CHAPTER II
LITERATURES REVIEWS

2.1 Electrospinning

2.1.1 Experimental Set Up

There are basically three components in electrospinning process: a high voltage supplier, a capillary tube with a pipette or needle of small diameter, and a metal collecting screen. In the electrospinning process, a high voltage is used to create an electrically charged jet of polymer solution or melt out of the pipette. Before reaching the collecting screen, the solution jet evaporates or solidifies, and is collected as small fibers. One electrode is placed into the spinning solution/melt and the other attached to the collector which is simply ground. The experimental setup of electrospinning process is showed in Figure 2.1. The electric field is subjected to the end of the capillary tube that contains the solution fluid held by its surface tension. This induces a charge on the surface of the liquid. Mutual charge repulsion and the contraction of the surface charges to the counter electrode cause a force directly opposite to the surface tension (Huang *et al.*, 2003).

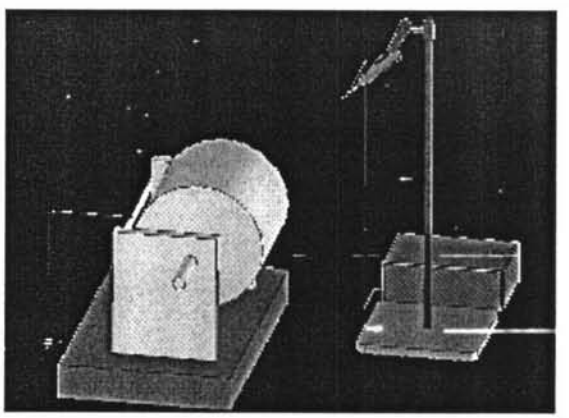


Figure 2.1 Schematic diagram to show polymer nanofibers by electrospinning

As the intensity of the electric field is increased, the hemispherical surface of the fluid at the tip of the capillary tube elongates to form a conical shape

known as the Taylor cone. Further increasing the electric field a critical valve is attained with which the repulsive electrostatic force overcomes the surface tension and the charged jet of the fluid is ejected from the tip of the Taylor cone. The discharged polymer solution jet undergoes an instability and elongation process, which allows the jet to become very long and thin. Meanwhile, the solvent evaporates, leaving behind a charged polymer fiber. In the case of the melt the discharged jet solidified when it travels in the air.

In general, the experimental setup can be divided into two types based on direction of polymer supplying nozzle including horizontally and vertically. The two kinds of polymer supplying nozzle are glass pipette and metal needle. For the experimental setup where glass pipette is used, the external potential was supplied by directly inserting a metal electrode to the capillary tube filled with a polymer solution (Reneker *et al.*, 1996).

Most nanofibers obtained so far are in non-woven form, which can be useful for relatively small number of applications such as filtration (Gibson et al., 1999), tissue scaffolds (Fertala et al., 2001), implant coating film (Buchko et al., 1999), and wound dressing (Jin et al., 2002). Efforts have been provided the way to made continuous single. A simple technique has been suggested that by rotating a cylinder collector at a very high speed up to thousands of rpm (round per minute), electrospun nanofibers could be oriented circumferentially. Researchers from Virginia Commonwealth University (Boland et al., 2001) have used this technique to obtain aligned electrospun poly(glycolic acid) (PGA) (at 1000 rpm rotating speed) and type I collagen (4500 rpm rotating speed) fibers.

2.1.2 Polymer Types

It has been reported in the open literature that more than fifty different polymers have been successfully electrospun into ultra fine fibers with diameters ranging from <3 nm to over 1 mm (Huang *et al.*, 2003). Several researchers investigated spinnibility of different polymers. Most of the polymers were dissolved in some solvents before electrospinning as the processing conditions involved are simple and straight forward.

It is noted that the same polymer dissolved in different solvents may all be electrospun into nanofibers. Common textile polymer such as nylon6 and

Nylon6 montmorillonite, high performance polymers such as polyimide (Reneker *et. Al.*, 1996), and biopolymer such as DNA have been electrospun (Fang *et. Al.*, 1997). Moreover, liquid crystalline polymer such as polyaramide and conductive polymers such as polyaniline have been also been reported as successful.

