

FOREIGN BODY REACTIONS TO NEURAL IMPLANTS IN THE BRAIN

by

PARISA LOTFI

Presented to the Faculty of the Graduate School of
The University of Texas at Arlington in Partial Fulfillment
of the Requirements
for the Degree of

MASTER OF SCIENCE IN BIOMEDICAL ENGINEERING

THE UNIVERSITY OF TEXAS AT ARLINGTON

August 2007

ACKNOWLEDGEMENTS

I take this opportunity to thank my supervising advisor D Liping Tang who supported me all the time in the process of writing this thesis. Without his advice nothing could have ever been accomplished.

I would like to appreciate all my friends and colleagues help in our lab specially Dr Shen with his support in surgical procedure and Ashwin Nair who provided an answer to the questions that I had.

And at the end, I want to thank my family; specifically my parents who inflamed the fire of curiosity in me from childhood and unfortunately are not here to share my happiness in progress in another stage of my life.

August 8, 2007

ABSTRACT

FOREIGN BODY REACTIONS TO NEURAL IMPLANTATS IN THE BRAIN

Publication No. _____

Parisa Lotfi, MS

The University of Texas at Arlington, 2007

Supervising Professor: Liping Tang, Ph.D.,

Neural implants have been used and studied for many decades. They are applied to record neural signals or to locally deliver drugs. Unfortunately, foreign body reaction and accompanied glial scar formation often lead to implant failure. The lack of knowledge about the molecular mechanism governing foreign body reactions in the brain significantly hinders the development of brain implantable devices with improved safety and biocompatibility. To study the immune responses to brain implants, a novel animal model has been developed and used for the following investigation. Our studies have established cell recruitment time courses of astrocytes, microglial cells, lymphocytes, monocytes and mesenchymal stem cells following implantation. The differential cell migration suggests an interesting interaction between these cells leading to glial scar

formation. In a parallel studies, the potential role of lymphocytes and fibrin deposition on foreign body reactions in the brain was also tested using Severe Combined Immuno Deficient mice and heparin therapy. Our results clear support that both T lymphocyte accumulation and fibrin deposition surrounding brain implants play a critical role in foreign body reactions to brain implants. It is our belief that the results obtained from this work will help the development of better and safer implantable brain devices.

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LIST OF ABBREVIATIONS

OEC.....	Olfactory ensheathing cells
BBB.....	Blood brain barrier
PMM.....	Polymethylidene malonat
SAIB	Sucrose acetate isobutyrate
NOS.....	Nitrogen oxidative species
IL-2	Interleukin-2
GFAP	Glial Fibrillary Acidic Protein
MHC	Major Histocompatibility Complex
TNF- α	Tumor necrosis factor alpha
IL-1 β	Interleukin 1-beta
CNS.....	Central nervous system
NGF.....	Nerve growth factor
TGF- β	Transforming growth factor
ECM.....	Extra cellular matrix
CNCT	Center for neural communication technology
CNF.....	Cornell nanoscale facility
IL-6	Interleukin-6
INF- γ	Interferon gamma
MPT	Mitochondrial permeability transition
SCID	Sever combined immunodeficiency

IgImmunoglobulin
IACUCInstitutional Animal care and use committees
PFParaformaldehyde
PBSPhosphate buffer salt
BSA.....Bovine serum albumin
SDStandard deviation
SSEA-4Stage specific embryogenic antigen-4
LMW-Hep.....Low molecular weight heparin

CHAPTER 1

INTRODUCTION

1.1 Neural implants for recording or stimulation

Neural probes with micromachined electrodes have been developed to facilitate the functional stimulation and recording of neurons in the peripheral and central nervous systems (Cui et al., 2002). Application of brain prosthetic devices (BPD) have been studied for treatment of some disease like Alzheimer, Parkinson's, and hearing deficits resulting from auditory nerve neuromas (Griffith et al., 2006; Benabid, 2003; Vitek et al., 2004; Otto et al., 2002 and Haberler et al., 2000). Multi-channel auditory brain stem implants to improve hearing problems associated with nerve impairment and deep brain stimulation prostheses for Parkinson's disease have been developed to the point of clinical application (Vitek et al., 2004; Shain et al., 2003; Benabid, 2003; Otto et al., 2002 and Haberler et al., 2000).

Although neural implants have been used extensively to treat central nervous system trauma, disease, and age-related degeneration for almost 30 years (Szarowski et al., 2003), almost all recording electrodes fail shortly after implantation (in a few weeks) (Zhong et al., 2007; Spataro et al., 2005; Szarowskie et al., 2003 and Turner et al.,

1999). It is widely accepted that implant failure is associated with the inflammatory tissue reaction and glial scar formation around the probe (Zhong et al., 2007; Spataro 2005). However, the processes underlying foreign body reactions to neural implants are mostly unclear.

1.2 Foreign body reactions to neural implants

For many years the brain was considered to be an immunologically privileged organ because of presence of blood brain barrier, lack of lymphatic system, low level expression of MHC (Major Histocompatibility Complex), and impaired rejection of implants in short term applications (Xiao et al., 1998 and Frohman et al., 1988). It is believed that, under normal conditions, lymphocyte traffic in the brain is relatively low compared to other organs. This can be due to the tight junction of endothelial cells in BBB (Blood Brain Barrier) or the inability of lymphocytes to adhere to endothelial cells in BBB (Xiao et al., 1998; Wang et al., 1993 and Frohman et al., 1988).

In response to injury, endothelial cells in blood vessels secrete cytokines and express adhesion molecules to facilitate the adhesion and penetration of circulating inflammatory cells (Xiao et al., 1998 and Wang et al., 1993). Cells migrating to the implantation site form a fibrotic sheath around the implant to separate it from the rest of the tissue (Zhong et al., 2007; Spataro et al., 2005; Szarowskie et al., 2003 and Turner et al., 1999). Cellular encapsulation isolates the prosthesis mechanically and electronically and disrupts its functionality (Zhong et al., 2007; Szarowski et al., 2003 and Turner et al.,

1999). The brain response to implants is characterized by high expression of GFAP (Glial Fibrillary Acidic Protein) in astrocytes and recruitment of microglia to the insertion site. A glial-fibroblastic scar is formed as a result of astrocyte activation and microglia migration (Korzhevskii et al., 2005 and Cui et al., 2003). This scar is a conductive barrier preventing the device from communicating with neurons and inhibits axon growth (Cui et al., 2003).

Several cells initiate immune responses in brain injury: glial cells (microglia, astrocytes and endothelial cells) and immune cells (T-cells and monocytes) (Xiao et al., 1998). Their specific responses can be summarized as follows.

Microglial cells: Microglial activation is the hallmark of inflammatory response in the brain. Brain macrophages, following injury, come from two different sources; parenchymal resident microglia and perivascular monocytes (Beschoner et al., 2002). Resident microglia are distinguished from perivascular monocytes by lack of expression of CD14, a surface molecule of monocytes, and high expression of CD11b coupled with low expression of CD45 (Pais et al., 2005; Wirenfeldt et al., 2005 and Beschoner et al., 2002). Some believe that microglial cells are bone marrow-derived cells that migrate and populate in the CNS (Central Nervous System) in early stages of embryonic development (Beschoner et al., 2002). After injury microglial cells change their morphology; resting microglia (ramified) become activated phagocytic (rounded) cells (Streit et al., 1999). Activated microglial cells are known for their ability to engulf cell debris and foreign materials (Matsumoto et al., 2007). They secrete cytokines including TNF- α (tumor

necrosis factor α) and NGF (nerve growth factor), which induce apoptosis in neurons (Bessis et al., 2007), and TGF- β (transforming growth factor) which promotes ECM (Extra-Cellular Matrix) deposition after injury (Aloisi et al., 2000 and Logan et al., 1999). Microglial cells express MHC molecules during inflammation. Studies suggest that microglial cells are the main candidate for antigen presenting cells in the brain (Aloisi et al., 2000; Aloisi et al., 1999 and Xiao et al., 1998).

Astrocytes: Astrocytes are the most abundant cells in the brain (Wikinson et al., 2006). They have a leading role in the formation of gliosis (Korzhevskii et al., 2005). They express vimentin and nestin before they activated and in the early stages of gliosis. Upon maturation, or following activation, they express an intermediate filament called GFAP (Glial Fibrillary Acidic Protein) (Alonso et al., 2005 and Eddleston et al., 1993). They reside in close contact with the BBB and secrete the essential chemokines for lymphocyte and microglial cell migration and activation (Wikinson et al., 2006; Korzhevskii et al., 2005 and Xiao et al., 1998). In addition, it is believed that they express MHC molecules in inflammatory sites and act as an antigen presenting cell to T-cells (Aloisi et al., 2000; Aloisi et al., 1999; Xiao et al., 1998 and Forhman et al., 1988). These cells secrete pro-inflammatory cytokines like TNF- α and IL-1 β (Interleukin 1- β) (Xiao et al., 1998 and Forhman et al., 1988). Glial scar formation in the CNS (Central Nervous System) is primarily composed of interwoven hypertrophic astrocytes and excessive deposition of extra cellular matrix (ECM) (Wang et al., 2007). Astrocytes are responsible for secretion of some ECM proteins like proteoglycan NG2, neurocan and collagen (Hirano et al.,

2004). Collagen IV produced by astrocytes participates in glial scar formation post injury (Hirano et al., 2004).

Microglial cells and astrocytes are the main resident cells involved in immune reactions in the brain, however interactions between them is complex and still uncovered (Streit et al., 1999).

Lymphocytes: Under normal physiological conditions, the brain has far fewer lymphocytes than any other organ (Xiao et al., 1998; Wang et al., 1993; and Frohman et al., 1988). After injury, trauma, or inflammation the endothelial cells in blood vessels are more permeable to lymphocytes. IL-2, the main cytokine essential for lymphocytes to become fully migratory, is secreted in brain after injury (Xiao et al., 1998).

Monocytes: There is an up-regulation in expression of CD14 after brain injury. This is due to migration of monocytes and activation of phagocytosis (Beschorner et al., 2002).

1.3 Improving the biocompatibility of implants

Implant-induced inflammatory responses are thought to affect subsequent glial scar formation. Many strategies have been developed and tested to improve neural biocompatibility aimed at reducing surgical induced trauma and inflammation. These methods include minimizing the size of the implant, improving the method of insertion (Bjornsson et al., 2006), application of more biocompatible materials like silicon as

electrodes (Cui et al., 2001), coating electrodes with electronically conductive polymers (polypyrrole) conjugated with cell adhesive biomolecules (fibronectin) (Cui et al., 2001), and by coating the electrodes with anti-inflammatory drugs (Dexamethasone and cyclosporine A) (Zhong et al., 2007 and Shain et al., 2003). Detailed description of these methods is listed below.

1.3.1 Minimizing the size of implant

It is believed that, by reducing the size of the medical implants, the implant-associated surgical trauma and subsequent tissue reactions may be substantially reduced. Nano and micro fabrication techniques are used to decrease the size of implant. Most of these probes are made of silicon fibers (Turner et al., 1995). Two major labs producing silicon fiber probes are CNCT (Center for Neural Communication Technology) (Zhang et al., 2007) and CNF (Cornell Nanoscale Facility) (Szarowski et al., 2003). These implants are capable of measuring small areas of brain and stimulating a small number of functionally related neurons and possibly single neurons (Turner et al., 1999, Zhong et al., 2007, Holecko et al., 2005, Spataro et al., 2004, Szarowski et al., 2003 and Cui et al., 2002 and 2003).

1.3.2 Applying biocompatible materials and coatings

Some common biocompatible materials in neural probe preparation are silicon fiber (Zhon et al., 2007, Spataro et al., 2005, Bjornsson et al., 2006, Szarowski et al.,

2003, Cui et al., 2003 and Turner et al, 1999), gold (Stauffer et al., 2005), platinum (Griffith et al., 2006), and stainless steel (Shain et al., 2003; Benabid, 2003; Otto et al., 2002 and Haberler et al., 2000).

For long-term implantation, surface modification is necessary and usually facilitated by mixing a conductive polymer (i.e. polypyrrole) with synthetic peptides (i.e. fibronectin, laminin) for cell adhesion (Cui et al., 2001 and Stauffer et al., 2005). This coating will increase the attachment of neurons to the probe while preserving conductivity and decreasing host responses (Cui et al., 2002, 2003).

1.3.3 Using drug therapy

Many anti-inflammatory drugs, including dexamethasone and cyclosporin A, have been used to reduce glial scar formation surrounding neural implants. The observations from these studies are summarized as follows:

Dexamethasone: Neurosteroids and their receptors on the neurons and glial cells increase after injury in central nervous system (Fournier et al., 2003). Dexamethasone is a synthetic glucocorticoid anti-inflammatory drug which can reduce inflammation in the central nervous system. It functions through the glucocorticoid receptors on the neurons and glial cells and inhibits astrocyte and microglial activation and proliferation (Wadhwa et al., 2005). Additionally dexamethasone reduces some pro-inflammatory cytokines

including IL-1 β , IL-6, INF- γ and TNF- α . Dexamethasone can be administered via either peripheral injection (Shain et al., 2003) or local release (Wadhwa et al., 2005).

Cyclosporin A: Cyclosporin A is another drug used to reduce inflammation in brain implants (Shain et al., 2003). It has been shown that application of Cyclosporin A after brain damage ameliorates the injury and enhances the survival (Li et al., 2000). Cyclosporin A inhibits the formation of MPT (Mitochondrion Permeability Transition Pore) and disrupts the mitochondrial membrane by inhibiting calcineurin-dependent processes (Shain et al., 2003 and Li et al., 2000).

1.4 Goals of this investigation

Although most of neural implants failed due to foreign body reactions (glial scar formation), the pathogenesis of neural implant-mediated foreign body reactions is mostly undetermined. The lack of this knowledge has substantially hindered the development of better and safer neural implants. To fill the gap, our first goal was to develop an animal implantation model which allows us to systematically study the cascade of immune and glial cell responses to implants (Chapter 2). Using the knowledge obtained from this basic study, we developed and tested new therapeutic approach to reduce neural implant-mediated foreign body reactions (Chapter 3). The detailed information of these studies is listed below.

CHAPTER 2

KINETICS OF FOREIGN BODY REACTIONS IN BRAIN

2.1 Introduction

Different types of cells have been found to participate in immune reactions in the brain. These cells can be categorized as glial cells (microglia and astrocytes) and inflammatory cells (T-lymphocytes and monocytes) (Xiao et al., 1998). Microglial cells are the resident brain macrophages (Beschoner et al., 2002). Activated microglia engulf cell debris and foreign materials (Matsumoto et al., 2007). They secrete some cytokines to interact with other cells and promote ECM deposition after injury (Bessis et al., 2007; Aloisis et al., 2000 and Logan et al., 1999). Astrocytes are the most abundant cells in the brain and have a leading role in glial scar formation (Wikinson et al., 2006; Korzhevskii et al., 2005). They secrete the essential chemokines for lymphocyte and microglial cell migration and activation (Wikinson et al., 2006; Korzhevskii et al., 2005 and Xiao et al., 1998). Glial scars in the CNS are composed of astrocytes and characterized by excessive deposition of ECM (Wang et al., 2007). Inflammatory cells (monocytes and lymphocytes), which are present in small numbers in the uninjured brain, are found in brain after trauma or injury (Beschoner et al., 2002; Xiao et al., 1998; Wang et al., 1993 and Frohman et al., 1988). However, the source and kinetic recruitment of

these cells in response to brain implants are not totally understood. Their potential interactions are also poorly characterized. Our laboratory has recently uncovered that inflammatory responses are often accompanied by stem cell recruitment. Interestingly, our preliminary results have also found that mesenchymal stem cells are recruited to implantation sites in the brain. The potential role of stem cells in glial scar formation is not clear. To study such complex reactions, first we developed a mouse brain implantation model which would allow us later to study the kinetic cell responses at 1, 2, 3 and 4 weeks post implantation.

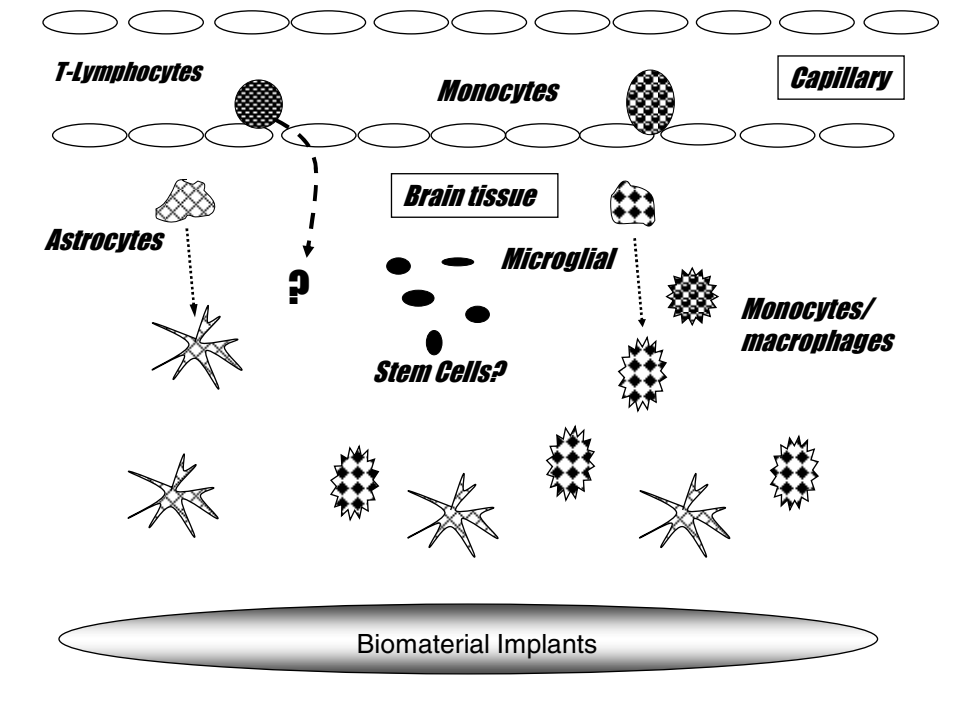


Figure 1 Schematic illustration of cells involved in the foreign body reaction to biomaterial implants in brain.

2.2 Materials and Methods

2.2.1 Materials

Brain probe implants: Stainless steel brain probes (1-3 mm) in the Brain infusion kit 3 (Alzet Osmotic Pumps Company, Cupertino, CA) were used as model brain implants.

