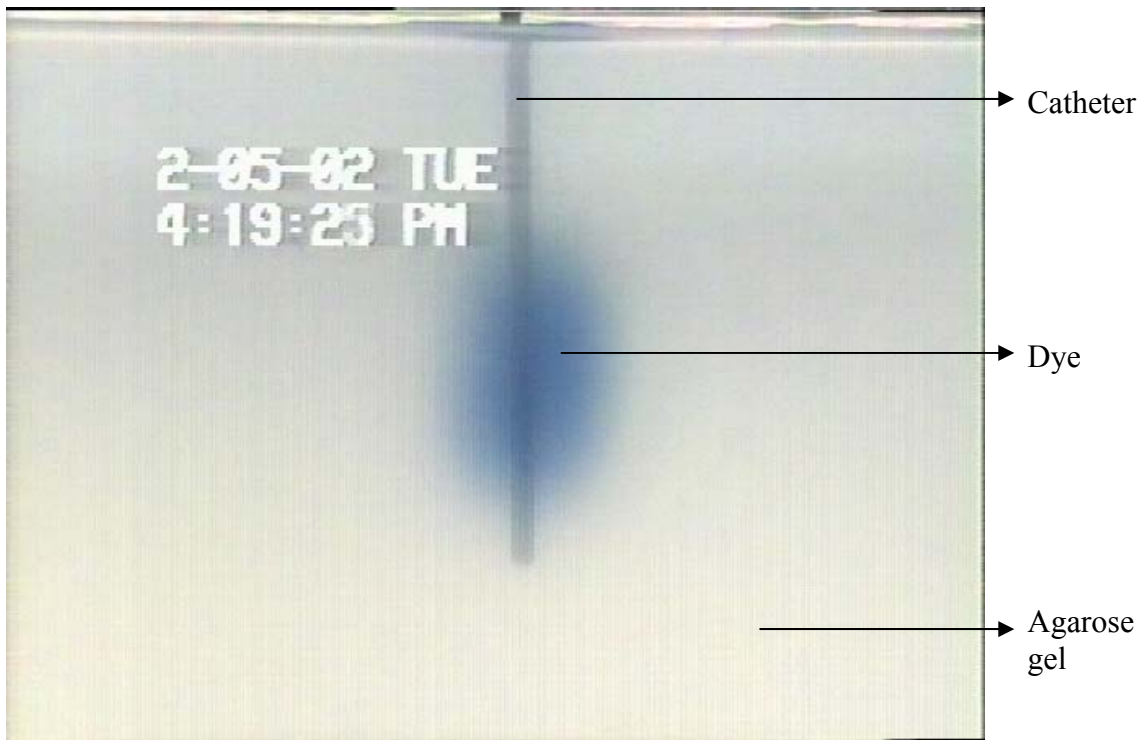
Figure 8. Dye experiments with 1.6 mm small bore catheter. Inner catheter filled and inserted into outer catheter and then into gel. Note no air bubble or backtracking of dye along catheter track.

 In the second set of experiments, the outer catheter was initially inserted in 0.6 %
agarose gel. The inner catheter was primed with the bromophenol blue dye and then
inserted into the outer catheter. Presence of air leak was noted and the dimensions of air
bubble were measured. With this combination, an air bubble was consistently observed
but there was no backtracking of dye noted. The maximum dimensions of the air bubble
produced were 0.3 x 1.0, 0.6 x 0.8 and 0.5 x 0.8. Air leak inside the brain has been
associated with multiple complications and is unfavorable. For statistical comparison, the
product of the maximum dimensions (visible surface area) of the air bubble was
computed and compared. The mean and standard deviation of the size of the air bubble
were 0.393 ± 0.09 cm². Fig. 9 shows a representative picture of infusion of gel with this
combination of catheters.
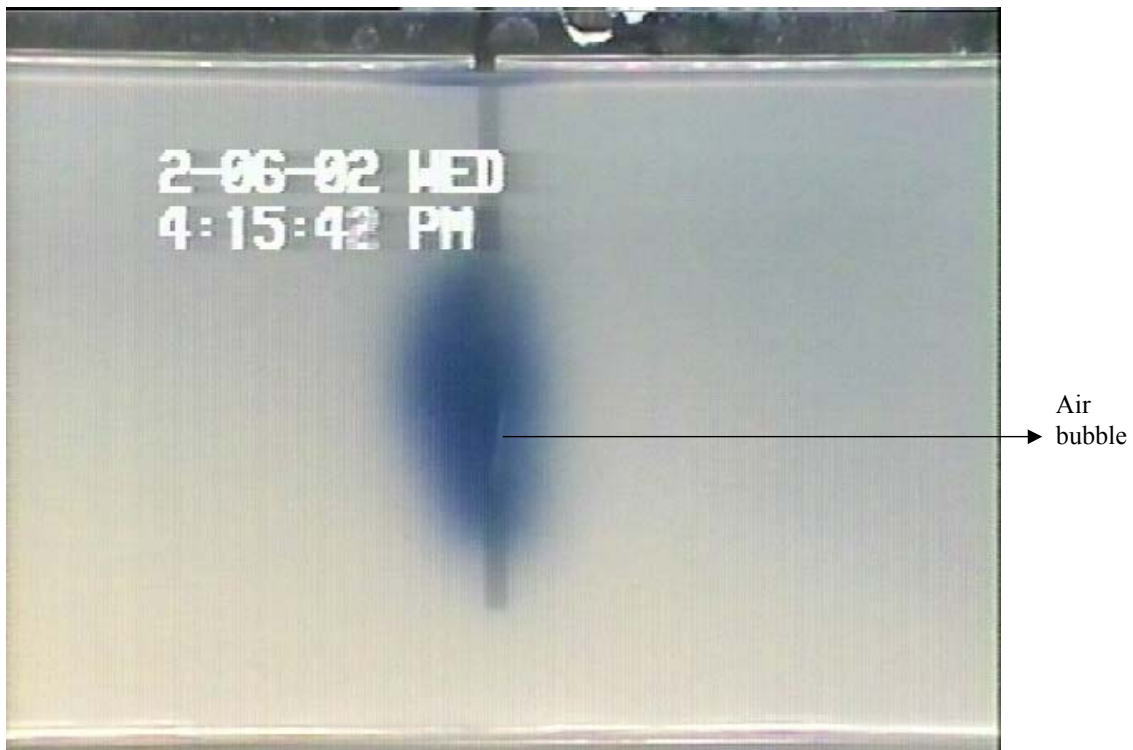
Figure 9. Dye experiments with 1.6 mm small bore catheter. Outer catheter inserted in the gel and then dye filled inner catheter. An air bubble is seen without any backtracking of dye.