Polymer, molten in high temperature, can also be made into nanofibers though elestrospinning. Instead of a solution, the polymer melt into the sapillary tube, the electrosping process for a polymer melt has to be performed in a vacuum condition, for example, polyethylene (PE) (Larrondo et al., 1981), polypropylene (PP) (Broda et al., 2003)

2.1.3 Microstructure and Morphology

The microstructure and morphology of electrospun fibers have been investigated through scanning electron microscope (SEM), Viscometer, Tensiometer, Conductivity Meter.

2.1.4 Applications

Due to the high surface area to volume ratio, high porosity and light weight of electrospun fibrous webs, a number of applications have been sight out such as composite, filtration application and biomedical applications.

2.2 Nerve Regeneration

When the nerve system loss of functioning neuronal circuits, it has little capacity for self-repairing and neurons are not capable of proliferating in their native environment. The standard therapies use for the restoration of neuronal function is the autograft technique by taking nerve tissue from a donor site and grafted into the injured site. However, this method is frequently associated with donor site morbidity and incomplete functional recovery. Fortunately, recent advances in the neural tissue engineering (NTE) provide optimism by creating a permissive environment for nerve regeneration also known as nerve guidance channel.

A variety of biomaterials have been investigated for their suitability in tissue engineering application. The recent studies focus on the use of directional nerve guidance with various advanced techniques in order to create complex guidance

channels, which precisely mimic a natural repairing process in the human body. For example;

Borkenhagen and coworkers (1998) prepared nerve guide channel by using phase-segregated multiblock copolymer prepared by co-condensation of telechelic, low molar mass poly[(R)-3-hydroxybutyric acid-co-(R)-3-hydroxyvaleric acid]-diol (PHB, forming crystalline domains) and poly[glycolide-co-(e-caprolactone)]-diol (present in amorphous domains), coupled with aliphatic, 2,2,4 trimethylhexamethylene diisocyanate (TMDI) as chain extender. Tubular structures were fabricated by using melt extrusion technique. Nerve regeneration results show the regenerated tissue cables centrally within the channel lumen and composed of numerous myelinated axons and Schwann cells and no significant difference in the degree of regeneration was observed between the various channel types.

Midha et al. (2003) reported the in vitro processing and in vivo application of synthetic hydrogel tubes that are filled with collagen gel impregnated with growth factors. Poly(2-hydroxyethyl methacrylate-co-methyl methacrylate) (PHEMA-MMA) porous tubes were used to repair surgically created the rat sciatic nerve. The inner lumen of the tubes was filled with collagen matrix alone or matrix supplemented. Nerve regeneration was improved in tubes in which growth factors were added, compared with empty tubes and those containing collagen gel alone (negative controls). Studies are in progress to assess the long-term biocompatibility of these implants and to enhance regeneration further.

Vleggeert-Lankamp et al. (2004) compared the adhesion and proliferation of human Schwann cells (HSCs) on different substrates. Attachment is a prerequisite for the SCs to survive and fast proliferation will yield large numbers of SCs in a short time, which appears promising for stimulation of peripheral nerve regeneration. On all coatings, attachment of HSCs was satisfactory and comparable, indicating that this factor is not a major consideration in choosing a suitable coating. Proliferation was best on fibronectin, laminin and PDL, and worst on collagen type I and PEI. Since nerve regeneration is enhanced by laminin and/or fibronectin, these are preferred as coatings for synthetic nerve grafts seeded with SCs.

Yuan et al. (2004) aimed to find out more about the biocompatibility between chitosan and peripheral nerve system. Interaction between cultured SCs and

biomaterials is of importance. The experimental results indicated that SCs could grow onto chitosan materials with two different shapes: spherical and long olivary. They contacted with the extensions. The long olivary cells inclined to encircle chitosan fibers up. It was also found that the cells on the chitosan fibers migrated faster than those on the chitosan membranes. There was a good biological compatibility between chitosan and SCs. Compared with the chitosan membranes, SCs migrated more easily onto the stereoframe of chitosan fibers.

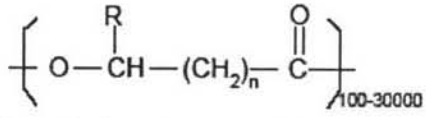
However, these advance technique as mentioned above take relatively long period of time to fabricate the nerve conduit. Thus, the electrospinning process seems to be the only method for fabricating fibrous scaffold which mimic the size of natural extracellular matrix (ECM). Recently, there are a few works on nanofibers electrospinnig scaffolds.