Osmotic pump: Micro-osmotic pumps (model 1002, Alzet Osmotic Pumps Company, Cupertino, CA) were used to locally release drugs to the tissue surrounding the implant. This pump is designed to secrete 0.25 μ l solution per hour for 14 days.

Animals: Balb/C mice were used for this experiment.

Antibodies: A variety of antibodies were used for immunohistological staining to distinguish different types of cells participating in foreign body reactions (as listed in Table 1).

Table 1 Primary antibodies and the labeled cells.

	Marker	Manufacture	Dilution	Species	Reactant	Target cells
Primary antibody	GFAP	Santa Cruz Biotechnology	1:50	Goat IgG	Mouse, Rat, Human	Astrocyte
	CD11b	BD Pharmingen	1:50	Rat IgG	Mouse	Microglial cells
	CD3	BD Pharmingen	1:50	Rat IgG	Mouse	T lymphocytes
	CD14	BD Pharmingen	1:50	Rat IgG	Mouse	Peripheral monocytes
	SSEA4	R & D systems	1:50	Mouse IgG	Mouse Human	Stem cells
	CD45	Santa Cruz Biotechnology	1:50	Rat IgG	Mouse	White blood cells

2.2.2 Experimental methods

Neural probe animal implantation procedures: All animal procedures were approved by The Institutional Animal Care and Use Committees (IACUC) at University of Texas at Arlington. Animals weighed between 20-30 g. The animals were anesthetized with a mixture of Isoflourane (1-3 %) and oxygen and immobilized. After shaving the hair, a

small incision (4-6 mm) was made in the skin on the skull. A hole was drilled to the skull 2 mm lateral to the mid line in right parietal. The stainless steel brain infusion cannulae (1-3 mm) was inserted into the hole and fixed with glue. In some animals the brain infusion probes were also connected to osmotic pumps with saline (as control) or drugs. The osmotic pumps were then implanted on the back of animals. The pump secreted 0.25µl per hour of normal saline for 14 days. At the end of the studies, animals were sacrificed, the implant sites were labeled with alcian blue (1mg/ml), and the brain tissues were carefully recovered for histological analyses.

Histological analyses: Whole brains were first subjected to a tissue fixing process (Figure 2) and subsequently embedded in OCT (Tissue-TEK 4583). The frozen blocks were sectioned and the sections finally underwent immunohistochemistry as depicted in Figure 3. The detailed experimental procedures for each step are listed below.

Brain tissue fixing process (modified from Zhang et al., 2007):

1. The whole procedure was done on ice bags to avoid the autolysis.
2. Following removal of the brain probe drops of alcian blue dye (1mg/ml, Richard-Alan Scientific) were placed on the cavity left by probes. Tissue around the cavity was stained blue for easy visual identification of the implantation sites.
3. Each brain was placed in 3 ml of 4 % paraformaldehyde (PF) in PBS at 4 degrees for 6 hours. Paraformaldehyde was made fresh from stock solutions of 8% paraformaldehyde by dissolving paraformaldehyde in PBS at a temperature of around 56 degrees C. Stock solutions were always kept in cold and dark conditions to avoid

the generation of autofluorescent properties. Brain tissues were then incubated with 30% sucrose in PBS for 6 hours.

4. After brief washing with PBS the brain tissues were dried with KimWipes, embedded in OCT (Tissue-TEK 4583), and kept at -80°C for more than 4 hours.

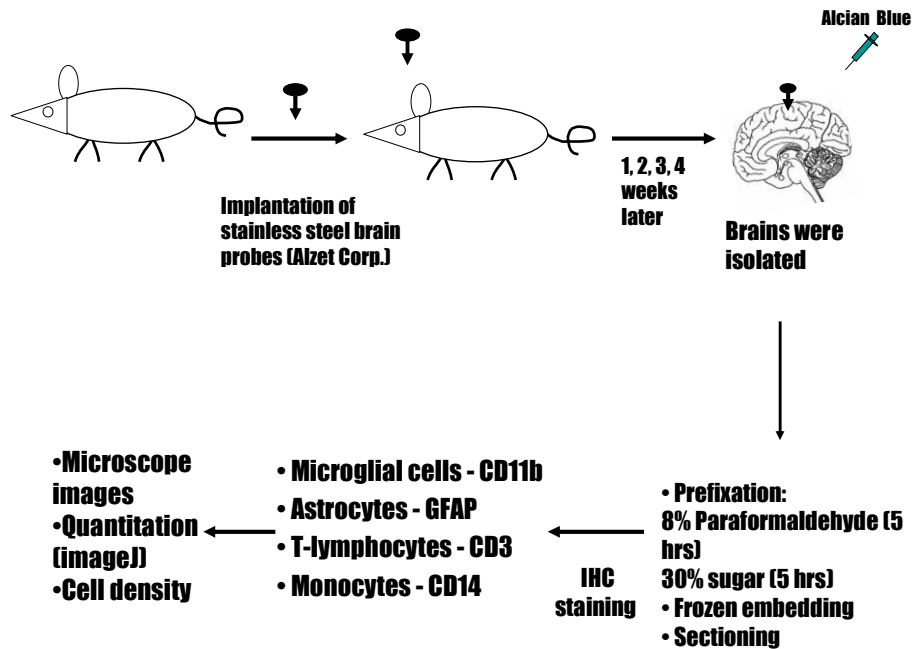


Figure 2 Schematic illustration showing the experimental surgical procedure.

Ten μm thick sections were sliced using a Leica Cryostat (CM1850) and placed on poly-L-lysine coated slides. To assess the immune responses to neural probes, the tissue sections were then stained for immunohistochemistry.

Immunohistochemical staining procedures' for brain tissues (modified from Zhong et al., 2007 and Matsumoto et al., 2007) are listed as follows (Figure 2). :

1. Slides were immersed in acetone at -20°C for 20 minutes.

2. Slides were then washed with PBS for 10 minutes at room temperature.
3. Slides were incubated with 1% Bovine Serum Albumin (BSA) for 20 minutes at room temperature.
4. After rinsing with PBS, tissue sections were then covered with primary antibodies (10 μ l) and incubated at 37°C for 2 hours.
5. After washing three times (5 minutes/time) with PBS, tissue sections were then incubated with 10 μ l of secondary antibody at 37°C for 1 hour.
6. Tissue sections were washed with PBS 3 times (5 minutes/time), air dried in the dark, mounted with 2 μ l antifading agent (Antifading agent, Invitrogen, Carlsbad, CA), and then covered with Coverslips.

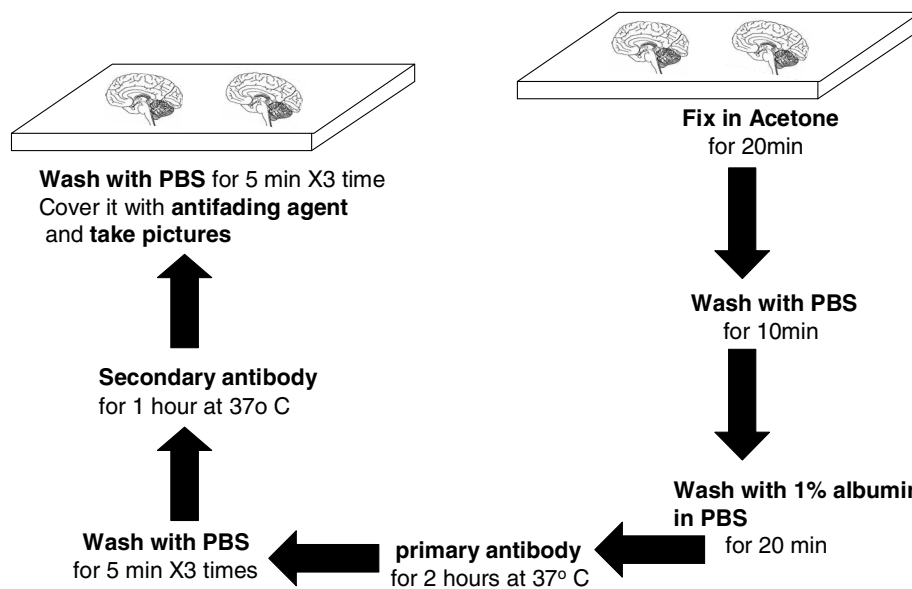


Figure 3 Schematic illustration of the Immuno- Histochemical (IHC) staining on brain tissues.

Tissue imaging and cell quantification: Immunohistochemically stained sections were observed under a fluorescent microscope (Leica DMLB, Leica, Wetzlar, Germany) with 20X magnification. Pictures were taken with Nikon E500 Camera (Nikon Corp., Japan) so as to cover the whole area surrounding the implantation site.

Cells were counted and the surface area of the section was measured using ImageJ software (Rasband, W.S., ImageJ, National Institutes of Health, Bethesda, Maryland, USA, <http://rsb.info.nih.gov/ij/>, 1997-2007). Data was adjusted to a normal population distribution for statistical comparison (achieved on the basis of cell population/ mm²).

Statistical analyses: For each parameter studied, the mean \pm SD was calculated. The normalized data (achieved on the basis of cell population/ mm²) was compared according to Student t- test. Differences were considered statistically significant when $p < 0.05$.

2.3 Results

Microglial cells and astrocytes are two major cell types which have long been associated with glial scar formation. To study the mechanisms of neural implant-mediated foreign body reactions, we first assessed the kinetic recruitment of microglial cells and astrocytes. We find that microglial cells and astrocytes migrate slowly to the implantation site after a few days post implantation compared to the control animal with no implant. Specifically, microglial cells (CD11b+ cells) were recruited to the implantation site slowly at one week. The cell density reached a maximum at week 2 and then slightly decreased at weeks 3 and 4 (Figure 4). The distribution of microglial cells in the implant-surrounding tissue was rather homogenous.

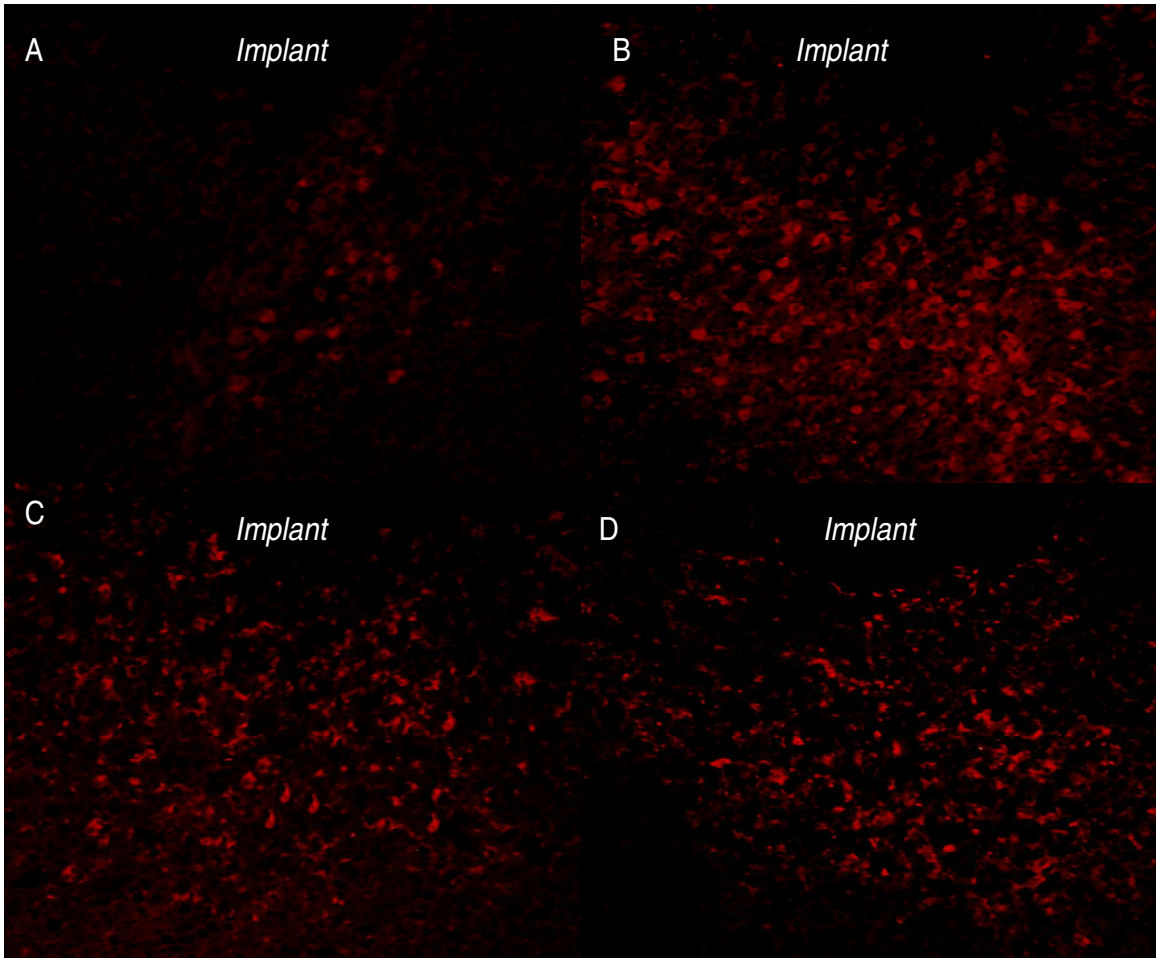


Figure 4 Immunohistochemistry staining of microglia (CD11b+ with red fluorescence) cells in tissues surrounding stainless steel probes which were implanted in Balb/C brains for (A) 1 week, (B) 2 weeks, (C) 3 weeks, and (D) 4 week.

Similar implant-induced cell recruitment can be found for astrocytes (GFAP+ cells) which appear in the tissue surrounding neural implants at one week. The astrocyte cell density achieved plateau at week 2 and only slightly decrease at weeks 3 and 4 (Figure 5). Astrocytes appear to be slightly concentrated in the tissue adjacent to the implants.

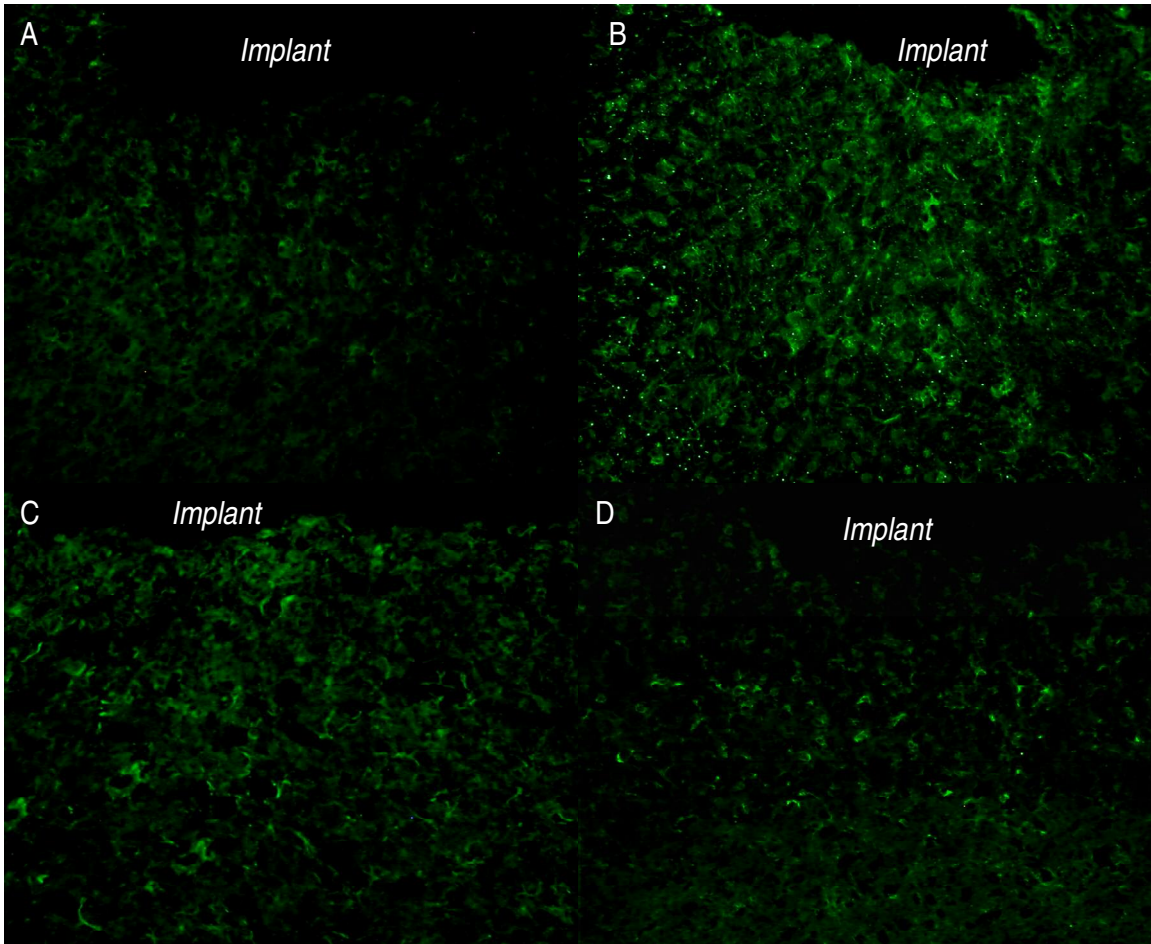


Figure 5 Immunohistochemistry staining of astrocyte (GFAP+ with green fluorescence) cells in tissues surrounding stainless steel probes which were implanted in Balb/C brains for (A) 1 week, (B) 2 weeks, (C) 3 weeks, and (D) 4 week.

Comparing the recruitment kinetics of both microglial cells and astrocytes, we found that both cells exerted similar responses to neural implants. The density of astrocytes is slightly higher than microglial cells in week 1, while there is not much of a difference in weeks 2, 3 and 4. Astrocyte and microglial cell density increases by more than 3 times from week 1 to week 2. In week 4, they both decrease to 1/2 of their maximum at week 2 (Figure 6).