In the third set of experiments, both inner and outer catheters were inserted in gel and then filled with bromophenol blue dye. The presence and maximum dimensions of air leak and backtracking of dye were measured. The entire experiment was repeated three times. An air bubble was consistently seen (1.1 x 1.5, 1.2 x 1.4, and 1.2 x 1.5 cm²) but backtracking of dye was seen only once. Mean and standard deviation of the size (visible surface area) of the air bubble were 1.71 ± 0.07 cm² respectively.   Size of the air bubble produced with this combination was the largest among the 3 combinations tested ($p < 0.05$). Backtracking of dye was seen with an air bubble of dimensions 1.1 x 1.5 cm², which was not the largest air bubble, observed. The results obtained after dye infusion using small bore catheter are presented in tables 4 and 5.

No air leak and backtracking of dye were noted when the inner catheter was filled with dye and then inserted with the outer catheter into the gel. The size of the air bubble produced with this combination was significantly smaller than the air bubbles produced with the other 2 combinations ($p < 0.001$). Further studies were conducted with the large bore catheter to corroborate these findings.

Table 4. Dye experiments with 1.6mm small bore catheter. Bromophenol blue dye was infused through the small bore catheter and presence and maximum dimensions of air bubble produced were measured. Backtracking of dye along the catheter track was also noted.

Method	Size of air bubble (cm²)	Back tracking of dye
Inner catheter filled with dye and inserted with outer catheter into gel	0 x 0	Absent
	0 x 0	Absent
	0 x 0	Absent
Outer catheter inserted in gel and then dye filled inner catheter	0.3 x 1.0	Absent
	0.6 x 0.8	Absent
	0.5 x 0.8	Absent
Both inner & outer catheters inserted in gel & then filled with dye	1.1 x 1.5	Present
	1.2 x 1.4	Absent
	1.2 x 1.5	Absent

Table 5.Graph of results of dye experiments with 1.6 mm small bore catheter. Table shows size of air bubble produced with different combinations of insertion of inner and outer catheters.
* Legend for combinations of insertion in dye of inner and outer catheters:
1 = Inner catheter filled with dye and inserted with outer catheter into gel
2 = Outer catheter inserted in gel and then dye filled inner catheter
3 = Both inner & outer catheters inserted in gel & then filled with dye

Mean air bubble produced with small bore cathter using different combinations of outer and inner catheters

Size of air bubble in sqcm

Combination of insertion in dye of inner and outer catheters*
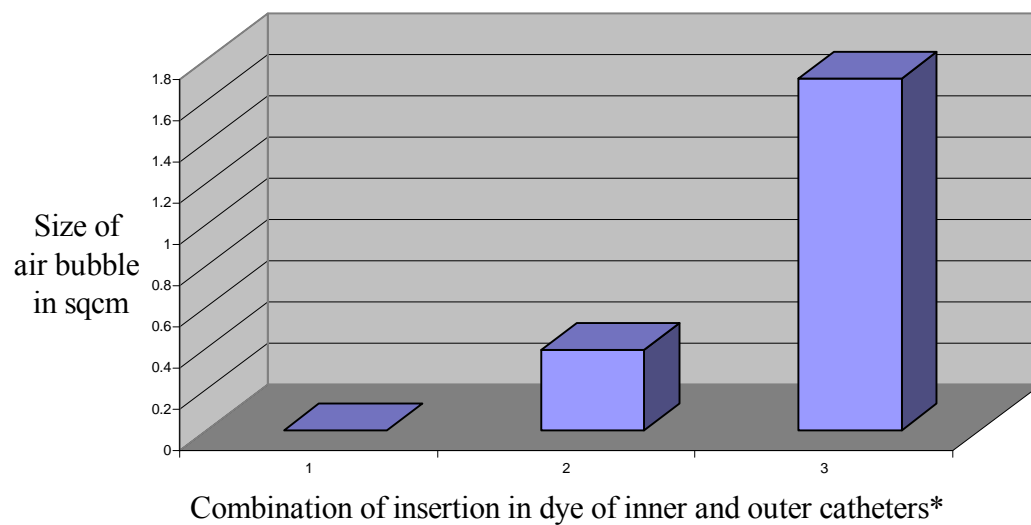
3.1.2 Large 2.0 mm bore catheter

The 2.0 mm large bore catheters were evaluated using the same 3 combinations of inner and outer catheters like with the 1.6 mm small bore catheter. In the first combination, the inner catheter was filled with dye and inserted into the outer catheter and then into the gel. In combination 2, the outer catheter inserted and then dye filled inner catheter. And finally both inner and outer catheters were inserted in gel and then filled with dye. In addition for combinations 2 and 3, the inner catheter was kept in locked or unlocked positions. Each experiment was repeated 3 times.

In combination 1, the inner catheter was filled with dye, inserted into the outer catheter and then inserted into the 0.6% agarose gel. Since the inner catheter was filled with dye before inserting in gel, it allowed the air between the inner and outer catheters to escape to the outside. We recognized that keeping the inner catheter locked or unlocked would not contribute to air bubble formation in gel, as the air would have already escaped between the catheters before insertion in gel. Similar to the results obtained with the 1.6 mm small-bore catheter, we found no air bubble and no back tracking of dye (table 6). The mean and standard deviation for size of air bubble were both zero cm². Using ANOVA analysis, we found the size of air bubble with this combination was significantly smaller than the air bubble produced with the other 2 combinations ($p < 0.001$). This combination proved to have the potential for eliminating the problems of air leak and backtracking of dye and because of this we decided to use this combination for cell delivery as described in subsequent sections.

