CHAPTTER II
THEORETICAL BACKGROUND AND LITERATURE REVIEW

2.1 Electrospinning

Nanotechnology has become an interesting topic to scientists and engineers in recent years where they have established research areas in many countries. The reduction of size to nano-meter range bring an array of new possibilities in terms of materials properties, in particular with respect to achievable surface to volume ratios.

The production of nano-scale dimension materials, one of simplest technique possibilities to reach small size is the mechanical elongation of melt material and production of thin wire or fiber. This technique is electrospinning, provide materials to be high porosity specific surface area, process for introduced to fabricate microfiber or nanofiber mat, from polymer solution.

2.1.1 Electrospinning Theory

Electrospinning or electrostatic spinning is a simple technique which utilizes high electrostatic forces for fiber production. Electrospinning uses high voltage (about 10-30 kV) to electrically charge the polymer solution for producing ultra-fine fibers. There are basically three components in electrospinning setup, which essentially consists of a high voltage power supplier, a conducting capillary tip, normally a needle of a small inner diameter, and an earthed collection screen. In addition, a metering syringe pump can be used to control the flow rate of the polymer solution. The experimental set up of electrospinning process is shown in Figure 2.1.

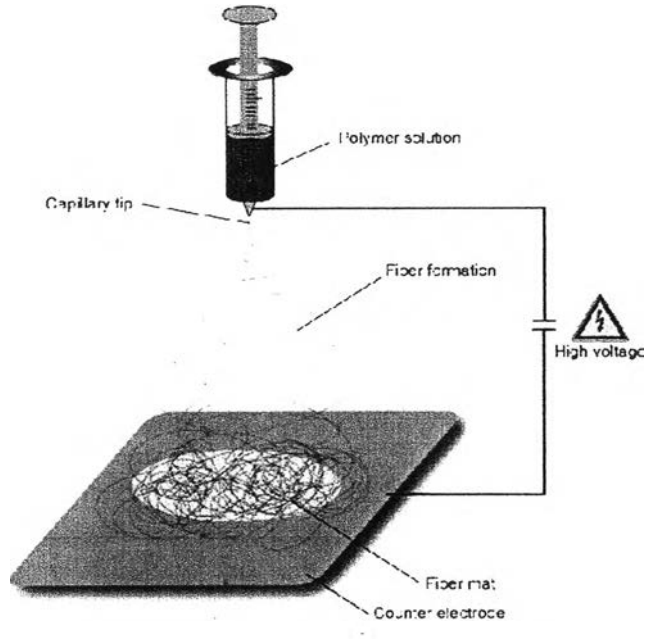

Figure 2.1 Schematic of the electrospinning set-up.

The influence of a strong electrostatic field, occurs when the electric forces at the surface of a polymer solution or melt overcome the surface tension and cause an electrically charged jet to be ejected. A thin liquid jet is observed to procure from an electrically charged pendent droplet. The use of electrostatic forces also leads to elongation of the liquid jet. The surface shape of charged droplet is called a "Taylor cone" and jetting will occur when critical angle is reached at the droplet tip. The charged liquid jet is submitted to a spiraling motion when accelerated towards the collector. Non-woven fiber mats is collected when solvent evaporates from the filaments leaving solid fibers to be formed.

2.1.2 Various Parameters

In general, the process of electrospinning is chiefly affected by two parameters, which are

1. *System parameter*, such as polymer molecular weight, molecular weight distribution and solution properties (e.g. viscosity, surface tension, conductivity).

2. *Process parameter*, such as flow rate, electrical potential, distance between capillary and collector, motion of collector.

2.1.3 Alignment Fibers and Fiber Collection Methods

The fibers can be collected on specifically designed collector systems as to obtain aligned fibers or array fibers. Aligned fibers have found many applications, such as tissue engineering, sensors, filters, electronic devices. Some commonly used techniques to produce aligned fibers are discussed in the subsection below.

2.1.3.1 Rotating drum collector

This method is commonly used to collect aligned fibers. Moreover, the diameter of the fiber can be controlled and tailored based on the rotational speed of the drum which is normally over 1000 rpm. The cylindrical drum is rotating at high speed and orienting the fiber boundaries where the linear rate of rotating drum should match the evaporation rate of the solvent.

