CHAPTER II

THEORY AND LITERATURE REVIEW

2.1 Biodegradable scaffold for tissue engineering

2.2.1 Tissue engineering

Tissue loss is one of the most common problems in human healthcare. Tissue engineering can be defined as the utilization of a combination of cells, engineered materials, and suitable biochemical factors to improve or replace biological functions in an effort to replace disfunctional tissues or organs [1,2]. A further description goes on to say that "understanding the principles of tissue growth, and apply this to produce functional replacement tissue for clinical use".

All of tissues and organs in our body are complexed biocomposites composed of microenvironments that include the extracellular matrix or ECM and cells. The ECM is composed of ground substance (i.e. proteoglycans) and fibrillar collagens which exist in three dimensional network structure in the order of 50-300 nm in diameter [3]. The functions of the ECM are to regulate cell activities, to provide mechanical support and act as main structural element of cell with an integrated vascular system for oxygen or nutrient supply. [3, 4, 5]

Tissue engineering solves problems about surgery by using living cells as engineered materials, such as fibroblasts as in artificial skins, and chondrocytes as in artificial cartilages. The cells are often categorized by their sources as follows.

- Autologous cells from the same body as that to which they will be reimplanted;
- Allogenic cells from another body;
- Xenogenic cells from another species.

Due to some disadvantage of invasive surgery such as limited availability of donor tissues and morbidity related to surgically chronic wound, three dimentional engineered materials have recently been used as scaffolds for tissue regeneration. In tissue engineering, attempts have been made to use a variety of

synthetic and natural polymers and many processing methods to fabricate fibrous scaffolds similar to the ECM structure.

2.1.2 Engineered materials

Tissue engineered materials are usually referred as scaffolds: artificial structures that are capable of support three-dimensional tissue formation from the seeding of cells while nutrient flow is maintained throughout the matrix [1,6]. Many different scaffolds (natural and synthetic, biodegradable and permanent) have been investigated. Most of these materials have been known in the medical field before the advent of tissue engineering as a research topic, e.g., bioresorbable sutures. These materials are mainly devided as metals, polymers, ceramics and composites. Some examples for metals are titanium, cobolt and chromium; for polymers, they are collagen, poly(methyl mathacrylic acid) or some linear aliphatic polyesters; for ceramics or inorganic materials, they are aluminium oxide and hydroxyapatite; and, for composites, they are a combination between inorganic and polymer materials [7].

A major goal of tissue engineering is to regenerate tissues and restore organ function through implantation of cells that can replace the natural ECM until the host cells can repopulate and resynthesize a new natural matrix [4].

To achieve the goal, scaffolds must have specific requirements that are

- Biocompatibility and biochemical factor delivery.
- A high porosity and an adequate pore size to facilitate cell diffusion throughout the whole structure of both cells and nutrients.
- Mechanically hardy to maintain 3-dimensional structure for regenerating tissue and to resist contractile force exerted by tissue formation.
- Material's surface should support the seeding, adhesion, and proliferation of cells or tissue formation.
- Enhance structural properties.
- Appropriate size and shape to match the target tissue.
- Exert certain mechanical and biological influences to modify the behaviour of the cell phase.
- Sterilization for inflection controll.

2.1.3 Biodegradable scaffolds

Biodegradability of scaffolds is essential for engineered tissues. It is enticing to have the materials that can be absorbed by surrounding tissues after therapeutic treatment time is reached without the need for surgical removal. In such an aspect, the degradation rate of the scaffolds has to match the rate of tissue formation. The most commonly used materials as scaffolds range natural biopolymers to synthetic polymers [1,6]. Natural biopolymers comprise collagen, demineralized bone matrix, agarose, elastine, chitosan, hyaluronan, and alginate. On the other hand, synthetic polymers include degradable polyesters, such as poly(glycolic acid) (PGA), poly(lactic acid) (PLA), and their copolymers, e.g., poly (lactic acid-*co*-glycolic acid) (PLGA). These are polyesters which degrade within the human body to form lactic acid, a naturally occurring chemical which is easily removed from the body. Similarly, a more hydrophobic polycaprolactone (PCL) can also be used, but its degradation period is slower than that of PGA, PLA, and PLGA. The slow degradation makes PCL less attractive for general tissue engineering applications, but more attractive for long-term implants and controlled release applications [6]. Furthermore, inorganic materials such as hydroxyapatite (HA) and β-tricalcium phosphate (TPC) which are components of hard bone tissues can be incorporated within polymer matrices to impart both the improved strength and osteoconductivity for further development as scaffolds for bone regeneration.