Bini *et al.* (2004) studied the preparation of electrospin poly(L-lactide-*co*-glycolide) biodegradable nanofibres as nerve guidance channels. The nanofibres were collected on to a rotating Teflon mandrel and fabricated to tubes or conduits. The nanofibre nerve guidance conduits were flexible, permeable and showed no swelling. The feasibility of *in vivo* nerve regeneration was investigated in the rat sciatic nerve model. After implantation of the nanofibre nerve guidance conduit, there was no inflammatory response and showed successful nerve regeneration.

Yang et al. (2005) studied the efficiency of aligned poly(L-lactic acid) (PLLA) nano- and microfibrous scaffolds on neural tissue outgrowth and compare their performance with random PLLA scaffolds by using electrospinning technique. The results of cell culture after 1 day show that the direction of NSC elongation and its neurite outgrowth is parallel to the direction of PLLA fibers for ANF and AMF scaffolds whereas it was random for RNF and no significant changes were observed on the cell orientation with respect to the fiber diameters. However, the rate of NSC differentiation was higher for PLLA nanofibers than that of microfibers and it was independent of the fiber alignment.

2.3 Hydroxyalkanoate Biopolyesters (PHAs)

One class of biodegradable polymer that has been receiving increasing amount of attention in the last 10 years is the hydroxyalkanoates. These are thermoplastic polyesters based on hydroxyalkanoic acids that are manufactured by bacteria as energy storage molecules. (Griffin 1994) They are fully biodegradable by bacteria. The most common of the PHAs is polyhydroxybutyrate (PHB) along with polyhydroxyvalerate (PHV), another member of this class of polymers. PHB properties are similar to polypropylene, but PHB is stiffer and more brittle. The general structures of polyhydroxyalkanoates are shown below.



n =1	R =	hydrogen	poly (-3-hydroxypropionate)
		methyl	poly (-3-hydroxybutyrate)
		ethyl	poly(3-horoxyvalerate)
		propyl	poly (-3-hydroxyhexanoate)
		pentyl	poly (-3-hydroxyoctanoate)
		nonyl	poly (-3-hydroxydodecanoate)
n = 2	R =	hydrogen	poly (-4-hydroxybutyrate)
n = 3	R =	hydrogen	poly (-5-hydroxyvalerate)

Figure 2.2 The general structure of polyhydroxyalkanoates.

2.3.1 Physical Properties of PHB / PHBV

As mentioned above, PHB has comparable mechanical properties to polypropylene. Table 2.1 below compares some key properties of PHB, PP, and PHBV with 25 mole% HV units. As mentioned before, it is possible to attain a range of propertics by adjusting the fraction HV.

2.3.2 Degradation of PHB/PHBV

Degradation of PHB/PHBV by bacteria is an enzymatically catalyzed pathway similar to a reversal of the synthesis pathway whereby the chain is broken back down to liberate stored energy and return to CO_2. This degradation can occur intracellularly or it can be catalyzed extracellularly by secreted enzymes.

Table 2.1 Comparison of some key properties of PHB/PHBV and polypropylene

Property	PHB	PHBV			PP
		3 mol%	14 mol%	25 mol%	
Melting point (°C)	175	169	150	137	176
Glass transition temp (°C)	15	-	-	-1	-10
Crystalline (%)	80	-	-	40	70
Young's modulus	3.5	2.9	1.5	0.7	1.7
Tensile strength (MPa)	40	38	35	30	34.5
Elongation to Break (%)	6	-	-	-	400
Impact strength (v/m)	50	60	120	400	45

2.3.3 Applications of PHB / PHBV

PHB and PHBV have been successfully used in bottles for shampoo and motor oil as well as in various personal hygiene items such as diapers and sanitary napkins. There is potential for these polymers to find uses in food packaging and beverage bottles where their good barrier properties would be an asset. PHBV coated paper for milk bottles has also been proposed. Medical applications, including the sutures, implants, and PHBV as a matrix for drug delivery, are other possibilities since the main breakdown product, hydroxybutyric acid, is a normal metabolic intermediate chemical already present in the human body.