2.1.3.2 Rotating disk collector

This method is a variant setup of the rotating drum collector and is used to obtain uniaxially aligned fibers. Disk collector over a drum is the most of fibers are deposited on the disk and are collected as aligned patterned nano-fibers (Yang, F *et.al.*, 2005).

2.1.4 Polymer Types

Electrospun materials are classified into two types.

1. Natural polymers, such as poly(lactic acid) (PLA), collagen, poly(ε-caprolactone) (PCL), poly(lactic-co-glycolic acid) (PLGA), and chitin or chitosan (Pakakrong, S., 2006) have high surface area to volume ratio and high porosity within the fiber mat.

In addition, characteristics of fibers have effect to applications for examples: Yang *et al.* (2005) produced nano/micro scale poly(lactic acid) scaffold in both aligned and random fibers for neural tissue engineering and found that both the fiber arrangements have influence on the cell behavior. The diameter also have influence on cell growth proliferation and differentiation. Jeong *et al.* (2005) produced e-spun collagen type I and found that collagen nanofibers showed good tensile strength. Bashur *et al.* (2006) produced poly(lactic-co-glycolic acid) scaffold and found that angular deviations of 31–60°

was effective to engineered ligament tissues and were promising materials for the repair of tears and ruptures.

2. *Synthesis polymers* conducting nanofibers such as polypyrrole (PPy), ployaniline(PANI), and poly(3,4-ethylenedioxythiophene) (PEDOT) were used as sensors to detect metal ions Fe^{3+} and Hg^{2+} (Dilek K *et.al.*, 1998).Furthermore, biosenser devices were created by integrating an enzyme into an electrode, and has been made to monitor and diagnosing metabolites (e.g., glucose, hormones, neurotransmitters, antibodies, antigens) for clinical purposes.

2.2 Conductive Polymers

Conducting polymers (CPs) are a group of organic materials that have both electrical and optical properties similar to metal and inorganic semiconductors. Structure of CPs have emerged aromatic in structure and exhibit good stabilities, good conductivities, and easy to synthesize.

Synthesis of conducting polymer can be carried out in two methods which are chemical or electrochemical.

1. Chemical synthesis including condensation polymerization, is a process carried out via small molecules such as hydrochloric acid or water,Furturemore addition polymerization, which used to process by radical, cation, anion polymerization.

2. Electrochemical synthesis is a common alternative for making CPs, by electrochemical polymerization. It is set-up equipmental by using a three-electrodes configuration (working, counter, and reference electrode) in monomer solution and electrolyte.

The difference between chemical and electrochemical synthesis is in the final product. Material that very thin, such as film with thickness of 20 nm, can be produced by using electrochemical technique, whereas powder or very thin film are typically produced by chemical polymerization (Gomez *et al.*, 2007). Each method has advantages and disadvantages which are summarized in Table 2.1

Table 2.1 Comparison of chemical and electrochemical CPs polymerization

Polymerization approach	Advantages	Disadvantages
Chemical polymerization	• Larger-scale production possible • Post-covalent modification of bulk CP possible • More options to modify CP back bone covalently	• Cannot make thin films • Synthesis more complicated
Electrochemical polymerization	• Thin film synthesis possible • Ease of synthesis • Entrapment of molecules in CP • Doping is simulaneous	• Difficult to remove film from electrode surface • Post-covalent modification of bulk CP is difficult

2.2.1 Conducting Polymer Used for Biomedical Applications.

Research on CPs used for biomedical application have expanded in many countries. Since materials were compatible with many biological molecules. Polypyrrole (PPy)-coated textile fabric was used in biosensors by studying for gas sensing capabilities. Conductivity changes found that increasing the cycle lifetime also increase the switching of time in PPy-coated textile f abrics can be using for reactive gas (Kincal *et al.*, 1998).

Apart from that, CPs were also shown in via electrical stimulation, to moderate cellular activity, including cell adhesion and migration. Specially, many of these studies involve nerve, bone, muscle, tissue engineering for examples:

Shi *et al.* (2004) produced electrically conductive biodegradable composite material of polypyrrole (PPy) nanoparticles and poly(d,l-lactide acid) (PDLLA) prepared by emulsion polymerization. The results showed growth of fibroblasts cell attached on the nanopaticles under applied voltages of 100 mV in direct current.