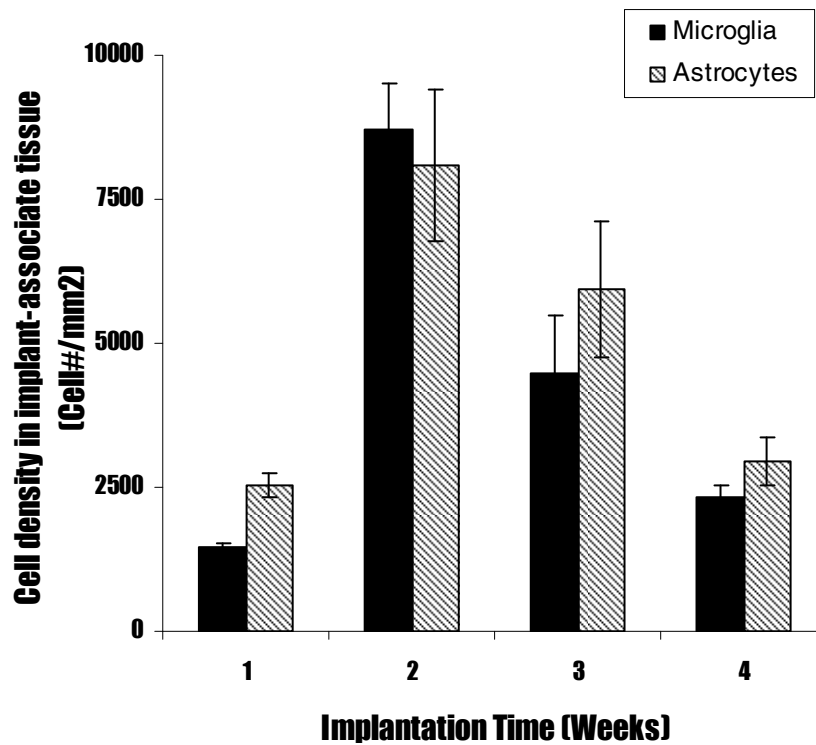


Figure 6 Microglial cell and Astrocyte density in implant-associated tissue (Cell#/mm²) in Balb/C mice at 1, 2, 3 and 4 weeks post implantation. Vertical lines denote ± 1 SD (n= 4 for each time point).

Since T-lymphocytes have been found to participate in inflammatory diseases (Aloisi et al., 2000), the recruitment of T-lymphocytes (CD3+ cells) to neural implant-surrounding tissues was also investigated. Similar to previous studies, immunohistological analyses was carried out on brain tissue from mice implanted with neural implants for different periods of time. We found that T-lymphocytes infiltrate to the implantation site at one week. Maximal T-lymphocyte cell densities were found in tissues of 2-week implants. Differing from astrocyte and microglial cells, the density of T-lymphocytes in the tissue reduced quickly at week 3 and T cells almost disappeared from the tissue at week 4 (Figure 7).

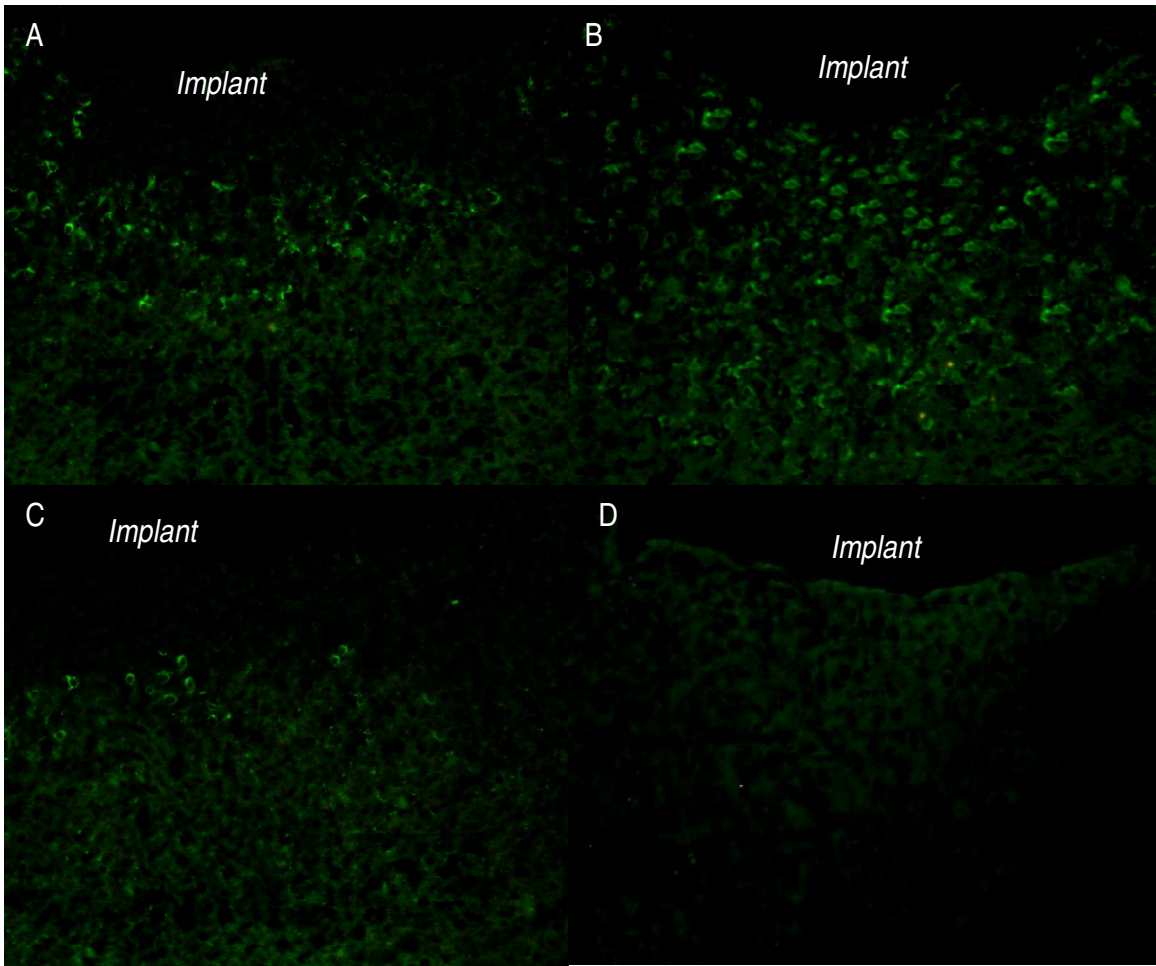


Figure 7 Immunohistochemistry staining of T-lymphocyte (CD3+ with green fluorescence) cells in tissue surrounding stainless steel probes which were implanted in Balb/C brains for (A) 1 week, (B) 2 weeks, (C) 3 weeks, and (D) 4 week.

The accumulation of monocytes/macrophages is the hallmark of inflammatory responses (Beschorner et al., 2002). To study the neural implant-mediated inflammatory responses, the accumulations of monocytes/macrophages (CD14+ cells) in tissue were also assessed. As expected, monocytes/macrophages infiltrate to the implantation site and achieve plateau at 7 days. The accumulation of monocytes/macrophages in tissue lasted for 2 weeks. The densities of monocytes/macrophages reduced dramatically in week 3. There was almost no CD14+ cells found in the tissue four weeks after implantation (Figure 8).

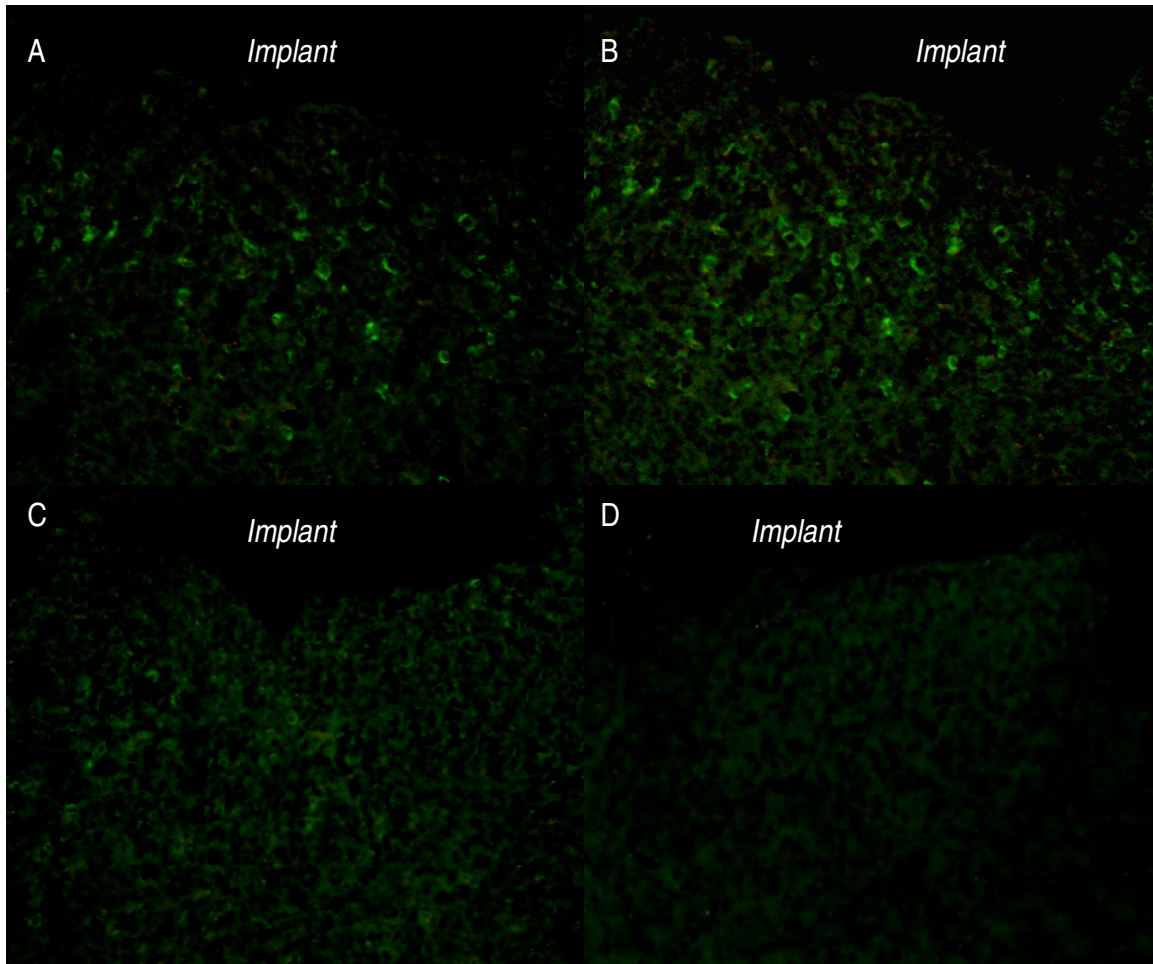


Figure 8 Immunohistochemistry staining of monocytes (CD14+ with green fluorescence) in tissues surrounding stainless steel probes which were implanted in Balb/C brains for (A) 1 week, (B) 2 weeks, (C) 3 weeks, and (D) 4 week.

The recruitment of T-lymphocytes and monocytes/macrophages in neural implant-surrounding tissues was quantified and compared at different time points (Figure 9). Our results show that T-lymphocyte population doubles in the second week compared to week 1 and then drops to almost 1/4 at week 3 compared to week 2. However monocyte density does not change significantly from week 1 to 2 then significantly drops in week 3 and almost disappears at week 4 (Figure 9).

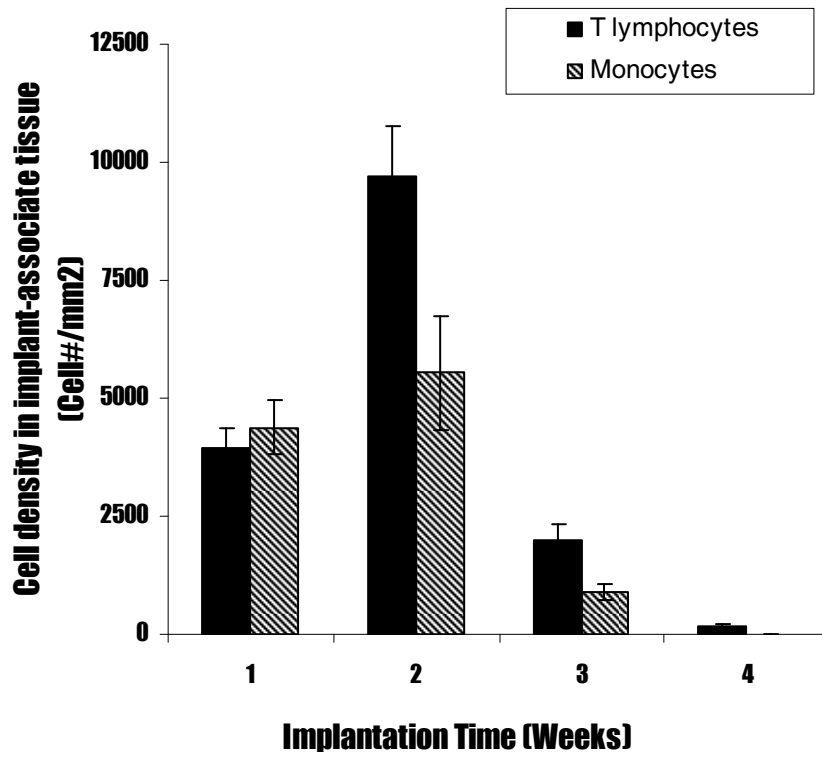


Figure 9 Cell densities of T-lymphocytes (CD3+) and Monocytes (CD14+) in implant-associated tissue (Cell#/mm²) in Balb/C mice at 1, 2, 3 and 4 weeks post implantation. Vertical lines denote ± 1 SD (n= 4 for each time point).

The recruitment pattern of resident glial cells (microglial cells and astrocytes) and inflammatory cells (T-lymphocytes and monocytes) were also compared (Figure 10). In general, both groups of cells were responding to neural implants with a similar time scale and trend. Monocyte/macrophage recruitment reaches a maximum density in 14 days. However, microglial cells, astrocytes and T lymphocytes reach maximal accumulation in week 2. In addition, microglial cells and astrocytes tend to stay in injured tissue for longer than 4 weeks. However, monocytes/macrophages and T-lymphocytes are not found in tissues of 4-week implants (Figure 10). They all reach a maximum in week 2 and decrease in weeks 3 and 4 (Figure 10).

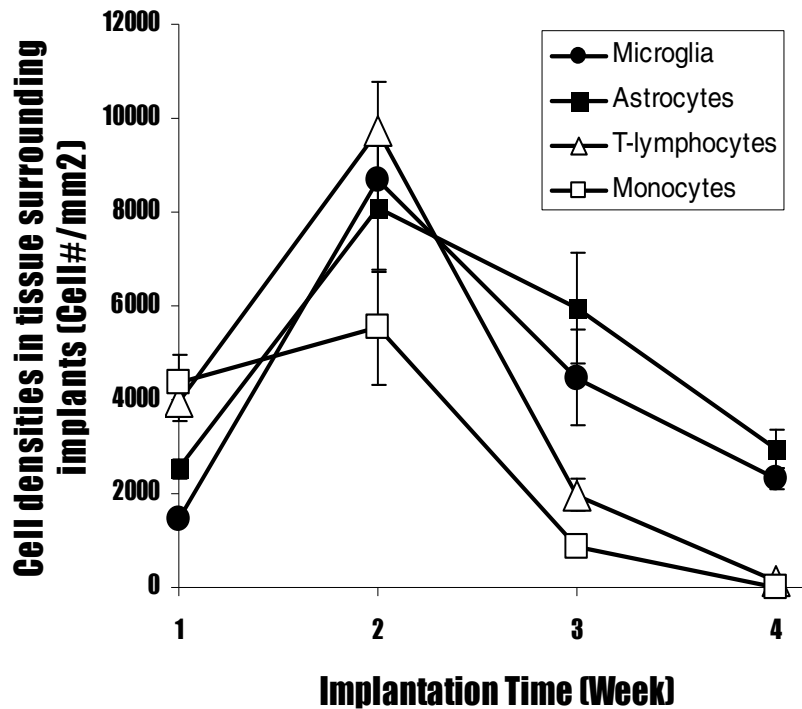


Figure 10 Cell densities of Microglial cells (CD11b+), Astrocytes (GFAP+), T-lymphocytes (CD3+), and monocytes (CD14+) in implant-associated tissue (Cell#/mm²) in Balb/C mice at 1, 2, 3 and 4 weeks post implantation. Vertical lines denote ± 1 SD (n= 4 for each time point).

Our recent studies have shown that mesenchymal stem cells were actively recruited to the injured tissues by inflammatory stimuli (M.S Thesis, University of Texas at Arlington). To determine whether mesenchymal stem cells (SSEA4+/CD45- cells) (Gang et al., 2007) were recruited to the neural probes implanted in brain tissues, immunohistochemical analyses was carried out on tissues of 2-week implants. Indeed, a large number of mesenchymal stem cells (SSEA4+/CD45-) were present in the implant-surrounding tissues (Figure 11). Most of the mesenchymal stem cells present were adjacent to the implants.

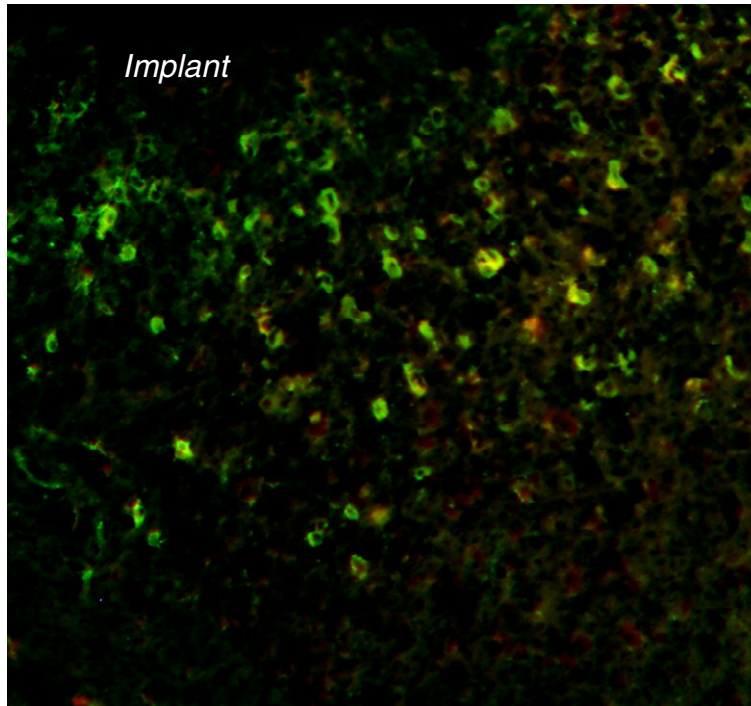


Figure 11 Presence of mesenchymal stem cells in brain tissues for 2 weeks after implantation. SSEA4 and CD45 were labeled with green and red fluorescence, respectively. Mesenchymal stem cells (SSEA4+/CD45-) are seen in this picture with green fluorescence. Cells with yellow fluorescence (SSEA4+ CD45+) are leukocytes.