Table 6. Results of dye experiments with 2.0 mm large bore catheters with inner catheter filled with dye and then inserted with outer catheter into gel.

Method	Size of air bubble (cm²)	Back tracking of dye
Inner catheter filled with dye and inserted with outer catheter in gel	0 x 0	Absent
	0 x 0	Absent
	0 x 0	Absent

In the second set of experiments, we inserted the outer catheter into the gel prior to inserting a dye filled inner catheter into the outer catheter. The position of the inner catheter was kept locked for 3 experiments and unlocked for another 3 experiments. We had hypothesized that the unlocked position of the inner catheter would allow air to escape between the catheters and prevent the air from going into the gel. However we found that an air bubble was produced in each experiment irrespective of the locked or unlocked position of the inner catheter. The maximum dimensions of air bubble produced with the inner catheter in locked position were 0.5 x 0.6, 0.4 x 0.4, 0.4 x 0.5 cm² and with the inner catheter unlocked were 0.1 x 0.1, 0.2 x 0.5, 0.6 x 1.2 cm² respectively (see table 7). The mean size of air bubble (surface area visible) with the inner catheter in locked position (0.22 ± 0.07 cm²) did not differ significantly ($p = 0.70$) with the mean air bubble size (0.30 ± 0.35 cm²) with the inner catheter in unlocked position. No backtracking of dye was seen in either position of the inner catheter (see table 7 and fig. 10).

Table 7. Results of dye experiments with 2.0 mm large bore catheters with outer catheter inserted and then dye filled inner catheter. An air bubble was consistently seen but no backtracking of dye was seen.

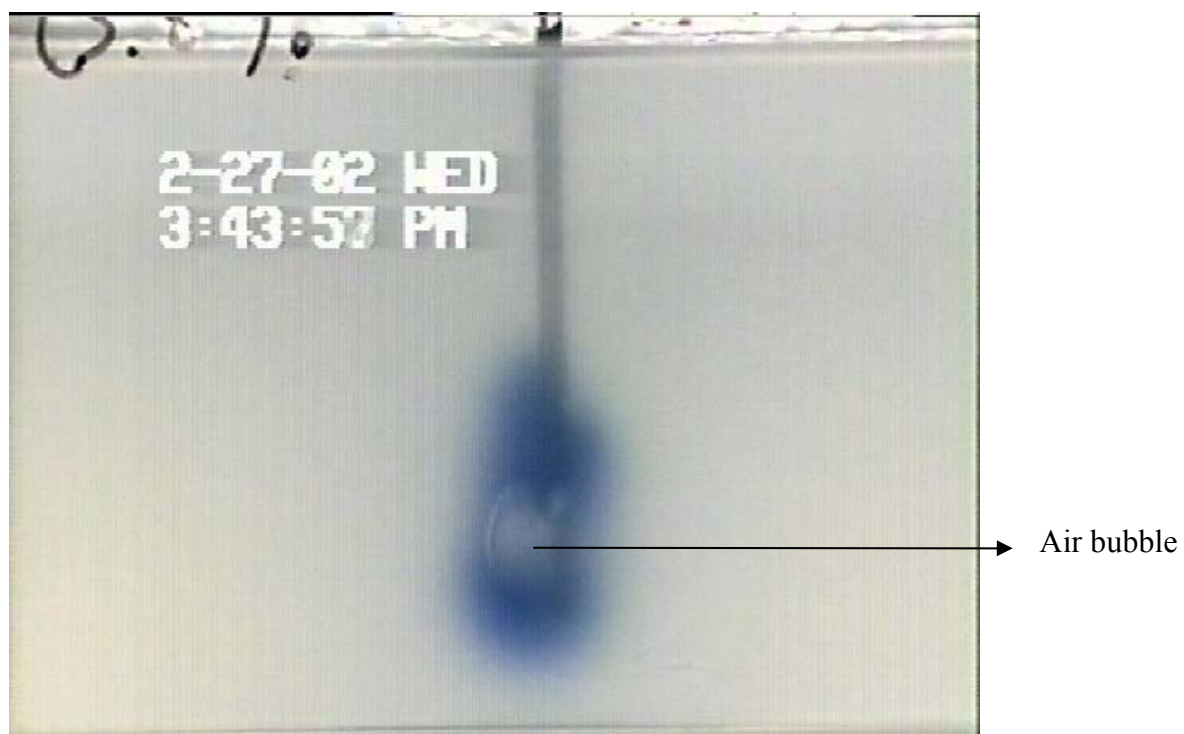| Method	Inner catheter	Air bubble (cm²)	Back tracking
Outer catheter inserted and then dye filled inner catheter	Locked	0.5 x 0.6	Absent
	Locked	0.4 x 0.4	Absent
	Locked	0.4 x 0.5	Absent
	Unlocked	0.1 x 0.1	Absent
	Unlocked	0.2 x 0.5	Absent
	Unlocked	0.6 x 1.2	Absent

Figure 10. Dye experiments with 2.0 mm large bore catheter. Outer catheter inserted and then dye filled inner catheter in unlocked position. Note air bubble produced without any backtracking of dye.