Martin *et al.*(2007) produced conducting polymer is poly(3,4-ethylenedioxythiophene) (PEDOT) that has ability to interact with neural cells by introduce process for polymerizing PEDOT around living cells. PEDOT coating on microelectrodes for hybrid conducting polymer-live neural cell electrode found that PEDOT can maintaining 80% cell attachment.

Lee *et al.* (2009) produced conductive meshes by coating polypyrrole (PPy) on random and aligned electrospun poly(lactic-co-glycolic acid) (PLGA) nanofibers, the study was to form electrically conductive nanofiber structures and to examine the combined effect of nanofiber structures and electrical stimulation. The result shows that PPy-PLGA electrospun meshes support the growth of rat pheochromocytoma 12 (PC12) cells and hippocampal neurons. Electrical stimulation studies showed that PC 12 cells stimulated with a potential 10 of mV/cm on PPy-PLGA scaffolds exhibited 40-90% longer neurite formation, whereas unstimulated cells on the same scaffolds exhibited neurite formation only 40-50% . In addition, stimulation of cells on aligned PPy-PLGA fibers resulted in longer neurites and more neurite-bearing cells than stimulation on random PPy-PLGA fibers, thus these scaffolds show potentials for neural tissue applications

Huang *et al.* (2009) applied electrical stimulation through polypyrrole/chitosan film not only enhanced the viability of Schwann cells but also significantly increase nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) level and protein expression.

Onada *et al.* (2009) fabricated PEDOT/PVA, and PPy/PVA composite films by electrochemical polymerization. The results suggestes that the materials are able to develop cell cuture system by introducing the fibroblasts cells on PPy-and PEDOT-coated ITO conductive glass plates for 7 days on the surface.

Previously, to realize those examples there are several conducting polymers suitable for biomedical application such as polypyrrole (PPy), Polyaniline (PANI), and poly(3,4-ethylenedioxythiophene) (PEDOT). Especially polypyrrole, due to it can support cell adhesion and growth in a number of different cell types, including rat pheochromocytoma (PC 12) cells, neurons and support cells (i.e.,

fibroblast). PPy, in its simplest unmodified form, can be synthesized to have small molecule anion as a dopant, ablitity optimize interactions with specific cell types and other materials due to non-covalent or covalent modification of polypyrrole.

In several studies have reveal the role of surface topography for CPs. For example, admicellar polymerization has been used to coat precisely control thickness polypyrrole. In further, refer admicellar polymerization technique for coated materials.

2.3 Admicellar Polymerization

Admicellar polymerization is an *in situ* polymerization reaction based on using physically adsorbing surfactant bilayer onto the substrate surface to apply a thin polymeric film.

There are four steps in admicellar polymerization: admicelle formation; monomer solubilization; polymeric film or *in situ* polymerization of adsolubilized monomer; and surfactant removal as illustrated in Figure 2.2