Comparing the density of different cell types in implant-associated tissue, we found that large numbers of microglial cells, astrocytes, and T-lymphocytes were present in the 2-week implant tissue. The cell density of monocytes is slightly less than microglial cells, astrocytes and T-lymphocytes. However, mesenchymal stem cells have the lowest cell density when compared with the other cells mentioned above (Figure 12).

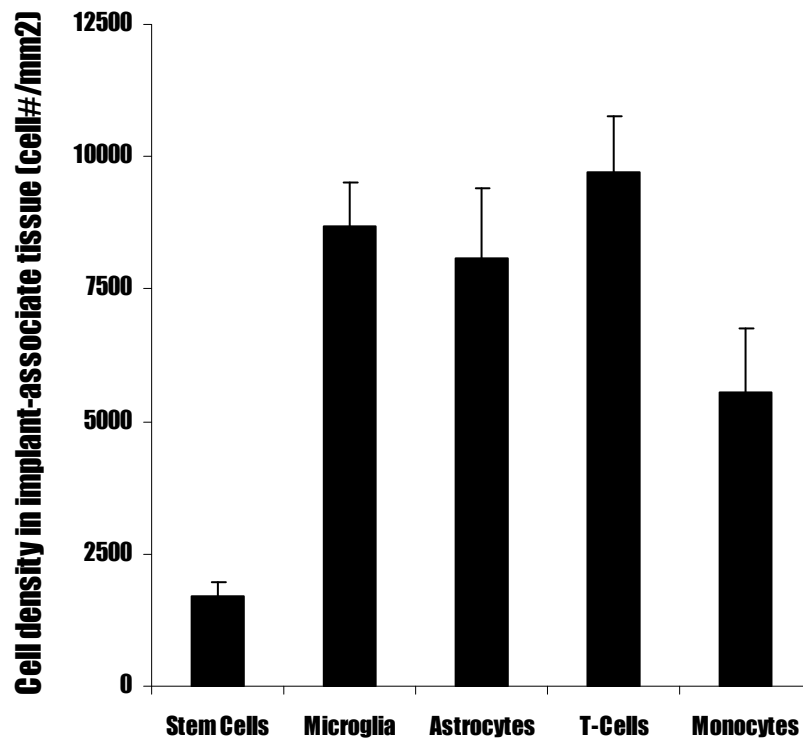


Figure 12 Density of mesenchymal stem cells (SSEA4+/CD45-), Microglial cells (CD11b+), Astrocytes (GFAP+), T-Lymphocytes (CD3+), and Monocytes (CD14+) in implant-associated tissue (cell#/mm²) of Balb/C mice 2 weeks after neural probe implantation. Vertical lines denote ± 1 SD (n= 4 for each time point).

2.4 Discussion

Scar formation generally is important in wound healing after injury. Following implantation or injury, glial scar (mostly astrocytes) would form in the injured spinal or brain tissue. Glial scars are composed of interwoven hypertrophic astrocytes and characterized by excessive deposition of ECM (Wang et al., 2007, Szarowski et al., 2003). Such fibrotic scar tissues serve as mechanical and chemical barriers which lead to nerve death and prevent axonal regeneration (Wang et al., 2007). However, studying other cells in brain immune reaction is necessary due to their complex interaction with microglial and inflammatory cells. The cells involved in scar formation secrete some cytokines to prevent the nerve regeneration close to the scar (Wang et al., 2007). Glial scar formation and cell encapsulation in the CNS are primarily responsible for long term rejection of brain implants.

Most of the early studies used rats as an animal model to evaluate the immune reactions and probe functionality in brain since the probes applied in their experiments were bigger (Zhong et al., 2007; Spataro et al., 2004; Shian et al., 2003; Szarowski et al., 2003; Turner et al., 1999). The benefits of using mice as implantation subject is that a variety of antibodies are available for immunohistochemistry. Rats are ten times bigger. Thus, more medicine is needed to achieve therapeutic effect. In other words, it is much cheaper to carry out inhibitor experiments in mice. We find that this mouse neural probe implantation model can be used to study not only the role of these cells in neural probe-

mediated immune responses but also different inhibitors in altering implant-induced glial scar formation.

Our studies have found that several cells are involved in immune reaction responses in responding to the brain implantation. In agreement with many early observations, these cells are the glial cells (microglial cell and astrocytes) and inflammatory cells (T-lymphocytes and monocytes) (Spataro et al., 2004; Zhong et al., 2007; Szarowski et al., 2003; Shain et al., 2003; Xiao et al., 1998).

We find that the recruitment of microglial cells and astrocytes achieves a plateau around 2 weeks and decreases at week 3 and stays at a very low cell density for at least 4 weeks. In week 2 the microglial cells are the most abundant cells which are different from what is seen at other time points and in the normal non-pathological brain (Wikinson et al., 2006). This result supports the idea that microglial cells are the main cells involved in brain inflammation after implantation (Beschoner et al., 2002). Substantial numbers of microglial cells and astrocytes are found in the implantation site for at least 4 weeks. The long term retention of microglial cells and astrocytes suggest that both cells play important roles in both acute and chronic foreign body reactions. This assumption is supported by many early studies (Zhong et al., 2007; Aloisi et al., 2000; Streit et al., 1999). These studies have observed that microglial cells and astrocytes are involved in capsule formation around implant (Zhong et al., 2007; Aloisi et al., 2000; Streit et al., 1999). There is little known about the potential interaction between microglial cells and astrocytes (Streit et al., 1999). However, it is known that cytokines

and growth factors are important mediators of inter-glia communication (Streit et al., 1999).

Microglial cells are involved in control of neural apoptosis and synaptic properties after injury caused by implantation or disease (Bessis et al., 2007). They are usually in quiescent or inactivated mood, after activation they proliferate and express Fas ligand (FasL) and secrete some cytokines including Tumor necrosis factor- α (TNF- α) and interleukin-1 β (IL-1 β) to promote neurons death (Bessis et al., 2007). In fact, recent study has found that the administration of IL-1 receptor antagonist substantially reduce the neural death following injury (Lozada et al., 2005). One of the most remarkable properties of microglia is to respond to signals from other cells (signals from stressed or damaged neurons or signals from T-cells) and direct their response to repair the tissue or conduct a protective immune reaction (Aloisi 2001). Based on some in vitro studies, microglia is considered the major CNS source of cytokines that stimulate humoral and cell-mediated immune responses. These cytokines include IL-6, IL-18, IL-12 and IL15 (Frei et al., 1989; Becher et al., 1996; Aloisi et al., 1997 and Hanisch et al., 1997).

One of the characteristics of astrocytes is their powerful response to various neurological stimuli, which induce their proliferation and activation. Astrocytes are activated after injury and express an intermediate filament cytoskeletal protein called Gliall Fibrillary Acidic Protein (GFAP) (Hirano et al., 2004). Astrological responses to brain damage happen rapidly within hours after injury (Eddleston et al., 1993). Prominent astrocytosis is seen in AIDS dementia, Alzheimer and brain injury (Eddleston

et al., 1993). Chondroitin sulfate proteoglycans (CSPGs) such as NG2, neurocan, versican, aggrecan and brevican are upregulated after CNS injuries (Morgenster et al., 2002; Asher et al., 2002; Stiche et al., 1999; Iseda et al., 2003 and Eddleston et al., 1993). Some in vivo studies have shown that CSPG inhibits neurite growth by changing the properties of ECM. Astrocytes are responsible for production of CSPG molecules. Type IV collagen produced by activated astrocytes participate in glial scar formation. However; it is still controversial if collagen type IV promotes or inhibits regeneration of axons after injury (Hirano et al., 2004). Reactive astrocytes interact with ECM and other cells in CNS and influence blood immune cells recruitment by secreting different cytokines (Eddleston et al., 1993). Transforming Growth Factor- β 1 (TGF- β 1) is increased in reactive astrocytes after CNS stab wound (Eddleston et al., 1993). TGF- β 1 may attract fibroblasts into the lesion site and play an important role in angiogenesis in scar and tissue repair (Eddleston et al., 1993). Both nerve growth factor (NGF) and basic fibroblast growth factor (bFGF) secreted by astrocytes promote axonal regeneration and extension in some types of cultured neurons (Eddleston et al., 1993).

The brain has very few lymphocytes compared to other organs (Xiao et al., 1998; Wang et al., 1993 and Frohman et al., 1988). Monocytes also do not present in the normal pathological brain tissue they have been shown to migrate to the brain after injury induced by implantation (Beschoner et al., 2002). Our study finds that T-lymphocytes and monocytes migrate to the vicinity of the implant and reach their maximum population at around 2 weeks. The increase in inflammatory cells density can be due to the disruption of blood vessels and impairment of the BBB after implantation of neural

probes (Szarowski et al., 2003, Xiao et al., 1998; Wang et al., 1993 and Frohman et al., 1988). It should be noted that both T-lymphocytes and monocytes/macrophages decrease tremendously at week 3 and almost disappear at week 4. T-lymphocytes recruitment in acute phase of immune reaction to implants has been studied before (Fee et al., 2002; Ling et al., 2006 and Fournier et al., 2003). However, its recruitment pattern has never been studied for a long period of time. Their short term appearance suggests that both cells participate in the early stage of neural-mediated foreign body reactions. The function of monocytes and T lymphocytes in brain is mostly unknown. It has been suggested that monocyte cells wall off the implant after a time depending on the material and implantation site in brain. They are responsible for phagocytosis activities to remove the implant or separate it from the normal tissue (Aloisi et al., 2000 and Beschorner et al., 2002). Moreover they secrete some cytokines including interleukine-1 (IL-1), interleukine-6 (IL-6) and Tumor necrosis factor- α (TNF- α) which intensify the inflammation (Aloisi et al., 2000). T lymphocytes have been found to participate in many fibrotic reactions (Hubeau et al., 2004). It has been shown that T lymphocyte secretes IFN- γ to induce activation markers (CD45 and Fc receptor) and MHC II on microglial cells in the brain. These results strongly support that antigen presenting property of microglial cells are induced by interaction between T-lymphocytes and microglial cells (Aloisi et al., 2000 and ling et al., 2006).

Very excitedly, we have also uncovered that substantial numbers of mesenchymal stem cells (SSEA4+ and CD45-) cells were recruited to the tissues surrounding neural implants. Most of mesenchymal stem cells present are adjacent to the implant. The role

of mesenchymal stem cells at the implantation site has yet to be determined. Since mesenchymal stem cells can also be differentiated into variety of neural cells, it is possible that mesenchymal stem cells participate in glial scar formation by differentiation into astrocytes and microglial cells (Redmond, et al., 2007 and Alvarez-Buylla et al., 2002). This assumption is supported by several recent findings. Specifically, some stem cell markers have been found on glial cells in other studies (Buylla-Alvarez et al., 2002, Zhang et al., 1997 and Corti et al., 2002).

CHAPTER 3

THERAPEUTIC STRATEGIES TO REDUCE GLIAL SCAR FORMATION

3.1 Introduction

The molecular mechanism of foreign body reactions in brain is mostly undetermined. The lack of such knowledge hinders the development of neural probes with improved safety and biocompatibility. Based on the previous work (Aloisi et al., 2000 and Akassoglou et al., 2004), we have hypothesized that foreign body reactions in brain involve the following sequence of events: (1) shortly after implantation, host proteins adsorbed onto implant surfaces. (2) Phagocytes and T-lymphocytes are recruited to the implantation sites and then interact with the neo-epitopes displayed by adsorbed fibrinogen. (3) After interaction with adsorbed fibrinogen, immune cells are activated to produce many pro-fibrotic cytokines and growth factors. (4) Upregulation of tissue factors by adherent phagocytes leads to the formation of fibrin clots on implant surfaces, providing a foundation for fibroblast immigration. (5) The release of pro-fibrotic cytokines and growth factors by immune cells prompts microglial cells proliferation, collagen production and glial scar formation (Figure 13). Because lymphocyte responses and fibrin formation have been associated with glial scar formation, we thus hypothesize

that “By diminishing T-lymphocyte responses and fibrin accumulation, the extent of neural implant-mediated glial scar formation may be substantially reduced”.

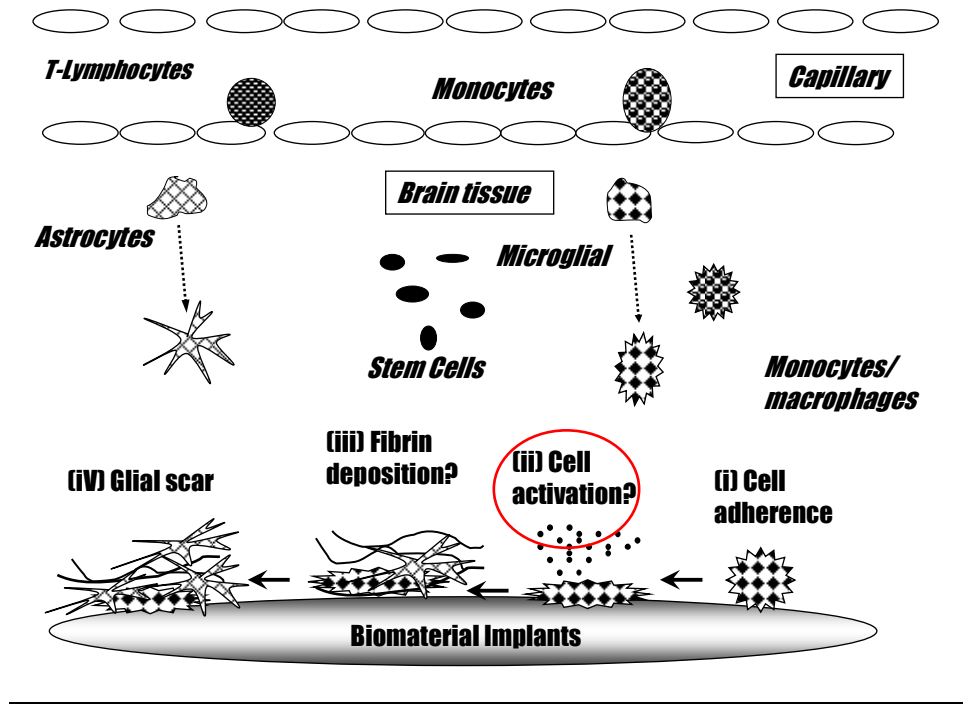


Figure 13 Schematic diagram of the possible mechanisms involved in foreign body reaction and glial scar formation in the brain post implantation.

3. 2 Study 1: Lymphocyte inactivation

Our study in the second chapter showed that there is a relatively high population of T-lymphocytes after implantation of neural probes (Figure 7, 9, 10 and 12). However, the potential role of T lymphocytes in foreign body reactions in the brain has not been determined. Using Severe Combined ImmunoDeficient (SCID) mice, our goal was to investigate the effect of T-lymphocytes on foreign body

reactions in the brain. This special characteristic of SCID mice has made it a very good model for many studies of inflammatory responses, immune reactions, and many diseases (Parham, 2005)

3.2.1 Materials and Methods

SCID mice have been widely established to have impaired T lymphocyte function.

Male SCID mice (Taconic Farm Inc., Germantown, NY) weighing 20 grams were used in this investigation. Stainless steel probes were implanted in the brain of SCID and control Balb/C mice for 2 weeks. The implantation procedures were identical to the methods described in Chapter 2.2.2. At the end of the study, mice were sacrificed and the brain tissues were isolated for histological analyses as mentioned in Chapter 2.2.2.

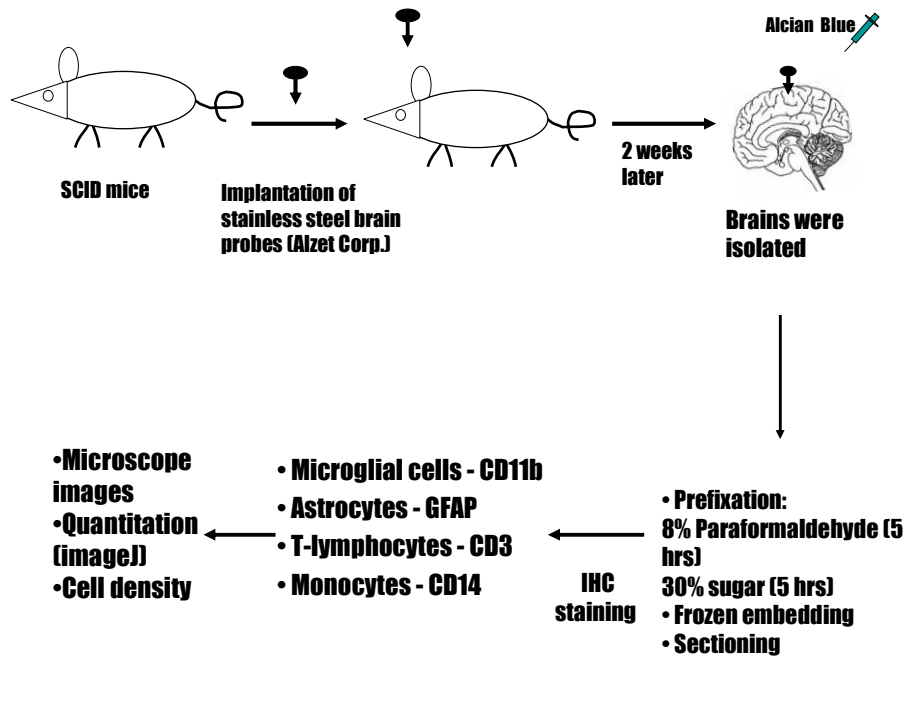


Figure 14 Schematic illustration showing the experimental surgical procedure in SCID

3.2.2 Results

Supporting the hypothesis, our results have shown that neural probes trigger substantially reduced foreign body reactions in SCID mice than in normal control (Balb/C mice). Specifically, we found that substantially fewer microglial cells and astrocytes were recruited to the implantation site in SCID mice than in control (Figure 15). However, T- lymphocyte deficiency does not affect the distribution pattern of microglial cells and astrocytes in the implantation sites.

