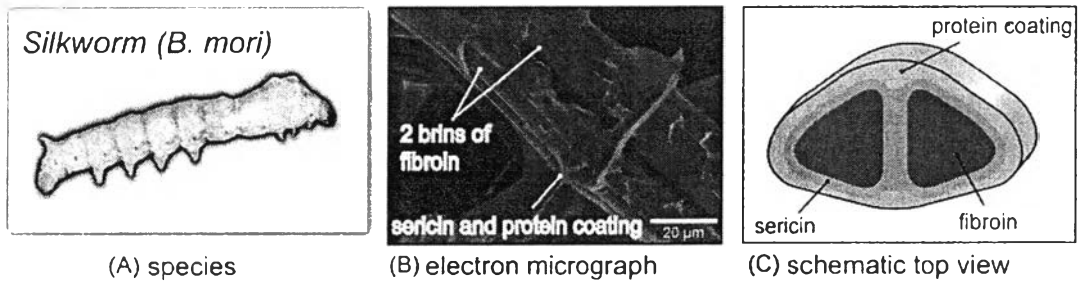
CHAPTER II
THEORETICAL BACKGROUND AND LITERATURE REVIEW

2.1 Silkworm (*B. Mori*) Silk

Mankind has farmed silk for thousands of years. The domesticated silkworm (B. mori) is well known as the represent of species that produce silk fiber which are about 10–25 mm in diameter and consist of two proteins: a light chain (25 kDa) and heavy chain (325 kDa) which are present in a 1:1 ratio and linked by a single disulfide bond (Gregory H, 2003). These fibers are coated with a family of hydrophilic proteins called sericins (20–310 kDa). The disulfide linkage between the Cys-c20 (20th residue from the carboxyl terminus) of the heavy chain and Cys-172 of the light chain connects the fibroin together and a P25 glycoprotein (25 kDa) is non-covalently linked to these proteins (Tanaka K, 1999). Silk sericin can be eliminated from fibroin by boiling silk cocoons in an alkaline solution. There is 25-30 % of the silk cocoon mass that is sericin, which is removed during the de-gumming process.



(A) species (B) electron micrograph (C) schematic top view
sketch not to scale

Figure 2.1 A) Photograph of a Bombyx mori silkworm. B) Electron micrograph of partially degummed B. mori silkworm cocoon fibers, in which the two brins of fibroin and the coating of sericins C) Schematic cross-section of the composite structure of a cocoon fiber, in which the two brins of fibroin and the coating of sericins. Adapted with the permission of both the authors and publisher, Copyright 2008, Elsevier Science Ltd., Oxford, UK. (John G, 2010)

2.1.1 B. mori Silk Fibroin Structure (Charu V, 2007)

There are many amino acid components composed in silk fibroin that consists primarily of glycine (Gly) (43%), alanine (Ala) (30%) and serine (Ser) (12%). The heavy chain consists of 12 domains that form the crystalline regions in silk fibroin, which are combined with primary sequence that is non-repetitive and thus forms fewer organized domains in the fibers. The crystalline domains in the fibers consist of Gly-X repeats, with X being Ala, Ser, Threonine (Thr) and Valine (Val). The crystalline forming domains consist of an average of 381 residues (596 in size in the seventh domain to 36 in the 12^{th} domain). Each domain consists of sub-domain hexapeptides including: GAGAGS, GAGAGY, GAGAGA or GAGYGA where G is glycine, A is alanine, S is serine and Y is tyrosine. These sub-domains end with tetrapeptides such as GAAS or GAGS. The less crystalline forming regions of the fibroin heavy chain, also known as linkers, are between 42 and 44 amino acid residues in length. All the linkers have an identical 25 amino acid residue (non-repetitive sequence), which is composed of charged amino acids can found in these regions. The primary sequence results in a hydrophobic protein with a natural co-block polymer design. Efficient secretion of fibroin is believed to be due in part to the formation of a disulfide bond between the heavy and light fibroin chains. A naked pupa mutation in B. mori has been mapped to the same locus as of the light chain on the 14th chromosome. The resulting fibroin light chain does not have a disulfide bond where the fibroin heavy chain and the cocoon have less than 0.3% in fibroin protein content.