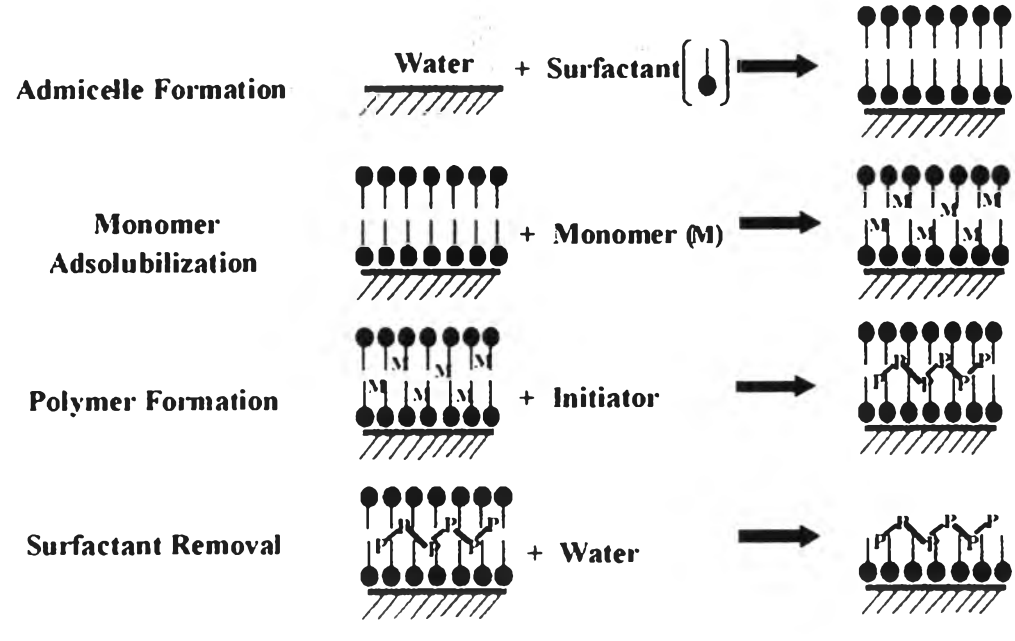

Figure 2.2 Schematic of the four steps of admicellar polymerization technique.

Step 1: Admicelle formation is adsorption of surfactant from aqueous solution to the surface is prevalent phenomenon. The initial feed concentration of surfactant is generally chosen close to or equal to critical micelle concentration (CMC) to avoid emulsion polymerization in the micelle and maximize admicelle formation. This is the concentration in region III of the typical adsorption isotherm of ionic surfactant on substrate (Figure 2.3). The point of zero charge (PZC) of the surfactant influences the choice of the surfactant. At pH below PZC, the surface becomes positive but it becomes negative at pH above PZC. Add salt can reduce the repulsion between head groups of surfactant and causes the surfactant molecules to come closer together, hence admicelle formation is enhanced.

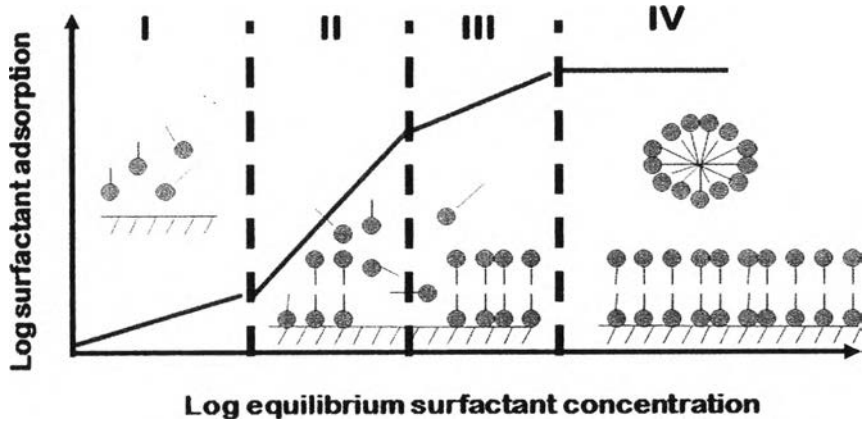

Figure 2.3 Typical adsorption isotherm of ionic surfactant on a substrate.

Step 2: Monomer solubilization is adsolubilization of monomer occur in the bilayer micelle. The monomers will diffuse from aqueous solution and solubilize in the hydrophobic interior of the micelle.

Step 3: Polymeric film or in situ polymerization of adsolubilized monomer in this step, the polymerization of the monomer takes place in the admicelle. Once the initiator is added, the polymerization is initialed in the admicelle that acts as two-dimensional reaction solvent for polymerization. The polymerization mechanism is similar to that occurs in the convention emulsion polymerization.

The polymeric film or *in situ* polymerization of adsolubilized monomer process is controlled by several parameters including the characteristics of the substrate surface, the type of surfactant and monomer, and the conditions used such as pH, and amount of electrolyte.

Step 4: *surfactant removal* is expose of the films by washing several times away excess surfactant and residual monomer.

Admicellar polymerization has been used in numerous studied to improve the interfacial adhesion of composite in various systems. For example:

Yuan *et al.* (1998) produced polypyrrole (PPy) coating via admicellar polymerization increase the conductivity of nickel-filled LDPE above the percolation threshold by approximately three orders of magnitude. This increasing may due to the formation of "molecular wires" created by polypyrrole entanglements at the particle-particle interface. These entanglements probably decrease the particle-particle contact resistance by increasing the contact area of the filler particles and may also reduce the tunneling resistance by decreasing film thickness. The large-scale structure of PPy-coated nickel flake are similar. The flake size is approximate 40 μm. The film-like and strand-like structures observed in STM image of PPy-coated nickel confirm the existence of PPy on the nickel flake. The machanical and thermal properties of the composite were not affected by the addition of PPy coating. The tensile modulus increased with increasing filler concentration, but the ultimate stress and elongation at break decreased with increasing filler concentration owing to poor polymer-filler adhesion.