The third combination for large bore catheter consisted of insertion of both the tubes in 0.6% agarose gel and then infusing bromophenol blue dye into the inner tube. The inner catheter was kept locked or unlocked inside the outer catheter. An air bubble was consistently seen irrespective of the locked or unlocked position of the inner catheter. The maximum dimensions of the air bubble produced with the inner catheter locked were 0.7 x 1.1, 0.6 x 0.4, 0.6 x 2.0 cm² and with the inner catheter unlocked were 0.2 x 0.2, 1.5 x 2.0, 0.4 x 0.7 cm² respectively (see table 8). The mean size of air bubble with the inner catheter in locked position (0.73 ± 0.48 cm²) did not differ significantly ($p = 0.72$) from the mean size of air bubble produced with the inner catheter in unlocked position (1.1 ± 1.6 cm²). However, backtracking of dye was only seen when the inner catheter was locked (see fig. 11). This could be due to fact that the locked position of the inner catheter did not allow air to escape. This air was forced to escape in the gel. Table 9 summarizes the findings after dye infusion through 2.0 mm large bore catheter.

In all the dye experiments with 1.6 mm small bore and 2.0 mm large bore catheter backtracking of dye was noted only when an air bubble was produced. However not every air bubble, nor size of air bubble correlated with backtracking of dye. Size of air bubble produced did not correlate with presence of backtracking. Keeping the inner catheter of the catheter locked did not prevent air leak. Our dye experiments confirmed that when the inner catheter was filled with gel, inserted in the outer catheter and then into the gel, no air leak and backtracking of dye were observed. This combination had the potential to eliminate the problem of introducing air during neurosurgery.

Table 8. Results of dye experiments with 2.0 mm large bore catheter with both inner and outer catheters inserted in gel and then filled with bromophenol blue dye.

Method	Inner catheter	Air bubble (cm²)	Back tracking
Both inner and outer catheters inserted and then filled	Locked	0.7 x 1.1	Present
	Locked	0.6 x 0.4	Present
	Locked	0.6 x 2.0	Present
	Unlocked	0.2 x 0.2	Absent
	Unlocked	1.5 x 2	Absent
	Unlocked	0.4 x 0.7	Absent

Figure 11. Dye experiments with 2.0 mm large bore catheter. Both inner and outer catheters inserted and then filled (tight fit). Note presence of air bubble and backtracking of dye.

Table 9. Graph of results of dye experiments with 2.0 mm large bore catheter. Table shows size of air bubble produced with different combinations of insertion of inner and outer catheters.
* Legend for combinations of insertion of inner and outer catheters in dye:
1 = Inner catheter filled with dye and inserted with outer catheter into gel
2 = Outer catheter inserted in gel and then dye filled inner catheter in locked position
3 = Outer catheter inserted in gel and then dye filled inner catheter in unlocked position
4 = Both inner & outer catheters inserted in gel & then filled with dye in locked position
5 = Both inner & outer catheters inserted in gel & then filled with dye in unlocked

Mean size of air bubble produced with 2.0 mm large bore catheter using different combinations of inner and outer catheters
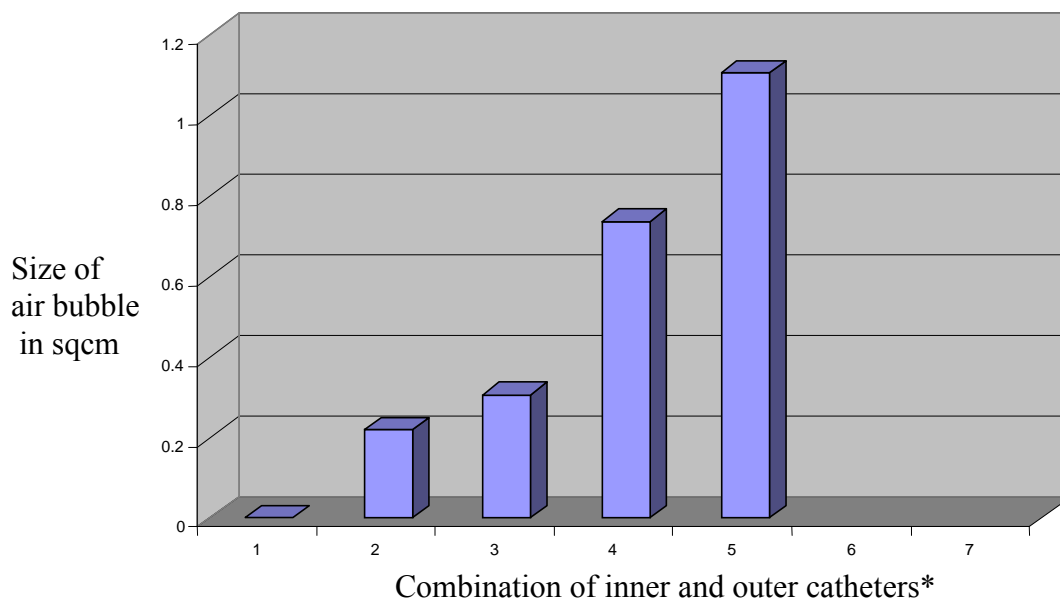
3.2   Cell Culture experiments

3.2.1 Needle Experiments

A series of cell infusion experiments were carried out using a 25-gauge syringe needle to optimize the microenvironment conducive for cell growth prior to catheter infusions. These experiments allowed determining the number of cells, quantity of gel, type of culture plate, inserts and growth factors. Pheochromocytoma (PC12) cells were infused through a 25-gauge syringe needle into collagen gel.