Recently, P. Wutticharoenmongkol *et al* [8] successfully fabricated ultrafine fibrous structure of polycaprolactone (PCL) containing calcium carbonate ($CaCO_3$) or hydroxyapatite (HA) by electrospinning technique. They evaluated the potential use of the electrospun fibrous scaffolds for in vitro bone regeneration by culturing human osteoblasts (SaOS2) on the scaffolds. The obtained results showed that all of the fibrous scaffolds exhibited much better adhesion and proliferation of cells than corresponding film scaffolds and polystyrene plates. Moreover, all of the fibrous scaffolds exhibited much greater tensile strength at yield than all of the corresponding film scaffolds.

2.1.4 Fabrication of biodegradable scaffolds

- **Solvent Casting & Particulate Leaching (SCPL)**: this technique allows the preparation of porous structures with regular porosity with a limited thickness. Firstly, the polymer has to be dissolved into a suitable organic solvent (e.g. poly(lactic acid) in dichloromethane), then the solution is cast into a mold filled with porogen particles, the small particles that can not be dissolved in both the polymer and the solvent. The porogen particles can be inorganic salts like sodium chloride, crystals of saccharose, gelatin spheres, or paraffin spheres. The size of the porogen particles will affect the size of the scaffold pores, while the polymer to porogen ratio is directly correlated to the amount of porosity of the final structure. After the polymer solution has been cast, the solvent is allowed to thoroughly evaporate, then the composite structure in the mold goes into leaching process by immersion in a liquid that is suitable for dissolving the porogen, such as water in case of sodium chloride, saccharose, and gelatin or an aliphatic solvent like hexane for paraffin. Whenever the porogen has been completely dissolved, a porous structure is obtained. Disadvantages of this method are getting the small thickness range of scaffolds and using of organic solvents which must be completely removed to avoid any possible toxic to cells seeded on scaffold.

H. Terai *et al.* [9] investigated the possibility of in vitro bone regeneration using osteoblastic cells derived from neonatal rats seeded into a 3D-PLGA scaffold. This scaffold was designed by using a solvent-casting and particulate-leaching technique. D-(+)-glucose was used as the porogen particles under a Rotational Oxygen-Permeable Bioreactor System or ROBS to reproduce dynamic and gas permeable culture conditions. The results showed that the mineralization of bone regeneration was observed within 2 weeks of culture and was shown throughout the scaffold at 7 weeks with embedded osteocytic cells. This study showed that a solvent-casting and particulate-leaching technique could be used to fabricated the scaffold for in vitro bone tissue engineering.

- **Emulsification/Freeze-drying**: this technique does not require to use of a solid porogen like SCPL. Firstly, a synthetic polymer is dissolved into a suitable solvent (e.g. polylactic acid in dichloromethane), then water is added and the two liquid phases are mixed in order to obtain an emulsion. Afterwards, the emulsion is cast into a mold and quickly frozen by means of immersion into liquid nitrogen. The frozen emulsion is subsequently freeze-dried to remove the dispersed water and the solvent, thus leaving a solidified or porous polymeric structure. Comparison of this technique to SCPL, emulsification and freeze-drying is faster than SCPL because it does not require a time during the leaching step but it still requires the use of solvents, furthermore pore size is relatively small and porosity is often irregular. Freeze-drying by itself can be emploed to fabricated porous scaffolds without the need for emulsion formation. In particular, it is used to prepare collagen sponges. Collagen is dissolved into acidic solutions of acetic acid or hydrochloric acid that are cast into a mold, frozen with liquid nitrogen then liophylized or freeze-dried.

- **Liquid-liquid phase separation**: this procedure requires the use of a solvent with a low melting point that is easy to eliminate. For example, dioxane can be used to dissolve poly(lactic acid), then adding a small quantity of water was added to induce phase separation. A polymer-rich and a polymer-poor phase are formed. It is followed by cooling below the solvent melting point and after a vacuum-drying to eliminate the solvent, a porous scaffold is obtained. Liquid-liquid phase separation presents the same drawbacks of emulsification/freeze-drying which are getting too small pore size and porosity is often irregular.

- **Gas Foaming**: a technique using gas as a porogen has been developed to overcome the necessity of using organic solvents and solid porogen. Firstly, the polymers are prepared into disc-shaped structures by compression molding using a heated mold. The discs are then placed in a chamber where they are exposed to a high pressure CO_2 for several days. The pressure inside the chamber is gradually restored to atmospheric levels. During this procedure the pores are formed by the carbon dioxide molecules that leaves from the polymer, resulting in a sponge like structure. The main problems related to such a technique are caused by the excessive heat used during compression molding (which prevents the combination of any

temperature labile material into the polymer matrix) and by the fact that the pores do not form into an interconnected structure.