A number of silk species have been reported, including the glandular state prior to crystallization (silk I), the spun silk state (crystallization state) which consists of the b-sheet secondary structure (silk II), a helical structure after an air/water assembled interfacial silk (silk III). The silk I structure is the water-soluble state that easy to heat or physical spinning converts to a silk II structure. The silk I structure is observed in vitro in aqueous conditions and converts to a b-sheet structure when immersed to methanol or potassium chloride. The b-sheet structures are asymmetrical with one side occupied with hydrogen side chains from glycine and the other occupied with the methyl side chains from the alanines that are the

hydrophobic domains. The β-sheets are arranged into crystals so that the methyl groups and hydrogen groups of opposing sheets interact by forming strong hydrogen bond and van der Waals forces to generate the inter-sheet stacking which is a thermodynamically stable structure. Between amino acids chain sequence, the inter- and intra-chain hydrogen bonds formed perpendicular to the axis of the chains and the fiber. The silk II structure resists water and is insoluble in several solvents including mild acid and alkaline conditions, and several chaotropes.

2.1.2 Control of Morphology of Silk Biomaterials

Nowadays, there are a lot of dry silk cocoon worldwide available for textile industry and thus for biomaterials applications. Several designs of material morphologies can be formed by aqueous or solvent system of the natural fiber form of silk for utilization in biomaterials or biomedical applications such as film, sponge, hydrogel, nanofibers, and more recently (Figure 2.2). The fibers must be firstly dissolved in aqueous systems, followed by reprocessing into desired material formats.

2.1.3 Utility in Biomedical Applications

One of the important concerns in assessing the biological responses reported to these silk fibers is the absence of detailed characterization of the fibers used. The various species of silk in each country, the level of extraction of the sericin, the chemical nature of wax-like coatings sometimes used, and many related processing factors also have to be known. This variability in raw material has resulted in confusion in the literature and potential concerns with this class of fibrous protein. For example, of greatest importance is that based on many studies it is clear that the sericin glue-like proteins are the major cause of adverse problems with biocompatibility especially in inflammatory reaction. The cellular responses to the fibroin fibers appear to be comparable to most other commonly used biomaterials by removing of sericin. Furthermore, there is clear evidence in the literature that silk protein is susceptible to in vivo proteolytic degradation and over longer time periods in vivo will slowly be absorbed (Gregory H, 2003).