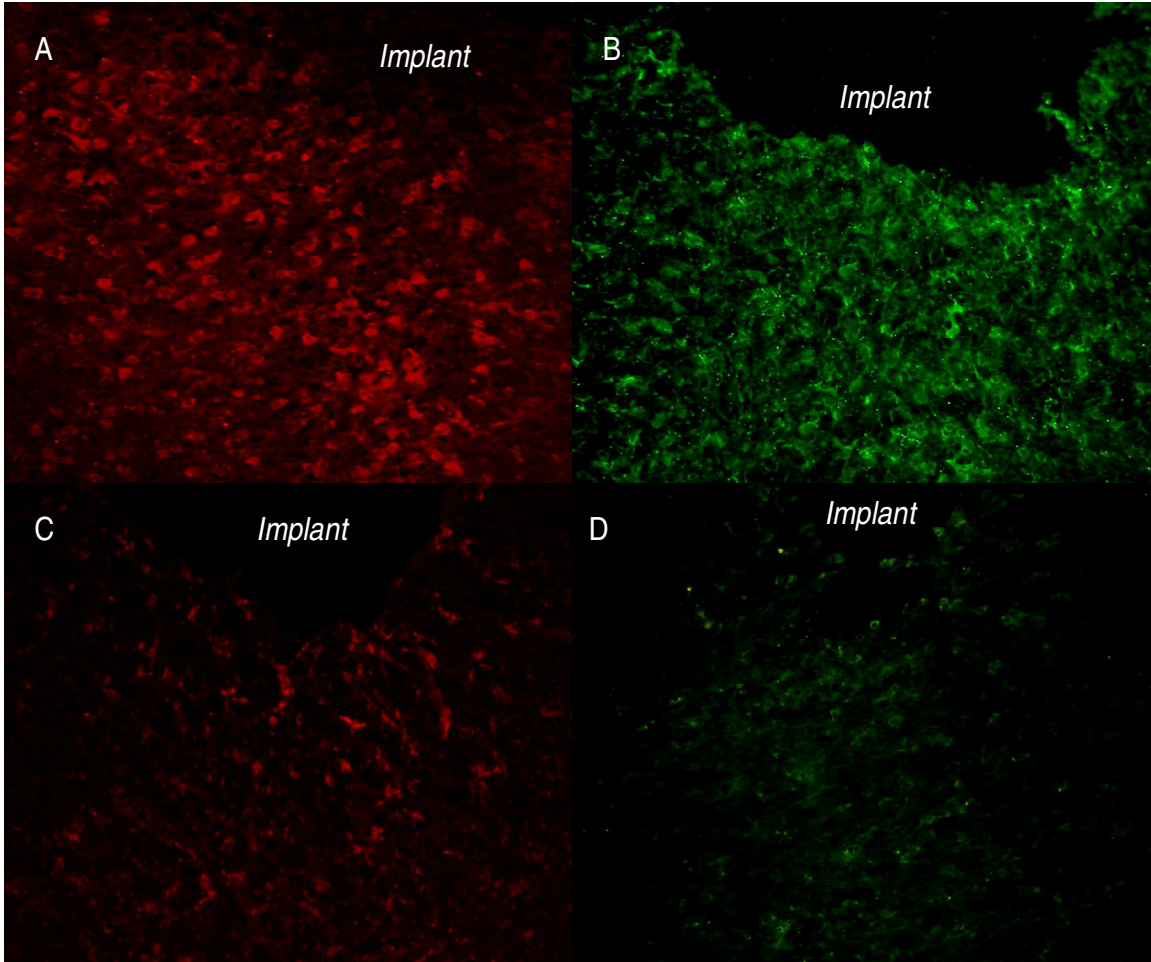


Figure 15 Accumulation of Microglia cells (CD11b+) and Astrocytes (GFAP+) in the brain tissue after 2-week implantation in Balb/C (control) and SCID mice. Substantially higher concentrations of microglial cells were found in Balb/C mice (A) than in SCID mice (B). Astrocytes in Balb/C (B) are also at a higher density than astrocytes in SCID mice (D).

The quantification of cell densities was carried out for both microglial cells and astrocytes. Both microglia and astrocyte populations are lower in SCID mice compared to Balb/C by a statistically significant margin. SCID mice have less than 30% and 25% of microglial cell and astrocyte accumulation compared to control animal, respectively (Figure 16).

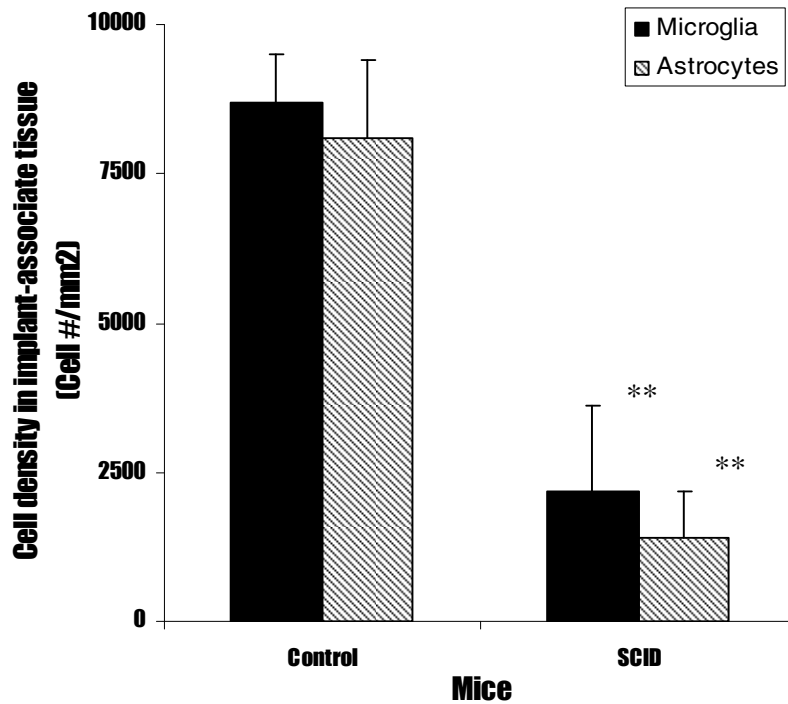


Figure 16 Cell density of Microglial cells (CD11b+) and Astrocytes (GFAP+) (Cell#/mm²) in neural probe-associated tissues of SCID mice and Balb/C mice (control) 2 weeks after implantation. Results are considered significant when $p < 0.05$. Vertical lines denote ± 1 SD ($n = 4$ for each group). Significance of differences among cell densities between SCID mice vs. control (Balb/C mice): **, $P < 0.01$.

T-lymphocytes are stained with CD3 (a cell surface marker) and Monocytes are stained with CD14 (a cell surface marker), both are then observed under green fluorescence. T-lymphocyte density in SCID mice brain tissue is much higher than control animal brain tissue (Figure 17). The increased accumulation of T-lymphocytes in SCID mice is rather unexpected, since under normal brain conditions there are very low densities of lymphocytes (Xiao et al., 1998; Wang et al., 1993 and Frohman et al., 1988). However, it should be noted that these T-lymphocytes have impaired immune function in SCID mice (Parham, 2005). On the other hand, the accumulation of monocytes/macrophages (CD14+) in SCID mice is much lower than in Balb/C after 2 weeks post-implantation (Figure 17).

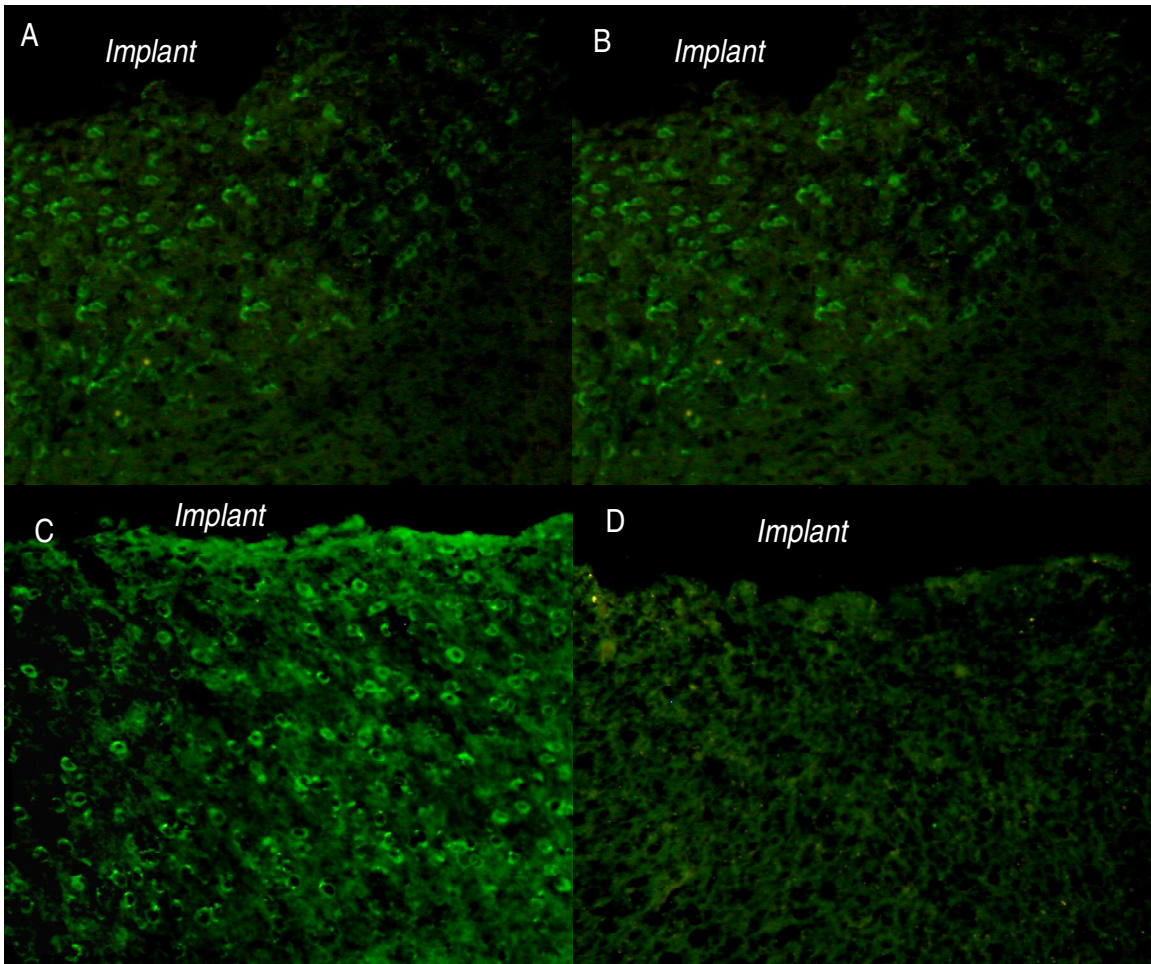


Figure 17 Accumulation of T lymphocytes (CD3+ cells) and Monocytes (CD14+ cells) in the brain tissue following 2-week implantation in Balb/C (control) and SCID mice. T-lymphocyte staining was carried out on tissue from Balb/C mice (A) and SCID mice (C). Monocytes/macrophages were also stained in tissue from Balb/C mice (B) and SCID mice (D).

Monocytes (CD14+) are much lower in SCID mice while T-lymphocytes (CD3+) are not. Monocyte population in SCID mice is less than 1/3 of monocyte in Balb/C mice and T-lymphocytes density is 3 times more in Balb/C mice at week 2 after implantation (Figure 18).

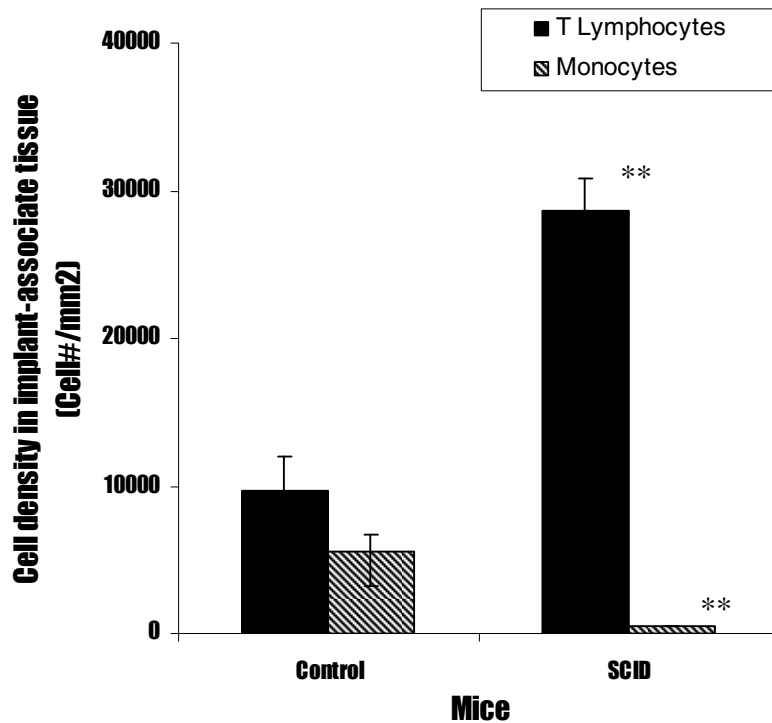


Figure 18 Monocyte (CD3+) and T-lymphocyte (CD14+) cell density (Cell#/mm²) in neural probe-associated tissues of SCID mice and Balb/C mice (control) 2 weeks after implantation. Results are considered significant when $p < 0.05$. Vertical lines denote ± 1 SD ($n = 4$ for each group). Significance of differences among cell densities between SCID mice vs. control (Balb/C mice): **, $P < 0.01$.

The influence of T lymphocyte function on mesenchymal stem cell recruitment was also evaluated histologically. The double stained tissues show

very few SSEA4+/CD45- (with green fluorescence) in the implantation sites in SCID mice (Figure 19-B) compared to Balb/C mice (Figure 19-A).

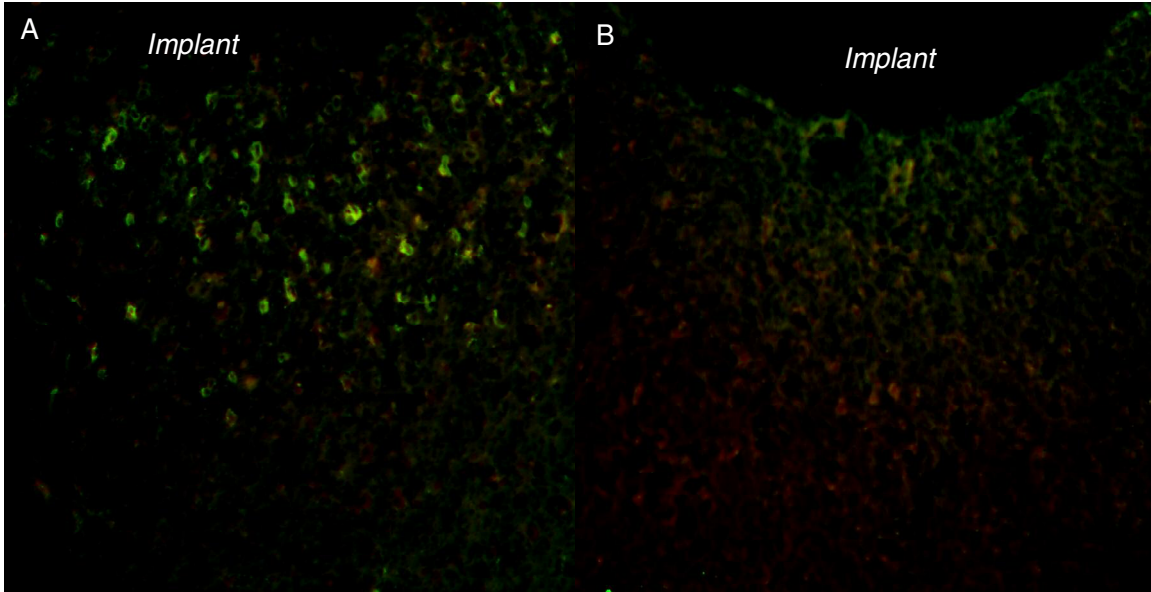


Figure 19 Presence of mesenchymal stem cells in brain tissues for 2 weeks after implantation. SSEA4 and CD45 were labeled with green and red fluorescence, respectively. Mesenchymal stem cells (SSEA4+/CD45-) are seen in this picture with green fluorescence. Many stem cells are seen in control (A) compared to SCID mice (B). Cells with yellow fluorescence (SSEA4+ CD45+) are leukocytes.

Mesenchymal stem cells density in SCID mice is less than 1/3 compared to Mesenchymal stem cells density in Balb/C. Its population in SCID mice is less than 1/3 of the microglial cells and astrocyte population (Figure 20).

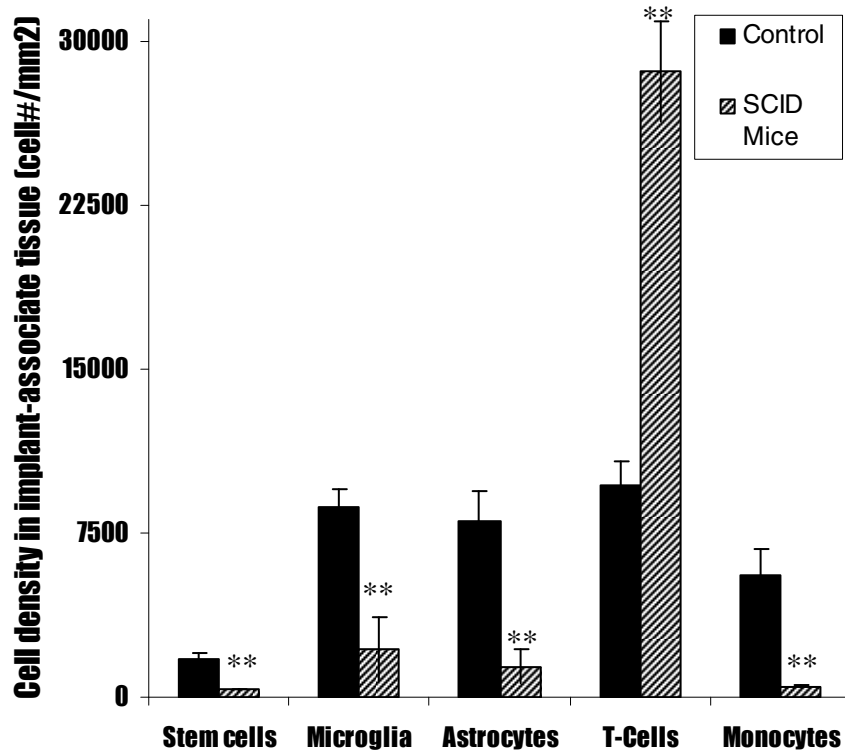


Figure 20 Density of mesenchymal stem cells (SSEA4+/CD45-), Microglia (CD11b+), Astrocytes (GFAP+), T-Lymphocytes (CD3+), and Monocytes (CD14+) in implant-associate tissue (cell#/mm²) of SCID mice compare to Balb/C mice (control) 2 weeks after neural probe implantation. Vertical lines denote ± 1 SD (n= 4 for each time point).