- **Textile technologies**: these techniques comprise all the procedures that have been succesfully employed for the preparation of non-woven meshes of different polymers such as electrospinning technique which can produce non-woven nanofiber. In particularly, non-woven polyglycolide structures have been tested for tissue engineering applications, such as fibrous structures which have been found useful to grow different types of cells. The main disadvatages are related to the difficulties of getting high porosity and regular pore size.

- **CAD/CAM Technologies**: since most of the above described techniques are limited when it comes to the control of porosity and pore size, computer assisted design and manufacture techniques have been introduced to tissue engineering. First a three-dimensional structure is designed using CAD software, then the scaffold is realized by using ink-jet printing of polymer powders or through Fused Deposition Modeling of a polymer solution. [6]

2.2 Electrospinning technique

Electrospinning has been recognized as a very beneficial and effective technique for the production of fibers with extremely small diameters in the range from several micrometers down to tens of nanometers, depending on types of the polymer and processing conditions such as a value of appiled electric field, a distance between a droplet of polymer and collector including the concentration, conductivity, surface tension, flow rate, and viscosity of polymer solution. In electrospinning process, a strong electric field is applied to a droplet of a polymer liquid at the tip of a syringe needle. If an electric field is higher than the surface tension of the polymer liquid, the shape of the droplet will change into a cone, known as the Taylor's cone. Further increase in the electric field causes an ejection of a continuous charged stream of the polymer liquid, i.e., charged jet. The jet accelerates in the direction of the counter electrode or the collector. The evaporation of solvent occurs during the transport of the jet to the counter electrode or collector. Finally, the jet solidify to form web of fibers on the collector .[10, 11, 12]

In 2003, K. Ohgo *et al.*[13] successfully prepared non-woven nanofibers of *Bombyx mori* and *Samia cynthia ricini* silk fibroins from hexafluoroacetone (HFA) solution using electrospinning method. In addition, they could recombine hybrid fiber between the crystalline domain of *B. mori* and non-crystalline domain of *S. c. ricini* silk fibroin.

Recently, K. Ohkawa *et al.*[14] succeeded for the first time in preparing of pure chitosan fibers which is a cationic polysaccharide by an electrospinning technique. They focused on the effect of the electrospinning solvent and the chitosan concentration on the morphology of the resulting nonwoven fabrics. The solvents tested were dilute hydrochloric acid, acetic acid, neat formic acid, and trifluoroacetic acid. None of them produced a visible jet as the electric field was applied excepted TFA. Only when TFA was used as the solvent, chitosan fibers were deposited onto the collector and the addition of dichloromethane to the chitosan-TFA solution improved the homogeneity of the electrospun chitosan fiber. As the chitosan concentration was increased from 5 to 8 % the morphology of the deposition on the collector changed from spherical beads to interconnected fibrous networks. Under optimized conditions, homogenous chitosan fibers with an average diameter of 330 nm were prepared.

Fig. 2.1 shows an electrospinning system which comprises of a syringe needle, syringe pump, high voltage power supply, and collector

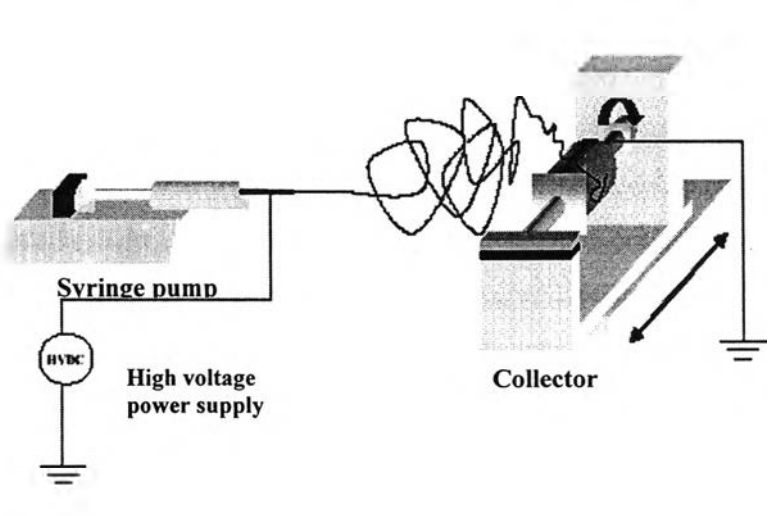


Fig. 2.1 The schematic of electrospinning system.

