# CHAPTER I
# INTRODUCTION

Tissue engineering is an emerging area in the contemporary human health care administration, in which the basic understanding of cellular biology and the application of bioengineering are harnessed together for developing feasible substitutes to aid in the clinical treatment. Nerve regeneration is a complex biological phenomenon. In the peripheral nervous system, nerves can regenerate on their own if injuries are small. Larger injuries must be surgically treated, typically with nerve grafts harvested from elsewhere in the body. Spinal cord injury is more complicated, as there are factors in the body that inhibit repair. Unfortunately, a solution to completely repair spinal cord injury has not been found. Thus, bioengineering strategies for the peripheral nervous system are focused on alternatives to the nerve graft, whereas efforts for spinal cord injury are focused on creating a permissive environment for regeneration. Fortunately, recent advances in neuroscience, cell culture, genetic techniques, and biomaterials provide optimism for new treatments for nerve injuries. [Schmidt C.E. *et al.*, 2003] A promising alternative for the repair of peripheral nerve injuries is the bioartificial nerve graft, comprised of a biomaterial pre-seeded with Schwann cells (SCs), which is an effective substrate for enhancing nerve regeneration. SCs appear to play an obligatory role in peripheral nerve regeneration by providing bioactive substrates on which axons migrate and release molecules that regulate axon proliferation. Interaction between cultured SCs and biomaterials is of importance.

A variety of biomaterials, in particular polymers, have been investigated for their suitability for applications in tissue engineering. The two basic components for structure supporting axonal migration, natural and synthetic materials are available. Natural materials appear to improve the biocompatibility, decrease the toxic effects and enhance the migration of support cells such as SCs. Although the use of these natural materials would be ideal, there are some inherent difficulties including undesirable immune responses, the potential for immuno-suppression and batch to batch variation in large scale isolation procedures. In an attempt to use natural materials, nerve grafts have been stored for prolonged periods of time. Because of the problematic potential of natural materials, synthetic materials have also been employed. Several synthetic materials, either nondegradable or biodegradable, have been used as a nerve conduit. The main objection for using nondegradable conduits is that they remain in situ as foreign bodies after the nerve has regenerated. A second surgery might then be necessary to remove the conduits, causing possible damage to the nerve. Therefore, biodegradable conduits seem to be a more promising apparatus to reconstruct nerve gaps. However, biodegradable conduits that degrade as a function of time may lose their functional capability as a structural cuff. Therefore, an ideal biodegradable conduit should maintain its structure integrity, permitting cell infiltration and subsequent tissue in growth during the regenerative processes.

Polyhydroxyalkanoate (PHA), a new class of microbial biopolymer, is a promising scaffold material due to its biocompatibility and biodegradability. Polyhydroxybutyrate (PHB) and polyhydroxybutyrate-*co*-hydroxyvalerate (PHBV) having complete biodegradability without any toxic byproducts can be biosynthesized by using a variety of microorganisms. These ecologically safe, environmentally friendly, polyesters have a high possibility to replace conventional non-biodegradable materials, and they have been extending their applications to surgical sutures, implant materials, drug carriers, and scaffolds for tissue engineering.

Ultra-fine polymer fibers exhibiting high surface area-to-volume and length-to-diameter ratios can be prepared via electrospinning. Due to such physical properties, these electrospun fibers are thought to enhance cell adhesion. Cell migration, proliferation, and differentiated function are dependant on adhesion and should be enhanced on an ultrafine fibrous structure. Such an ultrafine fibrous matrix could be formed by three methods self-assembly, phase-separation and electrospinning.

Electrospinning represents attractive approaches for polymer biomaterials processing with the opportunity for control over morphology, porosity, composition using simple equipment and attractive approach to the fabrication of fibrous biomaterials, which mimic the size scales of fibers composing the extracellular matrix of native tissues and organs. Hence this method represents an attractive approach to the fabrication of fibrous biomaterials for tissue engineering purposes.

In electrospinning, the polymer solution is placed in a container having a millimeter size capillary. When a strong electrostatic force is applied to the capillary, the polymer solution is ejected from the capillary and deposited as a non-woven fibrous structure on a template serving as the ground for the electric charges. The morphology of ultra-fine fibers produced by electrospinning strongly depends on (i) System parameters, such as polymer molecular weight, molecular weight distribution and solution properties (e.g. viscosity, surface tension, conductivity). (ii) Process parameters, such as flow rate, electric potential, distance between capillary and collector, motion of collector, etc. Therefore, these parameters should be carefully optimized while controlling fiber diameter and its alignment.

This work can be divided into two main experimental parts. The first is the electrospinning of Polyhydroxybutyrate (PHB) and polyhydroxybutyrate-*co*-hydroxyvalerate (PHBV). In this part, an attempt is made to fabricate biodegradable PHB and PHBV scaffolds for nerve regeneration application by using an electrospinning technique. The characteristics of the as-prepared solutions will be evaluated by a viscometer, a conductivity meter. Wettability of as spun scaffold is evaluated by contact angle. The effect of applied potential on morphological appear of as-spun fibers are thoroughly investigated by using scanning electron microscopy. Mechanical integrity of the fibrous scaffolds prepared will also be investigated. The second is study peripheral nerve regeneration by culture SCs on the scaffolds. In this part, the growth properties of SCs on fibers are investigated. The potential to use scaffolds in nerve guidance channel is evaluated in vitro by testing in cell biocompatibility, cell attachment and cell proliferation on scaffolds.