Yuan *et al.* (2002) produced polypyrrole thin films were chemically synthesized from an aqueous solution using admicellar polymerization. Measuring film thickness when polypyrrole was deposited on mica and graphite could be directly measured by atomic force microscopy (AFM) across the film edge. The result found that chemically deposited films on mica using admicellar polymerization gave a film 50 nm thickness.

Bunsomsit *et al.* (2002) produced polypyrrole coating on latex particles by using admicellar polymerization. For preparation of an electrically conductive polymer found that the natural rubber (NR) latex exhibited a point of zero charge

(PZC) at 3.9 so used sodium dodecyl sulfate (SDS) as anionic surfactant, was adsorbed onto NR latex particles. The result chosen with solution pH adjusted to 3.0. Pyrrole caused a decreasing in surfactant adsorption. Suitable contents of SDS and pyrrole for admicellar polymerization were 16 and 10 mM, respectively. The presence of a small amount of salt, sodium chloride, substantially improved the surfactant adsorption and pyrrole adsolubilization. A PPy-coated NR latex prepared in the absence of surfactant exhibited slightly higher conductivity than a PPy-coated latex prepared with surfactant with or without salt.

Lekpittaya *et al.* (2003) prepared conductive polymer-coated fabrics by admicellar polymerization. By this method, a thin layer of conductive polymers, namely, polypyrrole, poly(N-mrthylpyrrole), polyaniline, and polythiophene were formed on cotton and polyester fabrics via a surfactant template. The effects of monomer concentration, oxidant to monomer ratio and addition of salt on the resistivity of the resulting fabrics were studied. The results showed that the apparent surface and volume resistivity decreased with increasing in monomer concentration in the range 5-15 mM. There was not much change in resistivity when oxidant to monomer ratio was changed from 1:1 to 2:1. Addition of 0.5 salt was found to reduce the resistivity significantly. The lowest resistivity obtained with polypyrrole coated fabric having resistivity around 10^6 ohm. SEM micrographs of the treated fabric surface showed film-like polymer coating confirmed that the fabrics were successfully coated by admicellar polymerization.

Rungruang *et al.* (2005) produced polypropylene coated on the surface calcium carbonate ($CaCO_3$) was successful by using admicellar polymerization. In this technique used propylene gas as monomer for adslublized in sodium dodecyl sulfate bilayers adsorbed on the surface of $CaCO_3$ particles using sodium persulfate for *in situ* polymerization as the thermal initiator. Studying in non-isothermal crystallization occurs that incorporation of $CaCO_3$ particles of various surface characteristic shifted the crystallization peak toward at high temperature. Investigating at two types of c rystallization exotherm was exhibited at the single-peak and double-peak types found that the double-peak type filled isotactic polypropylene (iPP) only 30 wt %of untreated $CaCO_3$ particles, was effected to self-nucleation of iPP crystallites entrapped along the rough surface of untreated

$CaCO_3$ patrticles. Surface treatment of $CaCO_3$ particles by steric acid coated or admicelle-treated found that effect to reduced ability of nucleation particles. The mechanical properties coated iPP on $CaCO_3$ patrticles was investigated. In-corporation of steric acid coated or admicelle treated $CaCO_3$ patrticles decrease in the yield stress.

Ren *et al.* (2008) synthesized 3-(4′-vinylbenzyl)-5,5dimethylhydantion (VBDMH) as monomer used to coat cotton fibers by via admicellar polymerization. The admicellar polymerization of the monomer on cotton was performed using cetyl trimethyl ammonium bromide (CTAB) solution as cationic surfactant and using potassium persulfate (PPS) as initiator. The coated thin-film on cotton was characterized by FTIR and SEM. The result showed after chlorination with dilute sodium hypochlorite, the polymeric coated cotton inactived in both *S.aureus* and *E.coli* O157:H7 in relatively short contact times. The thin-film coatings were very stable, and most of the chlorine which lost after washing could be regenerated, upon rechlorination were very good as evidenced by standard washing test.