These nanofibers are of considerable interest for a broad range of applications because of their several useful properties such as high specific surface area, high surface area-to-volume ratio, and high porosity. Examples of applications are fiber membranes for filter, nanoreinforcements, tissue engineering, specialty protective clothing and templates for the formation of hollow fibers with inner diameters in the nanometer range, biomedical applications, wound dressing, biomedical applications including tissue engineering scaffold that has been used to culture many types of cells such as fibroblasts, bone cells, nerve cells, and schwann cells, etc.

In 2003, H. Yoshimoto *et al.*[15] reported the use of non-woven poly(ε-caprolactone) or PCL with diameters of the nanometer range that was fabricated by electrospinning technique as a scaffold for mesenchymal stem cells (MSCs) derived from the bone marrow of neonatal rats. The MSCs were cultured on electrospun PCL scaffolds for 4 weeks under dynamic culture conditions. The result showed that the construction of cell-polymer maintained the size and shape of the original scaffolds and the surfaces of the cell-polymer were covered with cell multilayers. The mineralization and type I collagen were also observed. They suggested that electrospun PCL is a potential candidate scaffold for bone tissue engineering because it has porous structure and a high specific surface area that is beneficial for tissue engineering.

2.3 Silkworm silk

Silk is a natural biopolymer produced by silkworms during the formation of their cocoon. There are two main types of the silkworms, mulberry silk or *Bombyx mori* which is also called cultivated silk, and wild silk such as *Samia cynthia ricini* or *A. pernyi*. Cultivated silks are fine, almost white and soft filaments with luster. The world silk production consists of nearly 80-85 % of cultivated silks. While wild silks are coarser, more irregular and brownish in appearance, they are never as white as the cultivated silk filament. Thai silk is one of the mulberry silkworm (*Bombyx mori*) silks but it differs somewhat in the appearance. It is yellower in colour, the filaments are coarser, and it has more silk gum. [16]

Silk consists of two natural macromolecular proteins, fibroin and sericin. Both of their molecular weights range from about 10,000 to over 300,000 Da. Fibroin is continuous protein with crystalline structure and is water-insoluble. It can be regenerated in several forms such as gel, powder, fiber, or membrane. Fibroin is one of the candidate materials for biomedical application because of its various characteristic such as good biocompatibility, good oxygen and water vapor permeability, and minimal inflammatory reaction. Additionally, fibroin has been used in cosmetic, medical materials for human health, and food additive. Sericin, or silk gum, is a globular protein, an amorphous structure, water soluble glue and it also has some impurities such as waxes, fats, and pigments. Sericin constitutes 25-30% of silk protein and it envelops the fibroin fiber with continuing sticky layers and that provide the cohesion of the cocoon by gluing silk threads together. Removal of sericin from silk fibroin is achieved by the process call degumming. When subjected to the degumming process, physically, chemically, or enzymologically, the sericin protein is degraded into polypeptide. The sericin peptide has excellent moisture absorption and releases a lot of biological activities such as antioxidant, anti-cancer activities and can be applied to degradable biomaterials. Furethermore, sericin is also used as a coating material for natural and artificial fibers which can prevent abrasive skin injuries [16,17].

The process of silk fiber formation can be described as follows [16]. Briefly, The mature silkworm builds its cocoon by extruding a viscous fluid or fibroin from two large glands in the body. The viscous fibroin is covered by another secretion that is sericin which flows from two other symmetrically-placed glands. Both of the two components solidify together when they are exposed to the air, coagulating and generating a continuous fibers.

Fig 2.2. presents the structure of raw silk filament, which contains microfibrils that are packed together to form the fibril bundle. The several fibril bundles produce a single strand of fibroin. Sericin envelops two single strand of fibroin together to form a raw silk filament.