Comparing results of different cell densities in SCID mice (Figure 20) with Balb/C mice (Figure 12) also shows a significant decrease in mesenchymals stem cells, microglial, astrocytes and monocytes density. This still supports the idea that stem cell differentiation to other cells in the site of inflammation.

3.2.3 Discussion

Our results have shown that SCID mice failed to prompt immune responses to neural implants. Specifically, microglial cell and astrocyte population is significantly lower in SCID mice compared to control. Monocyte numbers are reduced noticeably as well. The reduction of immune and microglial cell responses is likely caused by the impaired function of T lymphocytes, since null mice with defected T cells, show a remarkable reduction in microglial and astrocytes migration and proliferation in stab wound injury model (Wang et al., 2007). However, to the best of our knowledge, the mechanism in which T-lymphocytes interact with glial cells and other inflammatory cells in the brain is not completely known.

T lymphocytes have been found to play an essential role in immune responses in brain (Ling et al., 2006). The severity of inflammatory damages in the CNS is dependent on the density and localization of infiltrated T-cells (McGavern and Troung, 2004; McGaven et al, 2002). T-cells migration after injury induced by implant or disease increases the acute immune reactions (Ling et al., 2006). However evidence indicates that injury-directed migration of T-

lymphocytes would be beneficial in the chronic phase of brain healing processes (Serpe et al., 1999, 2000, 2003). This may be due to the secretion of multiple neurotrophic factors by T-lymphocytes which reduce the neural loss around injury sites (Hammarberg et al., 2000). TNF secreted by lymphocytes in acute immune reaction is deleterious. However; this cytokine may play a role in facilitating long-term recovery and histological repair after brain injury (Fee et al., 2003). T-lymphocyte secretes IFN- γ to induce activity in glial cells in the brain. This supports the interaction between T-lymphocytes and microglial cells (Aloisi et al., 2000 and ling et al., 2006) which may lead to scar formation.

Although the specific role of T lymphocytes in implant-induced glial scar formation has yet to be determined, many recent studies have revealed the important effect of T lymphocytes in the pathogenesis of various fibrotic diseases (Hubeau et al., 2004). They can also be involved in scar formation and implant encapsulation in the brain by secreting the cytokines including IFN- γ which activates glial cells (Ling et al., 2006 and Aloisi et al., 2000).

Contrary to the normal brain having low population of lymphocytes (Xiao et al., 1998; Wang et al., 1993 and Frohman et al., 1988), we found significantly higher populations of CD-3 positive cells (T-lymphocytes) present in the neural probe surrounding tissue in SCID mice. T lymphocytes in SCID mice tissue were homogenously spread. We failed to find any early report which document and explain this unique phenomenon. Our results suggest that these SCID mice T

lymphocytes possess “normal” migratory property in responding to inflammatory signals and lack of feedback inhibition lead to the over-population of T lymphocytes in SCID mice injured brain tissue. This hypothesis should be tested in the future work.

3.3 Study 2: Anticoagulant treatment

Anticoagulant drugs reduce fibrin deposition/formation and it is believed that can potentially reduce glial cells proliferation/ activation.

Fibrinogen is secreted by hepatocytes in liver and is one of the main proteins in blood. It has numerous biological functions including involvement in blood clotting. Thrombin cleaves fibrinogen and converts it to fibrin, the final product in clotting cascade, and plays a major role in blood clotting. Conversion of fibrinogen to fibrin plays a significant role in inflammation, infection, wound healing, and inhibition of regeneration process in some tissues (i.e.; muscle) (Schachtrup et al., 2007). Fibrinogen has a unique structure with several epitopes. It interacts with different receptors at different binding site (Schachtrup et al., 2007). Some studies have shown that depletion of fibrin decreases inflammation in the central nervous system for some diseases like multiple sclerosis (Akassoglou et al., 2003). Anticoagulant drugs (i.e. heparin) break the clotting cascade and stop the formation of fibrin.

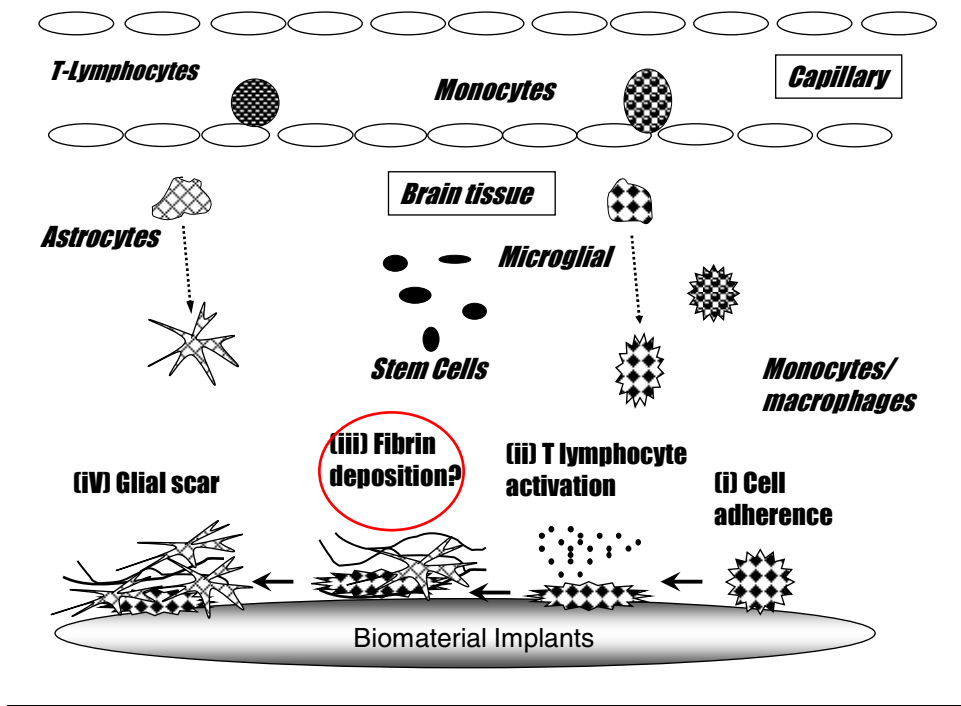


Figure 21 Schematic diagram of possible mechanisms involved in foreign body reaction and scar formation in the brain post implantation.

3.3.1 Materials and Methods

Animals: Four Balb/C mice were used in this experiment with weights between 25-30 g.

Heparin: Low molecular weight heparin (LMW-Hep) (Sigma) was used in this study. A solution of heparin in PBS was made with the concentration of 0.017

g/ml. All other materials were prepared as mentioned in chapter 2. The osmotic pumps were filled with drug and attached to a catheter tube. The osmotic pump delivered 4mg/kg every day for 14 day in each animal.

Surgery: The osmotic pumps were filled with drug and attached to a catheter tube. The osmotic pump delivered 4mg/kg per day (Pecly et al., 2006) for 14 day to each animal. Four Balb/C mice were subjected to the same surgical procedure and kept for 14 days. Animals were sacrificed after 14 days. All other experimental procedures are as mentioned previously in chapter 2.

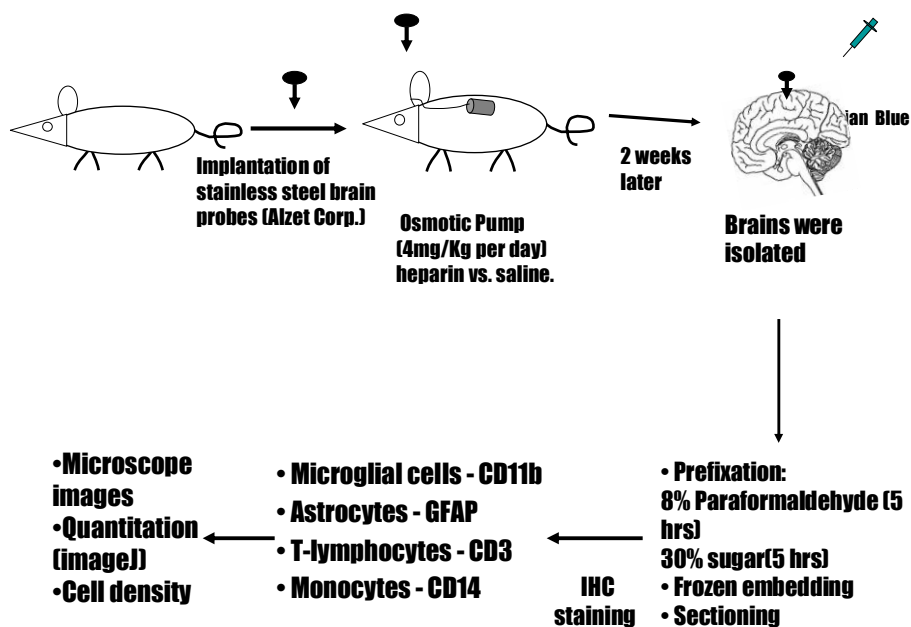


Figure 22 Schematic illustration showing the experimental surgical procedure in heparin treated mice

3.3.2 Results

Microglial cells and astrocytes recruit to the implantation site in heparin treated Balb/C mice the same as control animals with no treatment (Figure 23). However, they are much less, lower than 1/2 of their density, in heparin treated mice than control animals 2 weeks post implantation (Figure 23 and Figure 24). T-lymphocyte and monocyte density in animals with heparin treatment are much lower and are mainly located in areas close to the implant (Figure 25). They significantly decrease to almost 1/2 of their density in control animals with no treatment (Figure 24).

SSEA4+ cells are stained with green fluorescence and CD45+ cells are stained with red fluorescence. The double stained tissue shows very few SSEA4+/CD45- (mesenchymal stem cells (Gang et al., 2007) (green cells) close to implant (Figure 27). Comparing results of different cell densities in heparin treated Balb/C mice with Balb/C mice with no treatment (Figure 12 and Figure 28) we also see a significant decrease in mesenchymal stem cell, microglia, astrocyte, and monocyte density. This still supports the idea that stem cells can differentiate to any of those cells at the site of inflammation.

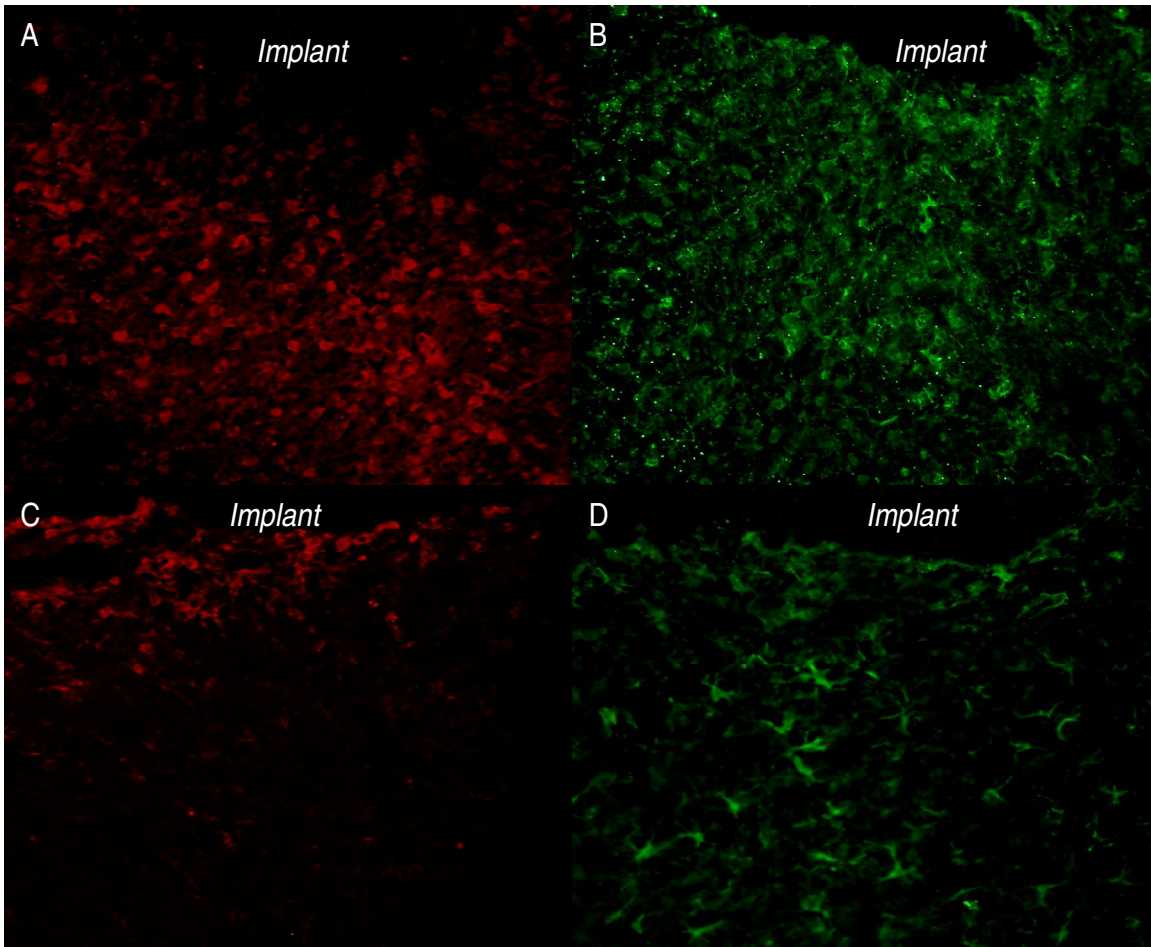


Figure 23 Microglia (CD11b+) and Astrocyte (GFAP+) accumulation 2 weeks post implantation in mice with heparin treatment compared to Balb/C mice (control). Higher concentrations of microglial cells were found in saline treated mice (A) than in heparin treated Balb/C mice (B). Astrocytes in control mice treated with saline (B) also have higher densities than astrocytes in heparin treated animal (D)

Quantification of cell densities shows that both microglia and astrocytes populations are statistically significant lower in Balb/C mice treated with saline compared to Balb/C treated with heparin. Heparin treated Balb/C mice have less than 25% and 20% of microglial cell and astrocyte accumulation, respectively (Figure 24).

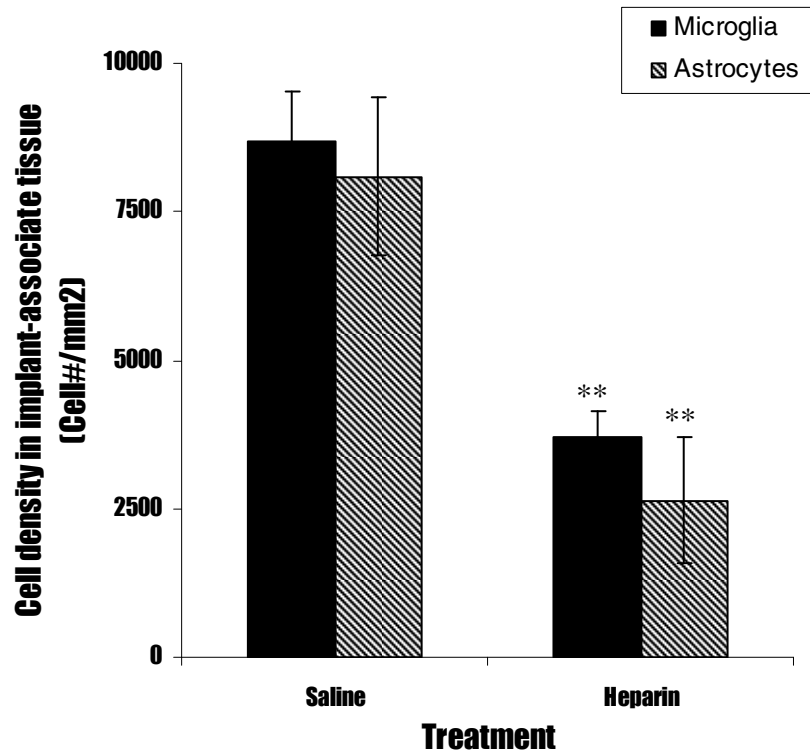


Figure 24 Microglial cells (CD11b+) and Astrocytes (GFAP+) cell density (Cell#/mm²) in neural probe-associated tissues of heparin treated Balb/C mice and saline Balb/C mice (control) 2 weeks after implantation. Results are considered significant when $p < 0.05$. Vertical lines denote ± 1 SD ($n = 4$ for each group). Significance of differences among cell densities between SCID mice vs. control (Balb/C mice): **, $P < 0.01$.