In the first set of experiments with a 25 gauge syringe needle, the tip of the needle was inserted directly into the collagen gel (made in a 6 well plate insert) and kept around 1.5 cm below the gel surface. The 25-gauge syringe needle was connected to a flow rate monitor and mounted on a stereo tactic assembly as mentioned in methods. Cell (conc. 1 million / cm³) were infused at a rate of 20 μl /min and continued for 3 min. The growth of cells was followed for a period of one week. We noted good cell growth after infusion of cells by the 25 gauge syringe needle. These experiments were repeated three times and cell growth was seen after each infusion. We conclude that PC12 cells at a concentration of 1 million / μl delivered by a 25 gauge syringe needle into collagen gel in a 6 well plate showed good growth potential. Figs.12 to14 show cell growth after 25 gauge syringe needle infusion in collagen gel.
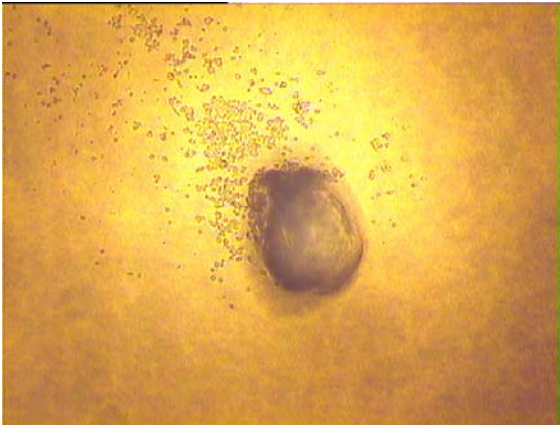
Figure 12. Infusion of PC12 cells by needle in collagen gel (Day 1). The central crater is created by the needle insertion in gel. Cells were infused through the needle. Cells spread on and below the surface of the gel and are visualized using high power microscopy (40X).

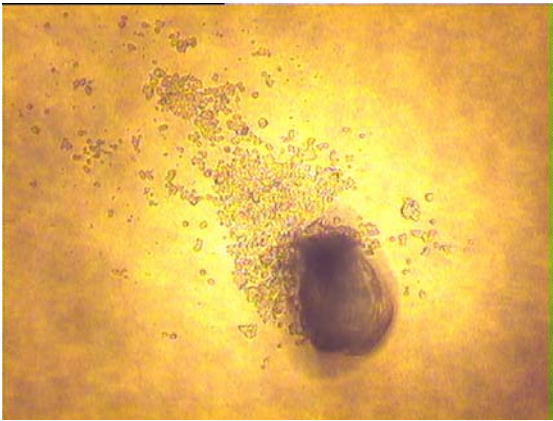Figure 13. Infusion of PC12 cells by 25 gauge syringe needle in collagen gel (Day 3). The cells appear to increase in number. 40X magnification.
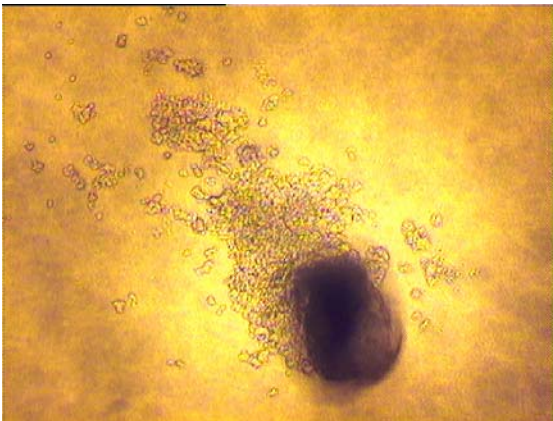

Figure 14. Infusion of PC12 cells by 25 gauge needle in collagen gel (Day 7). The cells continue to visibly increase in number. The collagen gel provided suitable microenvironment for growth of cells and also allowed their visualization. 40X magnification.

The 25 gauge syringe needle experiments confirmed that collagen gel provided a favorable medium for growth of PC12 cells in our lab. This provided vital background information for future studies with prototype catheters.

Additional cell infusion experiments were carried out using the 25 gauge syringe needle. The tip of the 25 gauge syringe needle was passed through a plastic guide cannula to ensure precise delivery of cells in the gel. The aim of the cannula experiments was to study cell growth patterns after vertical and lateral infusion of cells.

To study the growth of cells after vertical implantation in gel, a guide cannula was firmly implanted in the cover (lid) of the culture plate as mentioned earlier in methods section. The tip of the 25 gauge syringe needle was filled with PC12 cell suspension (1 million / μl) and infused through the vertically implanted guide cannula into the gel. The infusion was started at 1 μl /min and kept running for 3 mins. After dissembling, the cell growth in 6 well plate was followed over a period of 1 week. We observed a migration of cells towards the periphery. We conclude that cells infused vertically exhibited good growth and visualization of cell growth was also technically feasible (figures 15, 16 and 17).

Figure 15. Infusion of PC12 cells by 25 gauge syringe needle passed through vertical guide cannula on day1. Note spherical shape of the crater created by the needle. Cells were infused at a concentration of 1 million/μl and continued for 5 mins. 40X magnification.

Figure 16. Infusion of PC12 cells by 25 gauge syringe needle passed through vertical guide cannula on day 3. The cells appear to have migrated away from the center. 40X magnification.
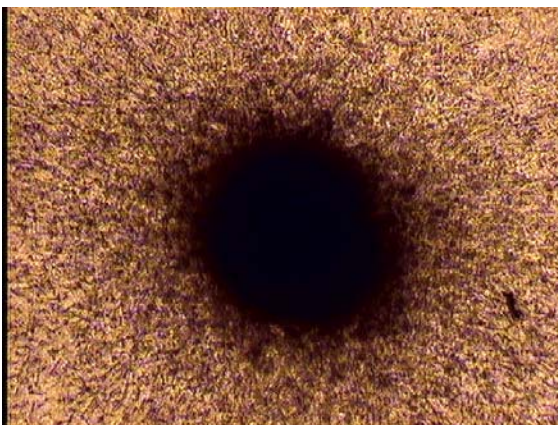
Figure 17. Infusion of PC12 cells by 25 gauge syringe needle passed through vertical guide cannula on day 7. Note cell migration. This method of cell infusion was found to be most favorable and was used to evaluate prototype cell delivery catheters. 40X magnification.

Another set of experiments were conducted to study the growth and migration potential of cells after lateral implantation in gel. Collagen gel was prepared in 6 well cell culture inserts as described above. The tip of the 25-gauge needle was inserted through the laterally implanted guide cannula into the 6 well plate. Cell growth and migration were followed over 1 week. Infusion through laterally implanted cannula revealed poor visible cell growth and migration (figs. 18 and 19). Taking cross sections of the gel and staining might provide more information on the patterns of cell growth after lateral implantation. The cells however showed excellent migration and growth potential with vertical implantation as mentioned earlier. For testing the prototype cell delivery catheters experiments we used vertical infusion delivery.