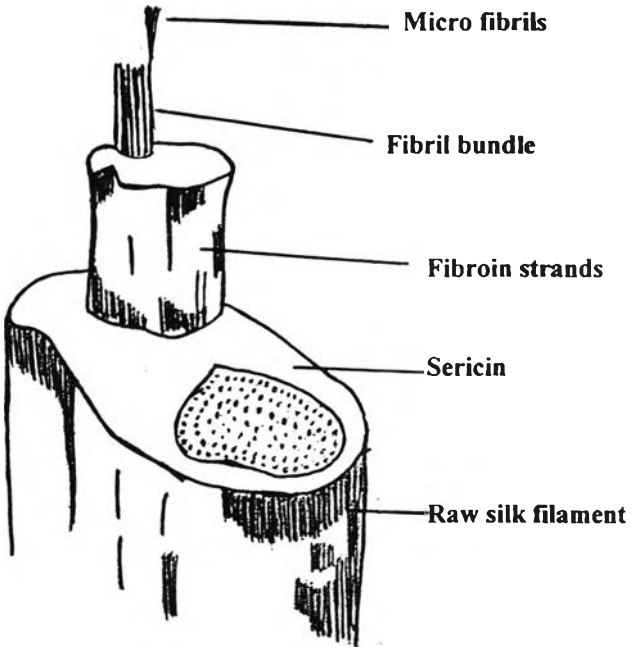


Fig. 2.2 The structure of a raw silk filament

Silk fibers after degumming are approximately 900 – 1,700 meters long and the diameter ranges from 9 to 11 microns. Silk fibers are extremely strong. A filament of silk is stronger than steel with an equal diameter, but the strength of the fiber is reduced greatly when it is wet (about 80-85 % of the dry strength). If silk fiber is heated at 140 °C, it will remain unaffected for a long period of time but it is decomposed very quickly at the temperature of 175 °C or higher. As shown in fig. 2.3, because the silk fibers have extensive hydrogen bonding, the abundant hydrophobic nature of their proteins, and the significant crystalline structure of the polypeptide chains in silk fibroin, so silk fibers are insoluble in most solvents, including water, dilute acid and alkali.

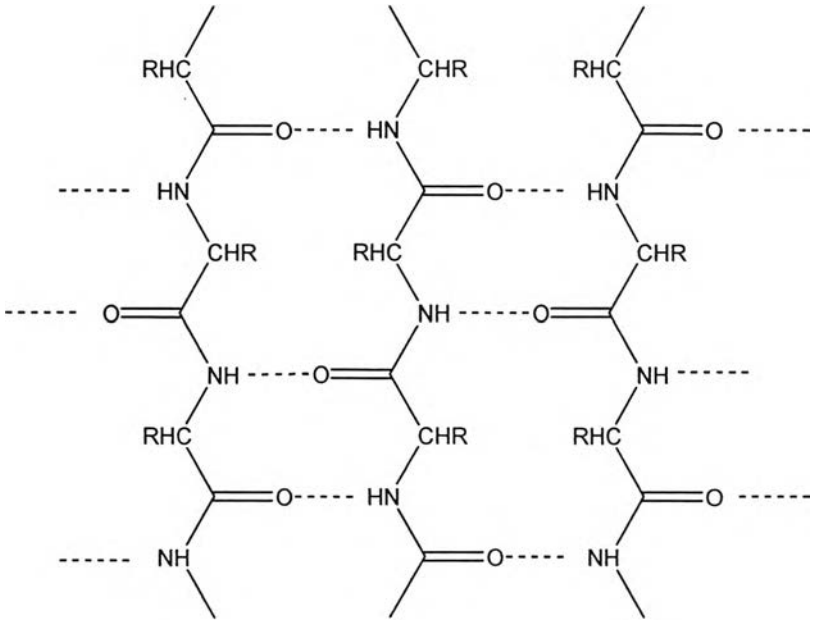
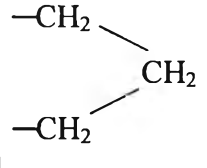


Fig.2.3. The crystalline structure of the polypeptide chains in silk fibroin (where - - - - represents hydrogen bonding)

Table 2.1 lists the composition of amino acid in silk fibers. There are different proportions of amino acid residues in fibroin and sericin. Fibroin has higher proportions of alanine, glycine, and serine than sericin leading to antiparallel *ß*-pleated sheet formation. The crystalline regions of silk fibers are composed mainly of repeating units of amino acid in silk fibroin chains. *Bombyx mori* and *A. pernyi* silk fibroin have a rather different chemical structure. The crystalline regions of *A. pernyi* silk fibroin are composed mainly of — (alanine - alanine) $_n$ — sequences, while — (glycine - alanine) $_n$ — repetitions are characteristic to *Bombyx mori* silk fibroin. A small amount of cysteine and methioinine residues gives a very small amount of sulfur in the fiber. [16,18]

Table 2.1 The composition of amino acid in silk fiber (%)