Tragoonwichian *et al.* (2008) modified surface a cotton fabric by admicel-lar polymerization for used to Ultraviolet protection property. A UV-absorbing agent, 2,4-dihydroxybenzophenone, was covalently bonded to monomer, acryloyl chloride, and the product, 2-hydroxy-4-acryloyloxybenzophenone(HAB),was polymerized on the cotton surface using sodium dodecylbenzene sulfate as the surfactant. Surfactant bilayer adsorbed on the fiber surface was used as the reaction site for the formation of polymer film. Surface characterization studies confirmed the existence of the poly (HAB) thin film on the fiber surface which significantly reduced ultraviolet transmission through the fabric. Ultraviolet Protection Factor (UPF) of the cotton fabric was greatly improved from a value of 4 plain fabric to greater than 40 after treatment with HAB at concentrations greater than 12 mM using the admicellar polymerization technique.

Barraza *et al.* (2008) produced electrically conducting polypyrrole (PPy) nano films were deposited on insulating mica plates by admicellar polymerization. Higher conductances were found in PPy thin films made by using the two-line probe method by drawing two lines of silver glue 8 mm apart on the sample surface. The current voltage curves of bare mica surface yielded a lateral conductance of 6.0×10^{-13} S.

In comparison, PPy thin films made using sodium dodecyl sulfate (SDS) and cetyl trimethyl ammonium bromide (CTAB) as surfactant templated showed conductance of 1.2×10^{-11} S and 7.7×10^{-12} S respectively. Based on the average film thickness, the lower-bound conductivities of PPy-SDS-mica and PPy-CTAB-mica were estimated as 4.0×10^{-3} S/cm and 2.6×10^{-3} S/cm respectively.

Sangthong *et* al. (2009) coated Sisal fiber with a poly(methyl methacrylate) film for enhance the interfacial adhesion of the fiber/polymer composite for mechanical property improved by admicellar polymerization. PMMA film coating on sisal fiber surface leaded to improve mechanical properties of the sisal fiber/unsaturated polyester composite due to the improvement of the interfacial adhesion of the composite. The composite with the best mechanical properties can be obtained by using 30 vol% fiber loading with fiber length of 30 mm and an MMA concentration of 0.075% v/v.

Tragoonwichian *et al.* (2009) produced bifunctional cotton fabric by using double coating via repeat admicellar polymerization. Dodecylbenzenesulfonic acid sodium salt (DBSA) used as the surfactant. HAB was coated on a cotton fabric in order to improve its ultraviolet protection and then methacryloxymethyl-trimethylsilane (MSi) was coated to create a water-repellent surface. The result showed adsorbtion of DBSA on the HAB-coated substrate was found to be less than on the untreated surface. Hence, the doubly coated fabric provided an excellent UV protection with a UPF value of around 40 and very good water repellency with the maximum contact angle 115.4° the optimum amount of monomers was found to be 1.5 mM HAB and 5 mM MSi.

The admicellar polymerization method offers several advantages more than the other methods for surface coating. This process is simple with minimal chemical usage as the coated film is ultrathin, in nano-scale and also no need for organic solvent.

2.4 Nerve Regeneration

Nerve regeneration is complex biological phenomenon. The nervous system is difficult recovery when compare with the other part of body because the nature of neuron do not growth undergo cell division. Hence, to enhance efficiency neuron regeneration, appropriate focused on designing nerve guidance channels. For developing nerve tissue engineering have components to consideration are including scaffold for extracellular matrix, proliferation, supporting cells, and growth factors.

2.4.1 Physiology of Nerve System

The nervous system consists of two parts, peripheral nervous system (PNS) and central nervous system (CNS). That two parts are different in their physiology and function. The central nervous system (CNS) includes the brain and spinal cord that consists of a vast number of neuron, astroglia, microglia and oligodendrocytes that act to coordinate, recognize, initiate, propagate and process signals from external and internal stimuli whereas the peripheral nervous system(PNS) contains only ganglia, motor neurons, sensory nerve and the bundles of neuron for connecting the CNS to other parts of the body.

A peripheral nerve consists of motor and sensory axons bundled together by support tissue into an anatomically defined trunk. A peripheral neuron consists of a cell body and a long process, or axon, which may reach one meter in length. (Figure 2.4) Short segments of the axon are wrapped with an insulating myelin sheath formed by Schwann cells, which also serve several important roles in the axon-regeneration process. Axons are grouped together into fascicles, several of which are enclosed in the epineurium to form a peripheral nerve.