Figure 18. Infusion of PC12 cells by a 25 gauge syringe needle passed through laterally implanted guide cannula into collagen gel (day1).

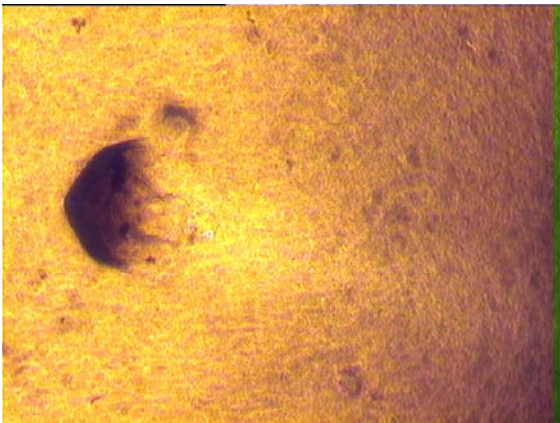Figure 19. Infusion of PC12 cells by a 25 gauge syringe needle passed through laterally implanted guide cannula into collagen gel (day7). Note poor visible cell growth after lateral implantation. This method of infusion was unfavorable and was not used to evaluate prototype catheters.

3.2.2 Cell culture experiments with prototype cell delivery catheters

The prototype cell delivery catheters are longer than the 25 gauge syringe needle and have 4 spiral side pores as opposed to a single beveled opening in the needle. To minimize the development of air bubble and backtracking of dye, the inner tube was filled with the cell suspension and then inserted with the outer tube. Our agarose gel experiments had indicated that this combination was least likely to produce an air leak and backtracking. Preliminary experiments using 2 ml and 4 ml of collagen gel in the 6 well plate resulted in poor cell growth in the 2 ml gel and poor cell visualization in the 4 ml gel insert. To overcome these problems we used a sandwich gel of 0.1% agarose and collagen gels. The optimum quantity of each gel was found to be 2.5 ml. We used 0.1% agarose as it is transparent and solidifies faster than 0.6% agarose. Agarose gel neither favors nor hampers cell growth.

PC12 cells (1 million / μl) were infused in each experiment. The catheters (both small and large bore) were attached to a flow rate monitor and cells were infused at a rate of 1 μl / min for 3 min. Cell growth was noted over a period of a week. We used 2 combinations of sandwich gel. We observed no significant cell growth when collagen gel was layered on top of agarose gel, 5 days after infusion. This combination did not provide a good environment for cell growth. The experiments were repeated 3 times to ensure reproducibility. When agarose gel was layered on top of collagen, we observed cell growth. The lower layer of collagen favored cell growth while the upper layer of 0.1% agarose allowed cell visualization. We repeated these experiments three times to confirm the findings. Both 1.6mm small bore and 2.0mm large bore catheters were evaluated.

Similar results were obtained after each experiment. Figures 20-22 show representative

pictures of results obtained after PC12 cell infusion through prototype catheters into

sandwich gel.

Figure 20. Large bore catheter infusion of PC12 cells with 0.1 % agarose gel on top of collagen gel, day 1. Note central cluster of cells and some migration around the periphery. Also note size of the crater created by catheter infusion was significantly larger than that made by the needle. 40X magnification.

Figure 21. Large bore catheter infusion of PC12 cells with 0.1 % agarose gel on top of collagen gel, day 3. 40X magnification.
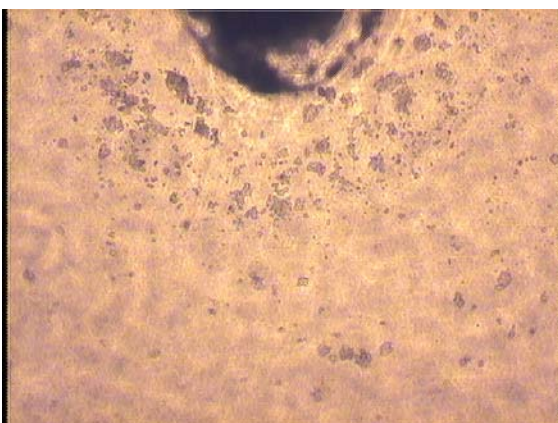

Figure 22. Large bore catheter infusion of PC12 cells with 0.1 % agarose gel on top of collagen gel, day 7. The prototype cell delivery catheters delivered PC12 cells into a sandwich gel with collagen gel on top and the cells appear to grow.

3.2.3 Neural Growth Factor

Neural growth factor (NGF) is known to promote differentiation of PC 12 cells in culture (52, 53). We wanted to test if NGF could differentiate PC12 cells following infusion using the prototype cell delivery catheters. This would have significant impact on the role of catheters for cell replacement strategies in which growth and differentiation factors could be delivered at some point following the infusion of cells. Both 1.6 mm small bore and 2.0 mm large bore catheters were used for cell delivery. Sandwich gel (0.1%agarose on top of collagen) was prepared in 4 cell culture inserts. PC 12 cells were infused at a concentration of 1 million / μl into sandwich gel in each of the 4 inserts separately. The duration of infusion in each insert was 3 mins. The 4 cell culture inserts were placed in a 6 well plate. A dilute concentration of NGF was prepared. The NGF solution was then added to 2 inserts and culture medium to another 2 inserts that served as control. Cell growth and most notably differentiation were recorded over a period of 4 days. We found significant differentiation of cells treated with NGF (figures 23 and 24) as opposed to controls. Within 2 days the cells with NGF were developing axons. When cells were delivered in the sandwich gel by catheters and NGF was not added, no cell differentiation was noted (fig. 25). The cells continued to grow but didn't develop into neurons. The entire experiment was repeated three times to ensure reproducibility. Similar results were obtained after cell infusion through small and large bore catheters. Cells delivered by the prototype cell delivery catheters had the potential to differentiate into neurons in the presence of NGF.
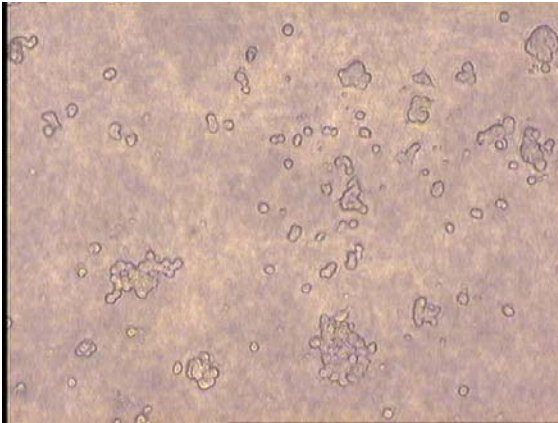
Figure 23. NGF added to sandwich gel after delivering PC12 cells with prototype catheter (day 1).