Type	Side group (R-group)	Amino acid	Proportion
Inert	—H	Glycine	46.39
	$-CH_3$	Alanine	44.55
	$-CH(CH_3)_2$	Valine	0.50
	$-CH_2CH(CH_3)_2$	Leucine	0.05
	$-CH(CH_3)CH_2CH_3$	Isoleucine	0.08
	$-CH_2C_6H_5$	Phenylalanine	0.02
Acidic	$-CH_2COOH$	Aspartate	0.17
	$-CH_2CH_2COOH$	Glutamate	1.03
Basic	$-CH_2CH_2CH_2CH_2NH_2$	Lysine	0.07
	$-(CH_2)_3NHC(NH)NH_2$	Arginine	0.17
Hydroxyl	$-CH_2OH$	Serine	7.17
	$-CH(OH)CH_3$	Threonine	0.15
	$-CH_2C_6H_4OH$	Tyrosine	0.14
Ring		Proline	0.02
Sulfur	$-CH_2$-S-S-CH_2-	Cysteine	0.02
	$-CH_2CH_2$-S-CH_3	Methioinine	0.04

A comparison of mechanical properties (Table 2.2) suggests that *Bombyx mori* silks provide a remarkable combination of strength and toughness. The distinguishing features of the silk fibroin filaments following extraction of sericin are the very high strength in combination with excellent elasticity in comparison with these other biomaterials. In additionally, these fibers display resistance to failure in compression that distinguishes them from other high performance fibers, such as Kevlar [19].

Table 2.2 Comparison of mechanical properties of silks (silkworm) to several types of biomaterial fibers and tissues commonly used today

Material	Tensile strength (MPa)	Modulus (GPa)	% Strain at break
B. mori silk (w/ sericin)[a]	500	5-12	19
B. mori silk (w/o sericin)[b]	610-690	15-17	4-16
B. mori silk[c]	740	10	20
Collagen[d]	0.9–7.4	0.0018–0.046	24–68
PLA[e]	28–50	1.2–3.0	2–6
Synthetic Rubber	50	0.001	850
Bone	160	20	3
Tendon (mainly collagen)	150	1.5	12
Kevlar (49 fiber)	3,600	130	2.7

[a] Bombyx mori silkworm silk — determined from multithread fibers naturally produced from the silk worm coated in sericin.

[b] Bombyx mori silkworm silk— determined from single individual fibroin filaments following extraction of sericin.

[c] Bombyx mori silkworm silk — average

[d] Rat-tail collagen Type I extruded fibers tested after stretching from 0% to 50%.

[e] Polylactic acid with molecular weights ranging from 50,000 to 300,000.

In a relative early study, H. J. Jin *et al.* [20] had prepared electrospinning fibers from *Bombyx mori* silk fibroin and poly(ethylene oxide) or PEO using hexfluoro-2-propanol and water as their solvents, respectively. The electrospun mats were successfully formed with less than 1 μm diameter fiber with the composition reflective of the solution concentration. Their results open new directions in the fabrication of silk based biomaterial scaffold for biomedical application.

Subsequently, H. J. Jin *et al.* [21] had successfully fabricated electrospun silk fibroin fibers blending with PEO, they recently showed the ability of fine electrospun silk fibroin fibers after PEO extraction in water with diameter of 700 ± 50 nm to support human bone marrow stromal cell (BMSC) attachment, spreading and proliferation in vitro. Due to this study, they suggested potential use of electrospun silk fibroin fibers as scaffolds for tissue engineering.

Recently in 2004, B. M. Min *et al.* [22] cultured normal human keratinocytes and fibroblasts in vitro in nonwoven silk fibroin (SF) nanofibers which fabricated by electrospinning technique using formic acid as a spinning solvent. To decrease the solubility in water, as-spun SF nanofiber mats were treated with 50% methanol solution. As-spun SF nanofibers had an average diameter of 80 nm and a porosity was decreased from 76.1% up to 68.1% after chemical treatment. In the cytocompatibility and cell behavior, the as-spun SF nanofibers was found to promote cell adhesion and spreading of type I collagen, because they provided a high level of surface area for cells attachment due to their three-dimensional feature and their high surface area to volume ratio. Their results indicated that the SF nanofibers may be a good candidate for the biomedical applications, such as wound dressing and scaffolds for tissue engineering.

2.4 Schwann cell

2.4.1 Nervous system

The nervous systems perform mainly two functions that are

1. Receptors, parts of the nervous system that receive sensory input or sense changes from internal and external environments. Most of receptors are on muscles and other internal organs. Sensory input can be in many forms, comprising taste, sound, pressure, light, pH, blood or hormone levels, that are converted to a signal and sent to the brain or spinal cord.