For CNS, both the spinal cord and the brain consist of white matter and gray matter. White matter is bundles of axons, each coated with a sheath of myelin. Gray matter is masses of the cell bodies and dendrites, each covered with synapses. In the spinal cord, the white matter is at the surface, the gray matter

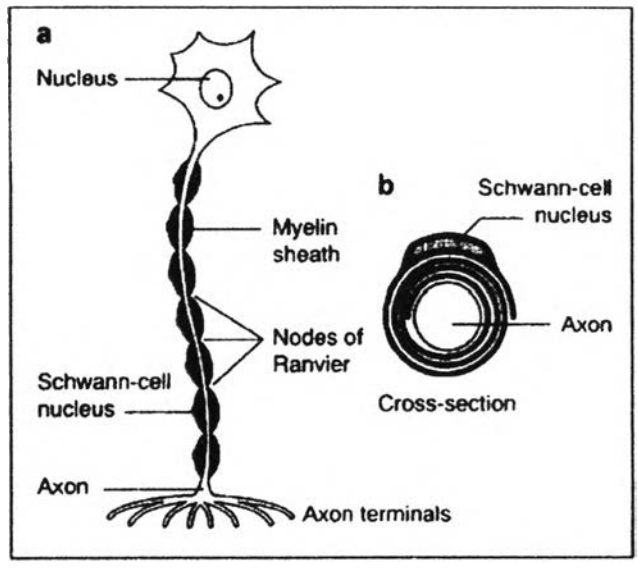

Figure 2.4 Peripheral motor neuron (a) the cell body (b) a cross section of the neuron.

2.4.2 Nerve Graft in Advance Fabrication Technique

In among to accurately mimic natural repair in the body, various fabrication techniques have been used to create three-dimensional channels or fiber structure. The fabricated properties morphology of nerve guidance channel have shown in Figure 2.5 a biodegradable and porous channel wall, the ability to deliver bioactive factors, the incorporation of support cell migration, intraluminal channels are included to mimic the structure of nerve fascicles, and electrical activities (Hudson *et al.*, 1998).

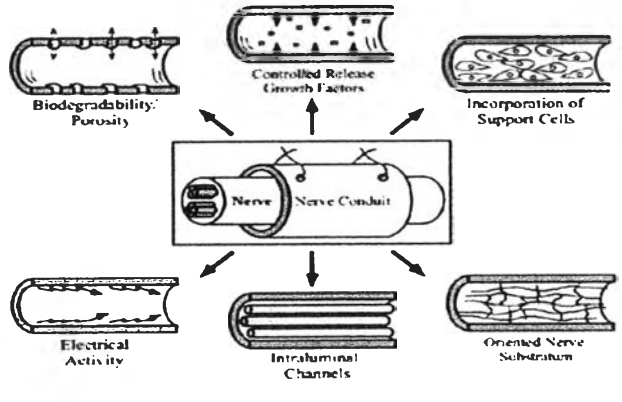

Figure 2.5 Properties of the ideal nerve conduits.

Another property which is important for nerve guidance channels is the alignment of polymer fiber, for example: Polymer blend of poly(ε-carprolactone)/ gelatin alignment nanofiber was exhibited to enhance the nerve stem (C17.2) cells and proliferation are neurite outgrowth on aligned nanofibrous scaffold is parallel to the direction of fibers (Ramakrisma *et al.*, 2008). Yang *et al.* (2005) studying in the efficiency of aligned poly (L-lactic acid) (PLLA) nano and micro fibrous scaffold by using electrospinning technique, The results of cell culture after 1 day showed that the direction of nerve stem cells elongation and its neurite outgrowth were parallel to the direction of PLLA fibers for alignment nano/micro scaffold.

2.4.3 Support Cells

Schwann cells produce many types of neurotropic factors. The factors effect to nerve regeneration . Four component of neurotrophin family are relevant in mammalian nervous system; NGF,BDNF, neurotrophin-3 (NT-3), and neurotrophin 4/5 (NT-4/5).

2.4.4 Extracellular Matrix

Extracellular matrix molecules such as laminin, fibronectin, and some of collagen, promote axonal extension, are excellent for incorporation into the lumen of guidance channels. The nerve grafts can place into molecules by physical coating, plasma treating, and chemical bonding through adhesion mediators.