Figure 24. NGF added to sandwich gel after delivering PC12 cells with prototype catheter (day 4). Note excellent differentiation of cells into neurons. Axons and dendritic processes are visible. This experiment showed that PC12 cells delivered by the prototype catheters had the potential of differentiating into neurons in the presence of NGF.

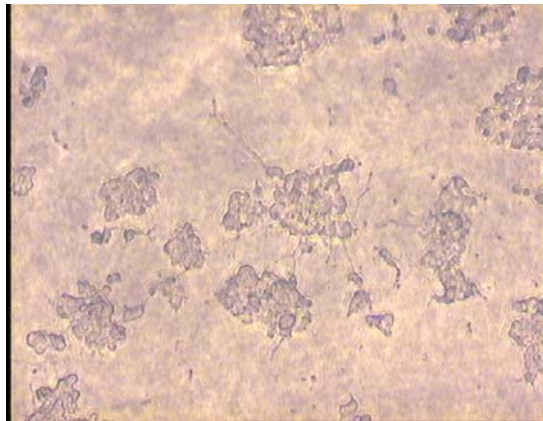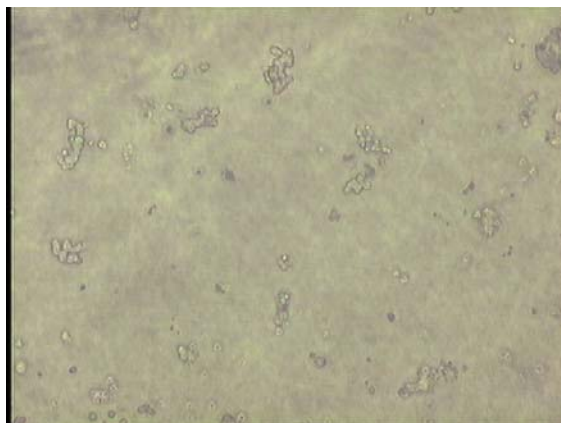Figure 25. No NGF added to sandwich gel after delivering PC12 cells with prototype catheter (day 4). Note lack of differentiation of cells in the absence of NGF.

IV   Discussion and Conclusions

4.1 Discussion

Neurodegenerative diseases including Parkinson's disease and Alzheimer's disease are prevalent worldwide. Medical treatment of these disorders is associated with complications and after a few years many patients fail to respond to therapy. Intracranial therapy of stem cells, drugs and growth factors has the potential to cure these diseases. There have been reported promising results following stem cell transplantation, dopaminergic producing cells and gene therapy for Parkinson's disease (55, 56).Wang, et al. showed that transplantation of neural stem cells into the prefrontal and parietal cortices of dementia induced mice, dramatically alleviated the cholinergic deficits and recent memory disruption (57).

Glioblastoma multiforme (GBM) is the most common and aggressive type of primary brain tumor, accounting for 52 percent of all primary brain tumor cases and 20 percent of all intracranial tumors (26, 27). Treatment can involve chemotherapy, radiotherapy and surgery; all of which are considered as palliative measures as they rarely provide a cure (58). There is extensive literature on the use of intracranial chemotherapy in the treatment GBM. Many new experimental protocols are under investigation. Pellagatta, et al demonstrated that intracranial injection of dendritic cells

increased survival in mice bearing intracranial glioblastomas (59). Aghi, et al reviewed the usefulness of gene therapy for improving survival in patients with GBM (60). In addition to these novel modalities infusion of cytokines, antiangiogenesis factors and monoclonal antibodies are under investigation (61, 62, 63).

Several techniques have been developed for delivering agents directly to the brain. Implantable miniature osmotic pumps, implantable microporous devices and macroencapsulation are some of the techniques for intracranial therapy. However results with these modalities have been variable. The prototype cell delivery catheters provide a novel method for delivering stem cells, drugs and growth factors into the brain parenchyma. Intracranial therapy offers the hope for potential cure of neurodegenerative disorders and brain tumors. The major advantage of intracranial therapy is the ability to overcome the blood brain barrier and achieve very high concentration of therapeutic agents inside the brain while minimizing systemic side effects. Air leakage during catheter infusion creates a significant problem during neurosurgery including backtracking of agent, need for higher infusion pressures, damage of tissues due to local pressure and rarely air embolism all of which may increase morbidity and mortality. Backtracking of therapeutic agents is undesirable as it not only reduces the concentration of agents at the intended site but also increases it at an unintended site. This might be particularly important during delivery of stem cells for neurodegenerative disorders and delivery of cytotoxic drugs in localized brain tumors (64, 65).

We evaluated prototype cell delivery catheters aimed to reduce the above mentioned complications. Each catheter has an inner catheter with one end pore and an

outer catheter with 4 spiral pores. Small bore (1.6 mm outer catheter) and large bore (2.0 mm outer catheter) catheters were evaluated using 0.6 % agarose gel and collagen gel experiments.