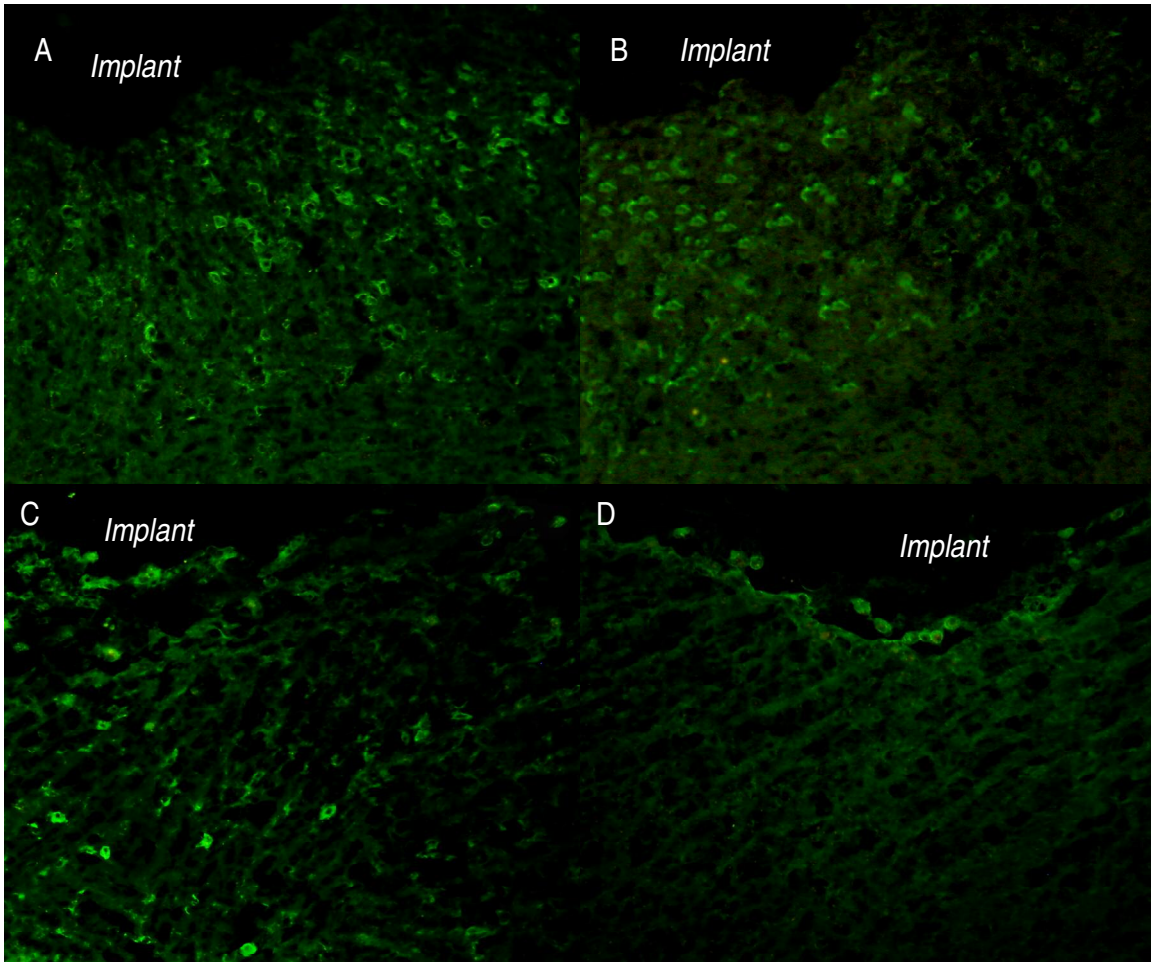


Figure 25 T lymphocytes (CD3) and Monocytes (CD14) 2 weeks post implantation in Balb/C mice with heparin treatment compare to Balb/C mice (control). (A) T-lymphocytes (CD3) in Balb/C (B) Monocytes (CD14) in Balb/C (C) T-lymphocytes (CD3) in Balb/C mice with heparin treatment (D) Monocytes (CD14) in Balb/C mice with heparin treatment.

T-lymphocytes are stained with CD3 (a cell surface marker) and monocytes/macrophages are stained with CD14 (a cell surface marker) and both are observed for green fluorescence. T-lymphocyte and monocytes/macrophages density in animals with heparin treatment are much lower compared to animals

with saline treatment and are mainly located close to the implant (Figure 25). T-lymphocyte and Monocyte density significantly decreased in animals with heparin treatment at week 2. The Inflammatory cell (both monocytes and t-lymphocytes) density in Balb/C with heparin treatment is almost 1/2 the density of that seen in control animals (Figure 26).

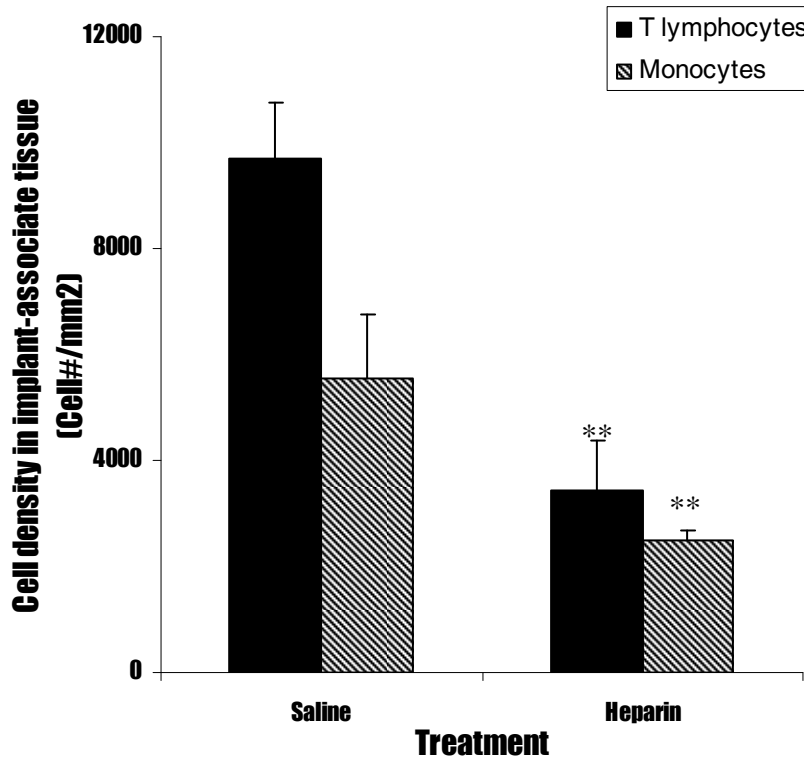


Figure 26 Monocyte (CD3+) and T-lymphocyte (CD14+) cell density (Cell#/mm²) in neural probe-associated tissues of heparin treated Balb/C mice and saline treated Balb/C mice (control) 2 weeks after implantation. Results are considered significant when $p < 0.05$. Vertical lines denote ± 1 SD ($n = 4$ for each group). Significance of differences among cell densities between heparin treated mice vs. control (saline treated): **, $P < 0.01$.

Mesenchymal stem cell recruitment was also evaluated histologically after heparin treatment. The double stained tissues show very few SSEA4+/CD45- (with green fluorescence) cells at the implantation sites in heparin treated mice (Figure 27-B) compared to saline treated mice (Figure 27-A).

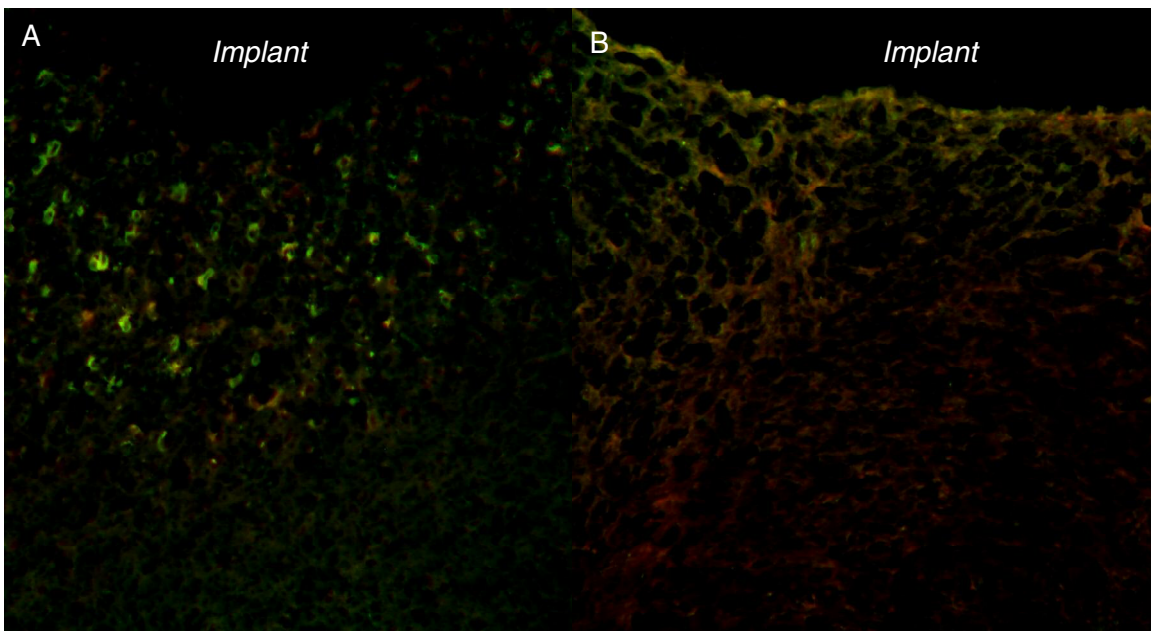


Figure 27 Mesenchymal stem cells in brain tissues for 2 weeks after implantation were studied histologically. SSEA4 and CD45 were labeled with green and red fluorescence, respectively. Mesenchymal stem cells (SSEA4+/CD45-) are seen in this picture with green fluorescence. Many stem cells are seen in saline treated Balb/C mice (A) compared to heparin treated Balb/C mice (B).

Comparing the results of different cell densities in Balb/C mice treated with heprin to Balb/C treated with saline (Figure 28), we see a significant decrease in mesenchymal stem cell, microglia, astrocyte, and monocyte density. This result supports the idea that stem cells can differentiate to any of the other cells at the site of inflammation. Mesenchymal stem cell density in heparin treated Balb/C mice is very low, less than 1/3 of microglial and astrocyte populations (Figure 28).

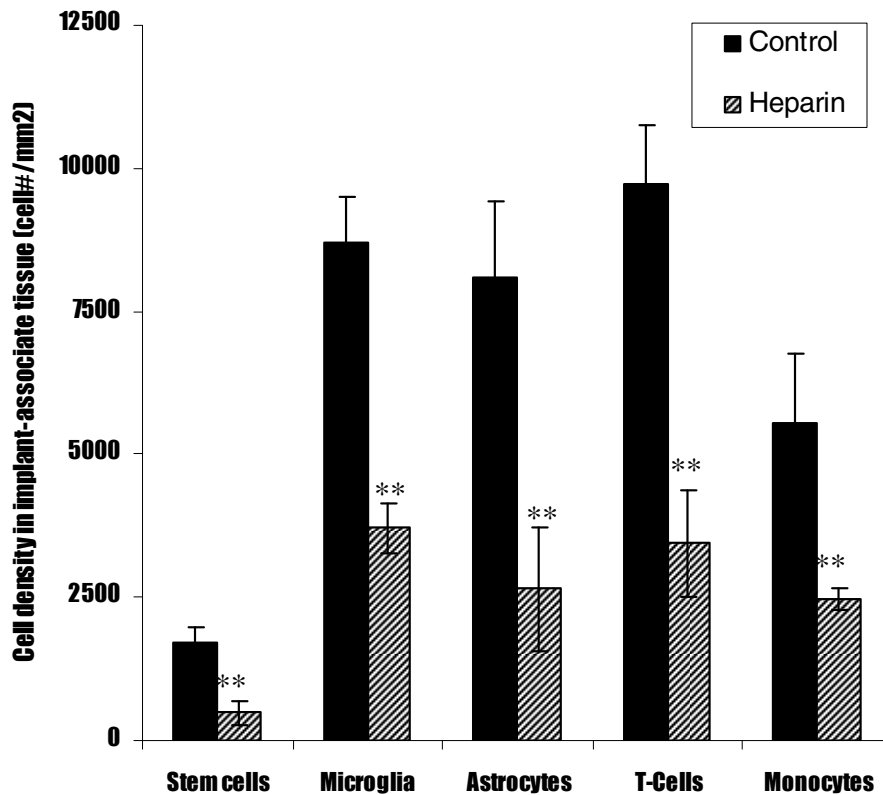


Figure 28 Mesenchymal stem cell (SSEA4+/CD45-), Microglia (CD11b+), Astrocyte (GFAP+), T-Lymphocyte (CD3+), and Monocyte (CD14) densities in implant-associated tissue (cell#/mm²) for heparin treated Balb/C mice 2 weeks post implantation. Vertical lines denote ± 1 SD (n= 4 for each time point): **, P<0.01.

3.3.3 Discussion

It is well established that fibrin deposition plays a significant role in inflammation, infection, wound healing, and inhibition of regeneration processes in many tissues (Schachtrup et al., 2007; Akassoglou et al., 2003). The ability of fibrin to mediate a wide range of biological effects is because of its unique structure with several nonoverlapping binding sites for different receptors such as

integrin and cadherin (Adams et al., 2004; Mosesson 2005). Fibrin only presents in brain after disease or injury (Schachtrup et al., 2007; Akassoglou et al., 2003). It is believed that fibrin accumulation in brain regulates the neuron function during degenerative and repair processes caused by implantation or disease in the central nervous system (Schachtrup et al., 2007). Different studies in the nervous system demonstrated that fibrin exacerbated sciatic nerve degeneration (Akassoglou et al., 2000) and promotes Schwann cells differentiating to a nonmyelinating state in some diseases (Akassoglou et al., 2002). Some studies have shown that fibrinogen increases inflammation and inhibits the neurite outgrowth and is deposited massively in the spinal cord after the brain injury (Schachtrup et al., 2007; Akassoglou et al., 2003). Studies on MS disease have shown that fibrin mediates inflammatory demyelination (Adams et al., 2007 and Akassoglou et al., 2004).

Since fibrin has been found to promote inflammatory responses and inflammation may determine fibrotic reactions, we have assumed that the depletion of fibrin deposition reduce neural implant-mediated immune reactions and subsequent glial scar formation. (Akassoglou et al., 2003). Transformin growth factor- β (TGF- β) monoclonal antibody has been used in some studies to inhibit fibrin deposition (Logan et al., 1999). In this study we used an anticoagulant drug to reduce fibrin deposition. Indeed, local administration of heparin substantially reduces the recruitment and accumulation of glial cells (microglia and astrocyte) and immune cells (T lymphocytes and monocytes).

These results support the idea that fibrin deposition is essential to the foreign body reactions in brain. In addition, the local anti-coagulation, including the local release of anticoagulant or anti-coagulant coating, can be used to improve the biocompatibility and function of neural probes by reducing inflammatory cell recruitment and glial cell proliferation. However the exact step of glial scar formation in which it interferes or interacts with the cells is not completely known.

Fibrin has been shown to activate microglia (Adams et al., 2007) and astrocytes (Leyton et al., 2001) via interacting with integrin receptors. The recognition of fibrin by members of three major families of integrin expressed on leukocytes, macrophages and monocytes, points out the importance of this molecule in adhesion, migration and activation of the major cells of inflammation (Ugarova et al., 2001). In fact, the early work from our laboratory has shown that CD11b (microglial cells marker) interact with fibrinogen P1 and P2 epitopes and such interaction lead to inflammatory reactions (Hu et al., 2001 and Tang et al., 1996). Fibrin induces macrophages activation and proinflammatory cytokines (Perez et al., 1999; Perez et al., 1995 and Fans et al., 1993) and chemokines (Sans et al., 2001).

Many studies have found large accumulation of fibrin nearby or on brain implants. The cause for fibrin deposition is not well known. However, some studies have shown that some cytokines like TGF- β secreted by damaged neurons

and glial cells induce a significant increase in fibrin deposition and scar formation (Logan et al., 1999).

CHAPTER 4

OVERALL CONCLUSIONS

1. The stainless steel neural probe implantation in mice is a good animal model to study and to evaluate foreign body reactions to brain implants.
2. In agreement with many previous observations, microglial cells and astrocytes were recruited to the implant-surrounding tissue shortly after implantations. The maximal recruitment for both cells is established in two weeks. Microglial cells and astrocytes stay in the implantation sites for more than 4 weeks. Our results suggest that both microglial cells and astrocytes participate in both acute and chronic immune responses to neural implants.
3. Similar to the inflammatory diseases in other organs, T lymphocytes and monocytes/macrophages present in the injured tissue adjacent to the brain implants. Both types of cells reached their maximal density (cell#/mm²) in week 2 but almost disappear from the implantation sites in 4 weeks. The quick immigration and disappearance suggest that both immune cells predominately engage in acute immune responses to brain implants.

4. One of the novel discoveries of this investigation is that mesenchymal stem cells were recruited to the implantation sites. Although the potential roles of mesenchymal stem cells in glial scar formation have yet to be determined, it has been suggested that recruited stem cells may differentiate into astrocytes and neurons in responding neural injury (Redmond et al., 2007; Chen et al., 2005).
5. Our limited studies have also shown that the defect function of T lymphocytes dramatically reduced brain implant-associated immune responses. These results suggested that localized T lymphocyte modulation may be used to reduce glial scar formation to brain implants.
6. We have also discovered that the localized administration of anticoagulant, such as heparin, substantially reduced immune cell accumulation in responding to brain implants. These results support that anticoagulant coating may be developed to substantially improve the biocompatibility of neural implants.

CHAPTER 5

FUTURE PERSPECTIVE

1. Although our studies have shown that microglial cells and astrocytes are recruited to the implantation sites following neural probes placement. However, the function and activity of microglial cells and astrocytes has yet to be determined.
2. The presence of T lymphocytes and monocytes/macrophages in the implant-associated brain tissues indicate that both cells may participate in glial scar formation. The role and activity of immune cells in glial scar formation should be further investigated.
3. The recruitment of mesenchymal stem cells in injured brain tissues provide an interesting therapeutic tools in which mesenchymal stem cells can be purposely differentiated into functional neural cells for restored neural function.
4. Both localized lymphocyte inactivation and anticoagulant release coating may serve as novel strategy to improve the longevity and function of neural implants. More studies are needed to develop and to test such coating technology.

REFERENCES

- Adams, RA, Bauer J, Flick, MJ, Sikorski, SL, Nuriel, T, Lassmann, H, Degen, JL, Akassoglou, K. “The fibrin-derived gamma 377-395 peptide inhibits microglia and suppresses relapsing paralysis in central nervous system autoimmune disease”. *J Exp Med*: 204: 571-582, 2007.
- Akassoglou, K, Adams, R.A, Bauer, J, Mercado, P, Tseveleki, V, Lassmann, H, Probert, L and Stricklan, S. “Fibrin depletion decreases inflammation and delays the onset of demyelination in a tumor necrosis factor transgenic mouse model for multiple sclerosis”. *PNAS*: 101: 6698-6703, 2004.
- Akassoglou, K, Yu, W-M, Akpinar, P and Strickland, S. “Fibrin inhibits peripheral nerve remyelination by regulation Schwann cell differentiation”. *Neuron*: 33: 861-875, 2002.
- Akassoglou, K, Kombrinck, KW, Degen, JL and Strickland, S. “Tissue plasminogen activator-mediated fibrinolysis protects against axonal degeneration and demyelination after sciatic nerve injury”. *J Cell Biol*: 149: 1157-1166, 2000.
- Aloisi, F. “Immune function of microglia”. *Glia*: 36: 165-179, 2001.
- Aloisi, F, Serafini, B and Adorini, L. “Glial-T cell dialogue”. *J. Neuroimmunology*: 107:111-117, 2000.