2. Integrate the input to be output, a signal is integrated and a response is generated by the sensory centers of the brain or in the spinal cord. The response or a motor output, the signal converted into some form of action, such as changes in heart rate, movement, release of hormones, etc.

The nervous system is subdivided into two division

1. The central nervous system (CNS) , receiving sensory input from receptors, comprises of the brain, spinal cord and mainly of interneurons.

2. The peripheral nervous system (PNS) , connecting the CNS to other parts of the body, and is composed of nerves (bundles of axons).

The nervous system is composed of nervous cells which are neurons and neuroglia or glial cells to form nervous tissue. Neurons transmit nerve messages or signal impulses from one part of the body to another, while neuroglia are in direct contact with neurons and often surround them to supporting neuron to work regularly.

Humans have about 100 billion neurons in their brain alone. Neurons have mainly two parts which are cell body and cell process as shows in Fig 2.4. The cell body which has pyramidal shape, contains nucleus and cytoplasm or other organelles typical of eukaryotic cells such as mitochondria, lysosome, microtubule, etc. It is the site of synthesis of virtually all neuronal proteins and membranes. The cell process comprises dendrites and axon. Dendrites, short fibers extending outward from the cell body that being the input zone of neuron to receive signal impulse from the axon terminus of other neuron then transmit the message to the cell body and is actively conducted down to the axon. Axon, a long single fiber that conducts the nervous signal away from the cell body toward the axon terminus. This means that,

axon may be a meter or more in length. The diameter may vary from a micrometer such as nerves of human brain to a millimeter in the giant fiber of squid. The speed of the nerve signal conduction is roughly proportional to its diameter which can be as fast as 100 meters per second.

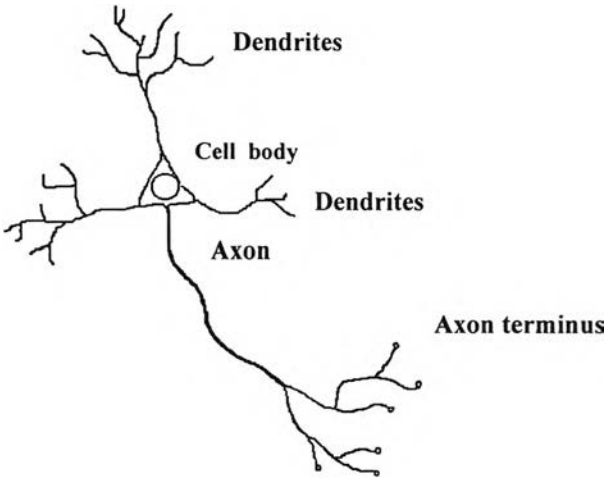


Fig. 2.4 The pyramidal cell – A common neuron

Some of axons are coated by a sheath of myelin, a stack of specialized plasma membrane sheets produced by glial cells that wraps its self around the axon. Each region of myelin formed by an individual glial cells is separated from the next region by an unmyelinated area call the node of Ranvier, which can increases the velocity of signal conduction. In the PNS, these glial cells are called Schwann cell while in the CNS they are calls oligodendrocytes. As shown in Fig.2.5, Schwann cells are a variety of neuroglia that wrap around axons in the peripheral nervous system (PNS), forming the myelin sheath