The 0.6 % agarose gel experiments were conducted to study the presence or absence of air leak and backtracking of dye after infusion of bromophenol blue dye by the catheters. Chen et al. has shown that 0.6 % agarose gel in brain box simulates the diffusion characteristics of the human brain (41). The small and large bore catheters were evaluated using 3 combinations of insertion of inner and outer catheters in the gel. The 2.0 mm large bore catheter in addition was evaluated with the inner catheter locked and unlocked inside the outer catheter.

The agarose gel experiments using the 1.6 mm small bore catheter demonstrated that when the inner catheter was filled with dye, inserted into the outer catheter and then into gel, no air bubble or backtracking of dye was seen. Using ANOVA analysis, we showed that this combination had significantly smaller size of air bubble indicative of air leakage as compared to the other 2 combinations. Therefore this combination has the potential to eliminate the problems associated with air leak during surgery. An air bubble was consistently seen but there was no backtracking of dye when the outer catheter was inserted in gel prior to the insertion of a dye filled inner catheter. When both inner & outer catheters were inserted in gel & then filled with dye, an air bubble was consistently observed but backtracking of dye was seen in only 1 out of 3 experiments.

The agarose gel experiments with 2.0 mm large bore catheter had similar results. When the inner catheter was filled and inserted with outer catheter, again no air bubble or

backtracking was observed. ANOVA analysis showed that the size of air bubble was significantly smaller with this combination of inner and outer tubes than the other combinations. Due to this reason, all cell culture experiments were conducted with this combination of inner and outer catheters. The inner catheter of the large bore catheter was kept either locked or unlocked for the other 2 combinations of inner and outer catheters. When the outer catheter was inserted in 0.6% agarose prior to insertion of dye filled inner catheter, irrespective of the lock between the 2 catheters (locked, unlocked) an air bubble was consistently seen. There was no backtracking of dye. In the third combination when both inner & outer catheters were inserted in gel & then filled with bromophenol dye, an air bubble was always observed. However in this paradigm, backtracking of dye was noted only when the inner catheter was locked inside the outer catheter. This could be due to the fact that the locked position of the catheters, does not allow air to escape to the outside and it forces air to pass through the spiral pore into the gel.

In all the 0.6% agarose gel experiments, backtracking of dye was seen only when an air bubble was produced. Size of the air bubble did not correlate with backtracking of dye. Further studies are needed to explore this phenomenon in detail. The timing and position of air bubble produced might be associated with the development of backtracking of dye.

Cell culture experiments were initially performed using a 25 gauge syringe needle to deliver pheochromocytoma cells (PC12) in collagen gel to optimize the baseline characteristics for cell growth and differentiation prior to using catheters. We found that

PC12 cells at a concentration of 1 million / μl delivered by a 25 gauge syringe needle into a collagen gel in a 6 well plate demonstrate growth of cells. These characteristics served as a good baseline for the catheter experiments. The 25 gauge syringe needle experiments proved that the collagen gel and microenvironment were conducive for cell growth.

Another set of experiments were performed to compare vertical and lateral infusion using a 25-gauge syringe needle passing through a cannula into collagen gel. The cannula was inserted either vertically or laterally in a 6 well culture plate. We observed that visualization of cell growth was better after vertical delivery as opposed to lateral delivery. This phenomenon could be due to actual decreased cell growth or poor visualization after lateral delivery. Taking cross sections and staining might help in better understanding this process.

Next we tested the prototype catheter found to be optimal for its lack of creating air bubbles and backtracking to deliver cells vertically into a collage gel. The inner catheter was primed with PC12 cells, inserted into the outer catheter prior to insertion of into a collagen gel. We observed minimum cell growth in 4 ml collagen gel. This was probably due to difficult visualization as 4 ml of collagen gel is not transparent. To overcome this problem we used a sandwich-type gel in which 2 ml of collagen was overlaid with 2 ml of 0.1 % agarose, which is transparent. Visible cell growth was observed with 0.1% agarose gel at the top and collagen gel at the bottom. PC12 cell infusion experiments were performed with the 1.6 mm small bore and 2.0 mm large bore catheters and consistent cell growth in sandwich gel was noted.

PC12 cells are known to differentiate in the presence of neural growth factor (51,

52). Using 1.6 mm small bore and 2.0 mm large bore catheters, PC12 cells were vertically infused into collagen gel. Neural growth factor was added to 2 inserts and the other 2 inserts served as controls. We observed significant differentiation of cells treated with NGF as opposed to controls. Within 4 days, the cells had developed axonal and dendritic-like processes. PC12 cells delivered by this prototype cell delivery catheter have the potential to grow and differentiate in the presence of NGF. Further studies with cell infusion and then NGF infusion through the prototype catheters would provide more information on the utility of catheters for cell delivery and differentiation.

4.2 Conclusions

The prevalence of neurodegenerative disorders and brain tumors is increasing. The Blood Brain Barrier (BBB) is a significant obstacle to the delivery of therapeutic agents to the brain administered systemically. These diseases would benefit from intracranial therapy. This method of treatment bypasses the BBB and allows direct delivery of therapeutic agents into the brain at the same time minimizing systemic side effects. We tested prototype cell delivery catheters using dye and cell culture models. With a primed inner catheter, minimal air leak and backtracking of dye was noted which would be beneficial during neurosurgical based treatments. Baseline characteristics of cell growth were optimized using cell infusion experiments with a 25 gauge syringe needle. The prototype cell delivery catheters demonstrate potential for delivering cells in collagen gel. PC12 cells delivered through prototype cell delivery catheters showed good differentiation in the presence of NGF. The common problems with neurosurgical

catheters are air leakage following catheter insertion and consequences secondary to this including tissue damage. Our studies show that the prototype cell delivery catheters tested could be used to overcome these problems during neurosurgery. Further studies including quantification of cell growth, migration of cells after delivery, infusion of growth factors and growth inhibitors through the catheters might shed more light on the therapeutic agent delivering potential of these catheters.

V   List of References

List of References

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Vita

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