- Aloisi, F, Simone, R.D, Columba-Cabezas, S, Penna, G and Adorini, L. “Functional maturation of adult mouse resting microglia into APC is prompted by granulocyte-macrophage colony-stimulating factor and interaction with Th1 cells”. *American association of immunologists*, 1999.
- Aloisi, F, Penna, G, Cerase, J, Menedez, Iglesias B, Adorini, L. “IL-12 production by central nervous system microglia is inhibited by astrocytes”. *J Immunol*: 159: 1604-1612, 1997.
- Alonso, G. “NG2 proteoglycan-expressing cells of the adult rat brain: possible involvement in the formation of glial scar astrocytes following stab wound”. *Glia*: 49: 318-338, 2005.
- Asher, R.A, Morgenstern, D.A, Sheare, M.C, Adcock, K.H, Pesheva, P, Fawcett, J.W. “Versican is upregulated in CNS injury and is a product of oligodendrocyte lineage cells”. *J. Neurosci*: 22: 2225-2236, 2002.
- Becher, B, Antel, JP. “Comparison of phenotypic and functional properties of immediately ex vivo and cultured human adult microglia”. *Glia*: 18: 1-10, 1996.
- Becher, B, Dodelet, V, Fedorowicz, V, Antel, JP. “Soluble tumor necrosis factor receptor inhibits interleukin-12 production by stimulated human adult microglial cells in vitro”. *J Clin Inv*: 98: 1539-1543, 1996.
- Beschorner, R, Nguyen, T.D, Gozalan, F, Pedal, I, Mattern, R, Schluesener, H.J, Meyermann, R and Schwab, J.M. “CD14 expression by activated parenchymal microglia/macrophages and infiltrating monocytes following human traumatic brain injury”. *Acta Neuropathol*: 103: 541-549, 2002.

- Bessis, A, Bechade, C, Bernard, D and Roumier, A. “Microglial control of neural death and synaptic properties”. *Glia*: 55: 233-238, 2007.
- Bjornsson, C.S, Oh, S.J, Al-Kofahi, Y.A, Lim, Y.J, Smith, K.L, Turner, J.N, De, S, Roysam, B, Shain, W and Kim, S.J. “Effects of insertion conditions on tissue strain and vascular damage during neuroprosthetic device insertion”. *J. Neural Eng.:* 3:196-207, 2006.
- Buylla-Alvarez, A, Seri, B and Doetsch, F. “Identification of neural stem cells in the adult vertebrate brain”. *Brain research bulletin*: 57(6): 751-758, 2002.
- Cui, X, Wiler, J, Dzaman, M, Altschuler, R.A and Martin, D.C. “In vivo studies of polypyrrole/peptide coated neural probes”. *Biomaterials*: 24:777-787, 2003.
- 8. Cui, X, Lee, V.A, Raphael, Y, Wiler, J.A, Hetke, J.F, Anderson, D.J and Martin, D.C. “Surface modification of neural recording electrodes with conducting polymer/biomolecule blends”.
- Cui, X and Martin, D.C. “Electrochemical deposition and characterization of poly(3,4-ethylenedioxythiophene) on neural microelectrode arrays”. *Sensors and Actuators B*: 89:92-102, 2002.
- Cizkova, D, Kakinohana, O, Kucharova, K, Marsala, S, Johe, K, Hazel, T, Hefferan, M.P and Marsala, M. “Functional recovery in rats with ischemic paraplegia after spinal grafting of human spinal stem cells”. *Neuroscience*•, 2007.
- Chen, N, Hudson, J.E, Walczak, P, Misiuta, I, Garbuzozova-Davis, S, Jiang, L, Sanchez-Ramos, J, Sanberg, P.R, Zigova, T and Willing, A. “Human umbilical cord blood progenitors: the potential of these hematopoietic cells to become neural”. *Stem cells*: 23: 1560-1570, 2005.

- Corti, S, Locatelli, F, Strazzer, S, Salani, S, Bo, R.D, Soligo, D, Bossolasco, P, Bresolin, S.N, Scarlato, G and Comi, G. P. “Modulated generation of neuronal cells from bone marrow by expansion and mobilization of circulating stem cells with in vivo cytokine treatment”. *Experimental Neurology*: 177: 443-452, 2002.
- Eddleston, M and Mucke, L. “Molecular profile of reactive astrocytes implications for their role in neurologic disease”. *Neuroscience*: 54: 15-36, 1993.
- Fee, D, Crumbaugh, A, Jacques, T, Herdrich, B, Sewell, D, Auerbach, D, Piaskowski, S, Hart, M.N, Sandor, M, Fabry, Z. “Activated/effector CD4+ T cells exacerbate acute damage in the central nervous system following traumatic injury”. *J. Immunol*: 136: 54-66, 2003.
- Fernandez, E, Mannino, S, Tufo, T, Pallini, R, Lauretti, L, Albanese, A and Denaro, L. “The adult paraplegic rat: treatment with cell graftings”. *Surgical Neurology*: 65:223-237, 2006.
- Fournier, E, Passirani, C, Montero-Menei, C.N and Benoit, J.P. “Biocompatibility of implantable synthetic polymeric drug carriers: focus on brain biocompatibility”. *Biomaterials*: 24: 3311-3331, 2003.
- Fournier, E, Passirani, C, Colin, N, Sagodira, S, Menei, P, Benoit, J.P and Montero-Menei, C.N. “The brain tissue response to biodegradable poly (methylidene malonate 2.1.2)-based microspheres in the rat”. *Biomaterials*: 27:4963-4974, 2006.
- Frei, K, Malipiero, UV, Leist, TP, Zinkernagel, RM, Scheab, ME, Fontana, A. “On the cellular source and function of interleukin 6 produced in the central nervous system in viral diseases”. *Eur J Immunol*: 19: 689-694, 1989.

- Friedmann, I, Yoles, E and Schwartz, M. “Thrombin attenuation is neuroprotective in the injured rat optic nerve”. *J. Neurochem*: 76: 641-649, 2001.
- Gang, E.J, Bosnakovski, D, Figueiredo, C.A, Visser, J.W and Perlingeiro, R. “Prospective isolation of MSC with SSEA-4”. *Blood*: 109: 1743-1751, 2007.
- Griffith, R and Humphery, D.R. “Long-term gliosis around chronically implanted platinum electrodes in the Rhesus macaque motor cortex”. *Neuroscience letters*: 406: 81-86, 2006.
- Hammarberg, H, Lidman, O, Lundberg, C, Eltaby, SY, Gielen, A.W, Muhallab, S, Svenningsson, A, Linda, H, Van Der Meide, PH, Cullheim, S, Olsson, T, Piehl, F. “Neuroprotection by encephalomyelitis: rescue of mechanically injured neurons and neurorephin production by CNS-infiltrating T and natural killer cells”. *J. neurosci.* : 20: 5283-5291, 2000.
- Hanisch, U-k, Lyons,,SA, Prinz, M, Nolte, C, Weber, JR, Kettenman, H, Kirchhoff, F. “Mouse brain microglia express interleukin-15 and its multimeric receptor complex functionally coupled to Janus kinase activity”. *J Biol Chem*: 272: 28853-28860, 1997.
- Hirano, S, Yonezawa, T, Hasegawa, H, Hattori, S, Greenhill, N, Davis, P.F, Sage, E.H and Ninomiya, Y. “Astrocytes express type VIII collagen during the repair process of brain cold injury”. *Biochemical and biophysical research communications*: 317: 437-443, 2004.
- Hu, W-J, Eaton, J.W and Tang, L. “Molecular basis of biomaterial-mediated foreign body reaction”. *Blood*: 1231-1238, 2001.

- Iseda, T, Nishio, T, Kawaguchi, S, Kawasaki, T, Wakisaka, S. “Spontaneous regeneration of corticospinal tract after transaction in young rats: collagen type IV deposition and astrocytic scar in lesion site are not the cause but the effect of failure of regeneration”. *J. Comp. Neurol*: 464: 343-355, 2003.
- Kamath, S. “Influence of polymer surface chemistry on the recruitment of stem cells in mice.” M.S. Thesis, University of Texas at Arlington. 2006.
- Kerr, A.K, Liado, J, Shamblott, M.J, Maragakis, N.J, Irani, D.N, Crawford, T.O, Krishanan, C, Dike, S, Gearhart, J.D and Rothstein, J.D. “Human embryonic germ cell derivatives facilitate motor recovery of rats with diffuse motor neuron injury”. *The J. Neuroscience*: 23(12): 5131-5140, 2003.
- Lee, J, Jallo, G, Penno, M.B, Gabrielson, K.L, Young, G.D, Johnson, R.M, Gillis, E.M, Rampersaud, C, Carson, B.S and Guarnieri, M. “Intracranial drug-delivery scaffolds: Biocompatibility evaluation of sucrose acetate isobutyrate gels”. *Toxicology and applied pharmacology*: 215: 64-70, 2006.
- Leyton, L, Schneider, P, Labra, CV, Ruegg, C, Hetz, CA, Quest, AF and Born, C. “Thy-1 binds to integrin beta (3) on astrocyte and triggers formation of focal contact sites”. *Curr Biol*: 11: 1028-1038, 2001.
- Li, P.A, Kristian, T, He, Q.P and Siesjo, k. “Cyclosporin A enhances survival, ameliorates brain damage, and prevents secondary mitochondrial dysfunction after a 30-min period of transient cerebral ischemia”. *Experimental Neurology*: 165:153-163, 2000.

- Ling, C, Sandor, M, Suresh, M, Fabry, S. “Traumatic injury and the presence of antigen differentially contribute to T-cell recruitment in the CNS”. *J. Neurosci.* : 26: 741-731.
- Logan, A, Green, J, Hunter, A, Jackson, R and Berry, M. “Inhibition of glial scarring in the injured rat brain by a recombinant human monoclonal antibody to transforming growth factor- β ”. *European J. Neuroscience*: 11: 2367-2374, 1999.
- Lozada, A, Maegele, M, Stark, H, Neugebauer, E.M.A and Panula, P. “Traumatic brain injury results in mast cell increase and changes of central histamine receptors”. *Neuropathology and neurobiology*: 31:150-162, 2005.
- Marsala, M, Kakinohana, O, Yaksan, T.L, Tomori, Z, Marsala, S and Cizkova, D. “Spinal implantation of hNT neurons and neural precursors: graft survival and functional effects in rats with ischemic spastic paraplegia”. *European journal of Neuroscience*: 20:2401-2414, 2004.
- Matsumoto, H, Kumon, Y, Watanabe, H, Ohnishi, T, Shudou, M, Li, S, Takahashi, H, Imai, Y and Tanaka, J. “Antibodies to CD11b, CD68, and lectin label neutrophils rather than microglia in traumatic and ischemic brain lesions”. *J. Neuroscience Research*: 85(5): 994-1009, 2007.
- McGravern, DB, Troung, P. “Rebuilding an immune-mediated central nervous system disease: weighing the pathogenicity of antigen-specific versus bystander T cells”. *J. Immunol.* : 173: 4779-4790, 2004.
- McGravern, DB, Homann, D, Oldstone, MB. “T cells in the central nervous system: the delicate balance between viral clearance and disease”. *J. infect. Dis*: 186: S145-S151, 2002.

- Mosesson, MW. “Fibrinogen and fibrin structure and functions”. *J. Thromb Haemost*: 3: 1894-1904, 2005.
- Parham, P. “The immune system”. Edited by Goatly, B, Blake, L and Lawrence, E. Garland Science Press, NY: 70-71, 2005.
- Pais, T.F and Chatterjee, S. “Brain macrophages activation in murine cerebral malaria precedes accumulation of leukocytes and CD8+ T cell proliferation”. *J. Neuroimmunology*: 163: 73-83, 2005.
- Pecky, I.M.D, Goncalves, R.G, Rangel, E.P, Takiya, C.M, Taboada, F.S, Martinusso, C.A, Pavao, S.G and Maurilo, L.Jr. “Effects of low molecular weight heparin in obstructed kidneys: decrease of collagen, fibrinogen and TGF- β , and increase of chondroitin/dermatan sulfate proteoglycans and macrophages infiltration”. *Nephrology dialysis transplantation*: 21(5):1212-1222, 2006.
- Perez, RL, Ritzenthaler, JD and Roman, J. “Transcriptional regulation of the interleukin-1beta promoter via fibrinogen engagement of the CD18 integrin receptor”. *J Respir Cell Mol Biol*: 20: 1059-1066, 1999.
- Perez RL and Roman, J. “Fibrin enhances the expression of IL-1 beta by human peripheral blood mononuclear cells. Implications in pulmonary inflammation”. *J Immunol*. 154: 1879-87, 1995.
- Redmond, D.E, Bjugstad, K.B, Teng, Y.D, Ourednik, V, Ourednik, J, Wakeman, D.R, Parsons, X.H, Gonzalez, R, Blanchard, B.C, Kim, S.U, Gu, Z, Lipton, S.A, Markakis, E.A, Roth, R.H, Elsworth, J.D, Sladek, J.R, Sidman, R.L and Synder, E.Y. “Behavioral improvement in a primate parkinson’s model is associated with multiple homeostatic effects of human neural stem cells”. *PNAS*: 104: 12175-12180.

- Sans, E, Delachanal, E and Duperray, A. “Analysis the role of ICAM-1 in neutrophil transmigration using a reconstititional mammalian cell expression model: implication of ICAM-1 cytoplasmic domain and Rho-dependent signaling pathway”. *J. Immuno:* 166: 544-551, 2001.
- Schachtrup, C, Lu, P, Jones, L.L, Lee, J.K, Sachs, B.D and Zheng, B. “Fibrinogen inhibits neurite outgrowth via $\beta 3$ integrin-mediated phosphorylation of the EGF receptor”. *PNAS:* 104(28): 11814-11819, 2007.
- Serper, CJ, Kohm, AP, Huppenbauer, CB, Sanders, VM, Jones, KJ. “Exacerbation of facial motoneuron loss after facial nerve transaction in severe combined immunodeficient (scid) mice”. *J Neurosci:* 19: RC7: 1-5, 1999.
- Serper, CJ, Kohm, CB, Sanders, VM, Jones, KJ. “Kinetics of facial motoneurons loss following facial nerve transaction in severe combined immunodeficient (scid) mice”. *J. Neuroscie Res:* 62: 273-278, 2000.
- Serper, CJ, Coers, S, Sanders, VM, Jones, KJ. “CD4+ T, but not CD8+ or B lymphocytes mediate facial motoneurons survival after facial nerve transaction”. *Brain Behav Immunol:* 17:393-402, 2003.
- Shain, W, Spataro, L, Dilgen, J, Haverstick, K, Retterer, S, Isaacson, M, Saltzman, M and Turner, J.N. ”Controlling cellular reactive responses around neural prosthetic devices using peripheral and local intervention strategies”. *IEEE Trans. Neural Syst. Rehabil. Eng.:*11:186-188, 2003.
- Spataro, L, Dilgen, J, Retterer, S, Spence, A.J, Isaacson, M, Turner, N.J and Shain, W. “DEX treatment reduces astroglia responses to inserted neuroprosthetic devices in rat neocortex”. *Exp. Neurol.:*194:289-300, 2005.

- Stauffer W.R and Cui, X. “Polypyrrole doped with 2 peptide sequences from laminin”. *Biomaterials*: 27:2405-2413, 2006.
- Stichel, C.C, Hermanns, S, Luhmann, H.J, Lausberg, H, Niermann, D, Servos, G, Hartwig, H.G, Muller, H.W. “Inhibition of collagen IV deposition promotes regeneration of injured CNS axons”. *Eur. J. Neurosci*: 11: 632-649, 1999.
- Streit, W.J, Walter, S.A and Pennel, N.A. “*Reactive microgliosis*”. *Progress in neurobiology*: 57: 563-581, 1999.
- Szarowski, D.H, Andersen, M.D, Retterer, S, Spence, A.J, Isaacson, M, Craighead, H.J, Turner, J.N, and Shain, W. “Brain responses to micro-machined silicon devices”. *Brain Res.*:983:23-35, 2003.
- Tang, L, Jennings, T.A and Eaton, J.W. “Mast cells mediate acute inflammatory responses to implanted biomaterials”. *Pro. Natl. Acad. Sci. USA*: 95: 8841-8846, 1998.
- Tang, L, Ugarova, T.P, Plow, E, Eaton, J. “Molecular determination of acute inflammatory responses to biomaterials”. *J. Clin. Inves*: 97: 1329-1334, 1996.
- Turner, J.N, Shain, W, Swarowski, D.H, Anderson, M, Martins, S, Issacson, M and Craighead, H. “Cerebral astrocytes response to micromachines silicon implants”. *Experimental Neurology*: 156:33-49, 1999.
- Ugarova, TP and Yakubenko, VP. “Recognition of fibrinogen by leukocyte integrins”. *Ann N Y Acad Sci*: 936: 368-85, 2001.
- Wang, Y, Moges, H, Bharucha and Symes, A. “Smad3 null mice display more rapid wound closure and reduced scar formation after stab wound to the cerebral cortex”. *Experimental Neurology*: 203: 168-184, 2007.

- Wadhwa, R, Lagenaur, C.F and Cui, X.T. “Electrochemically controlled release of dexamethasone from conducting polymer polypyrrole coated electrode”. *J. Controlled Release*: 110:561-541, 2006.
- Wilkinson, B and Landreth, G.E. “The microglial NADPH oxidase complex as a source of oxidative stress in Alzheimer’s disease”. *J. Neuroinflammation*: 3: 30, 2006.
- Wirenfeldt, M, Babcock, A.A, Ladeby, R, LAmbersten, K.L, Dagnaes-Hansen, F, Leslie, R, G, Q, Owens, T and Finsen, B. “Reactive microgliosis engages distinct responses by microglial subpopulations after minor central nervous system injury”. *J. Neuroscience research*: 82: 507-514, 2005.
- Xiao, B and Link, H. “Immune regulation within nervous system”. *J. Neurological science*: 157: 1-12, 1998.
- Zhang, S and Fedoroff, S. “Cellular localization of stem cell factor and c-kit receptor in the mouse nervous system”. *J. Neuroscience research*: 47: 1-15, 1997.
- Zhnong, Y, Bellamkonda, R, “Dexamehtasone-coated neural probes elicit attenuated inflammatory response and neural loss compared to uncoated neural probes”. *Brain Res.:* 1148:15-27, 2007.

BIOGRAPHICAL INFORMATION

Parisa Lotfi is born in Esfahan, a city in central area of Iran in 1974. She finished her high school in 1992. She got a bachelor in Pharmacy from Bahai Institute of Higher Education (BIHE) in Pharmacy in 2001. She started her master in Biomedical engineering in University of Texas at Arlington in 2005. She joined Dr Tang's lab in 2006 and finished her master's thesis in 2007. She is living in Arlington, TX.