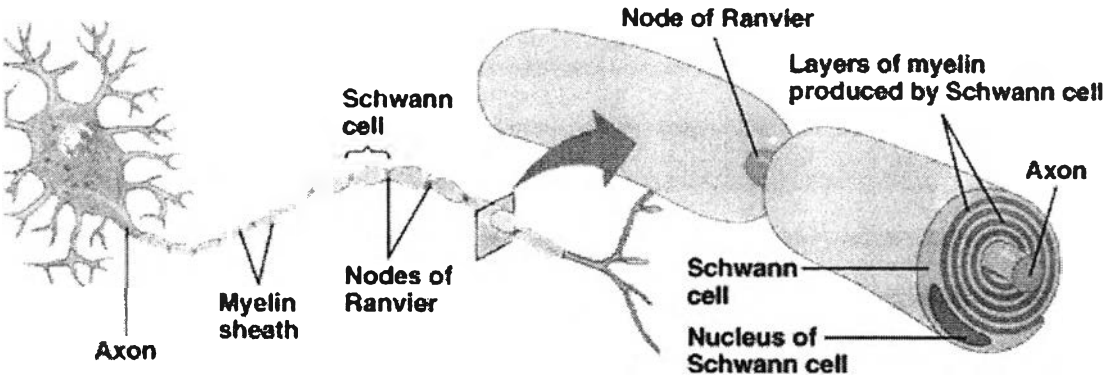


Fig. 2.5 An axon coated with myelin sheath

The formation and structure of myelin sheath was shown in Fig. 2.6. Schwann cells begin to form the myelin sheath in mammals during fetal development and work by spiraling their self around the axon then its cytoplasm is reduced. The structure of compact stacked plasma membrane is formed. A single Schwann cell can form a myelin sheath around multiple axons. A well developed Schwann cell is shaped like a rolled up sheet of paper, with layers of myelin in between each coil. Since each Schwann cell can cover about a millimeter (0.04 inches) along the axon, hundreds and often thousands are needed to completely cover an axon. Furthermore, Schwann cell can produce proteins essential for neuron growth [23].

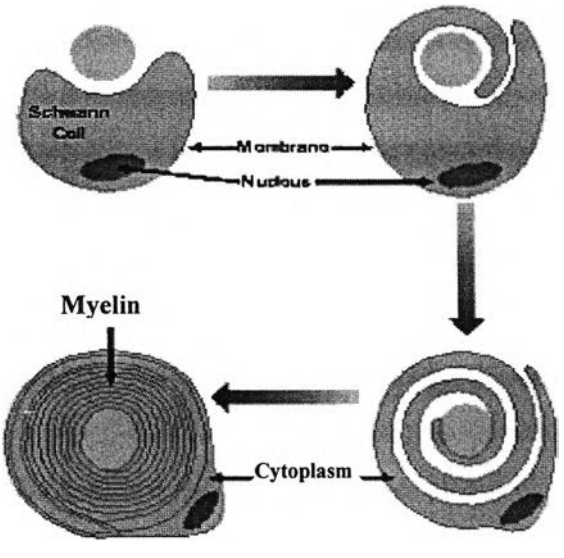


Fig. 2.6. Formation and structure of myelin sheath

In peripheral nervous system, a major problem is related to the treatment of peripheral nerve injury because the bridging of gaps between the cut ends of the transected nerve are larger. No satisfactory method currently exists for bridging neural defects. Autografts lead to inadequate functional recovery and risk of inflammatory surgery wound. A potential alternative for the repair of peripheral nerve injuries is an artificial nerve grafts. Because Schwann cells support nueron growth, they can be cultured on a biodegradable scaffold that is used as the artificial nerve grafts to enhance nerve regeneration.

From C. Miller *et al.*[24] work, they investigated the influence of substrate-mediated chemical and physical guidance on the growth and alignment of Schwann cells in vitro. They used poly(D,L-lactic acid) as biodegradable polymer substrates. Compression molding and solvent-casting were used to transfer microgroove patterns from quartz and silicon substrates onto the surface of poly(D,L-lactic acid) substrates. Laminin was selectively adsorbed onto the grooves and Schwann cells were seeded on the substrates. Laminin was found to improve adhesion of Schwann cells on the substrates and the microgrooves were found to cause the Schwann cells to align along the direction of the grooves. The degradation of the grooves in the solvent cast films was much slower than those in the compression-molded films. This approach offered that poly(D,L-lactic acid) substrates were potential significance promoting peripheral nerve regeneration.

Y. Yuan *et al.*[25] recently reported the biocompatibility between Schwann cells (SCs) and chitosan including chitosan membranes with 50 μm thickness and chitosan fibers with 15 μm diameter. Their experimental results indicated that SCs could grow onto chitosan materials with two different shapes which are spherical and long olivary. The long olivary cells inclined to encircle chitosan fibers up. Additionally, it was also found that the SCs on the chitosan fibers migrated faster than those on the chitosan membranes. These studied contribute information about biocompatibility of chitosan for nerve repair.

In very recently, C. A. Sundbacka *et al.* [26] examined the biocompatibility of a biodegradable elastomer, poly(glycerol sebacate) or PGS for neural reconstruction applications. They studied about the effect of PGS on Schwann cell activity, attachment and proliferation in vitro in comparison with poly(lactide-co glycolide) or PLGA. Both were fabricated in flate sheet formed. The results showed

that PGS had no negative effect on Schwann cell activity. The effects of PGS were similar to or superior to that of PLGA in vitro. In vivo, PGS demonstrated a favorable tissue response profile compared with PLGA, with significantly less inflammation and fibrosis and without detectable swelling during degradation. They suggested that PGS was an excellent candidate material for neural reconstruction applications.