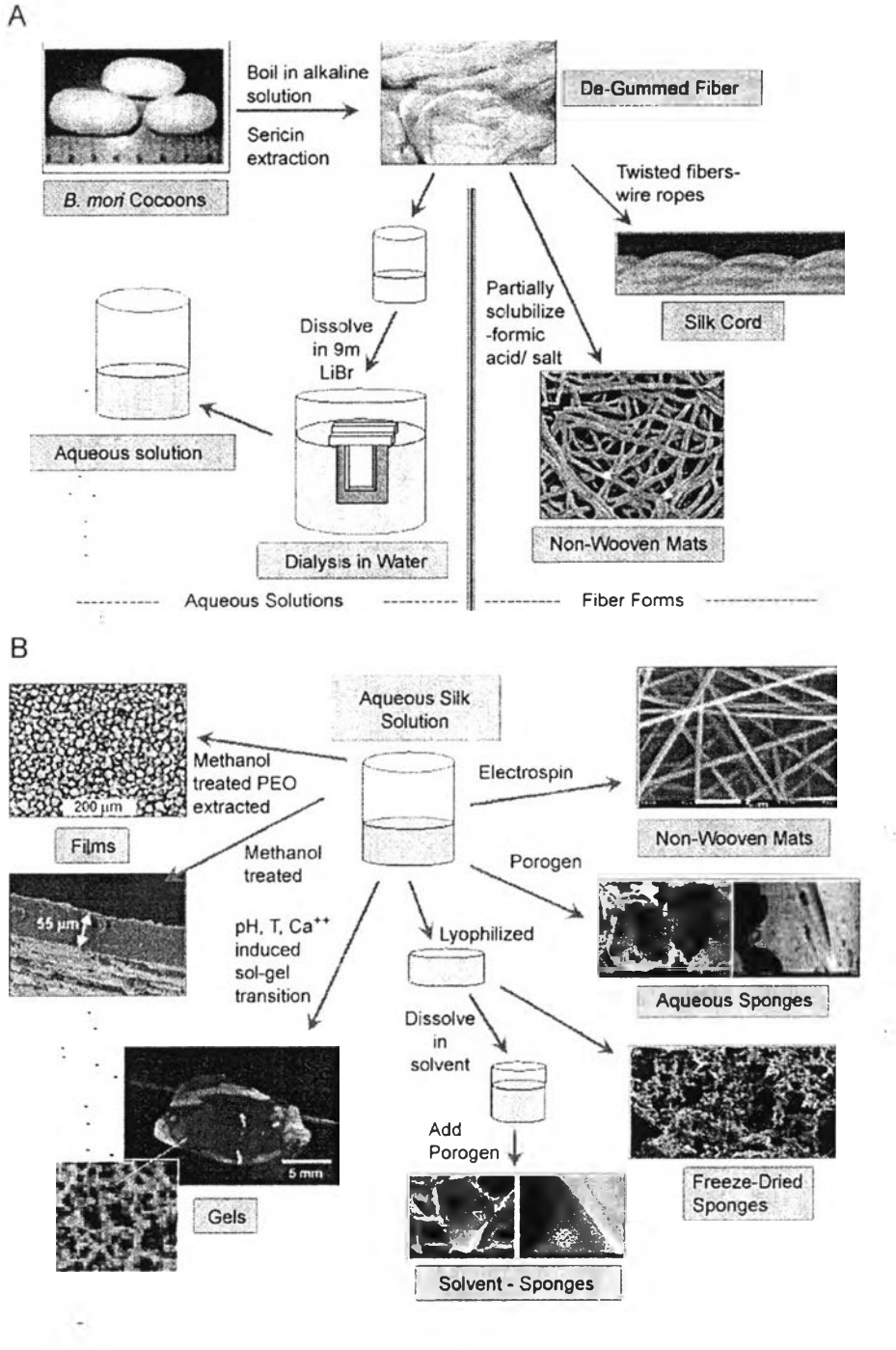


Figure 2.2 (A) Silk fibroin is purified from sericins by boiling in an alkaline solution. Non-woven silk mats by partial solubilization; or dissolved in lithium bromide, dialyzed and formed into aqueous silk fibroin solution for preparation of other material morphologies. (B) Processing of silk morphologies from aqueous silk fibroin solution into non-woven silk fibers; aqueous- and solvent-based porous sponges; hydrogels and films (Charu V, 2007).

2.1.4 Surface Modification of Silk Fibroin

To create bioactive functions, Surface modification is the one of important method that can be simply used to impact cellular activities. Due to the amino acid side chain of silk fibroin, Surface modification was simply achieved on the surface via mind chemical reaction. There are numerous works focus on cell attachment and proliferation after surface modification. In 1984, Pierschbacher MD studied the surface modification with the integrin recognition sequence RGDS that can increase cell attachment. And also in 1997, Gotoh studied on the modification of silk fibroin with poly(ethylene glycol) (PEG) which showed decreased hMSC attachment of fibroblasts contact angle, and protein adsorption related to the amount of PEG on the surface of the fibroin.

Surface modification can be classified mainly into physical adsorption and chemical immobilization of an active protein or ligand. Silk surfaces are hydrophobic, and attract and repel proteins depending upon the pI and hydrophobicity of the protein and pH of the solution. Differential adsorption of horseradish peroxidase (HRP) to silk fibroin porous sponges depended on the pH of the solution; HRP precipitated on the surface when the pI and solution pH were similar. Chemical immobilization of HRP increased the amount of HRP available on the silk fibroin surface (Vepari CP, 2006).

Silk fibroin can be immobilized via the amino acid side chain chemistry. The limitation to using silk fibroin for chemical modification is the limited total content of modifiable amino acid side chain groups: 3.3% of the amino acids contain carboxyl side groups compared with 9.5% found in bovine collagen. Carbodiimide chemistry, which uses amine or carboxyl groups on silk for modification, has been widely used to modify the surface of silk fibroin biomaterials or to react in solution followed by biomaterials formation. Glucose-oxidase was immobilized on silk fibroin films for use as a glucose sensor (Demura M, 1991). Arginine residues of silk fibroin were modified by 1,2-cyclohexanedione, altering cell attachment and proliferation (Gotoh Y, 1997). Silk fibroin modified with RGD peptide resulted in enhanced cell attachment and proliferation and subsequently increased collagen type I production by hMSCs and ligament fibroblast cells (Chen J,

2003). Covalent attachment of RGD to silk fibroin films increased the number of mineral modules formed by Saos-2 cells in osteogenic media (Sofia S, 2001). BMP-2 immobilized versus adsorbed on silk fibroin films showed increased inducement of bone markers from hMSCs, including alkaline phosphatase, calcium deposition, and transcripts for collagen type I, bone sialoprotein, osteopontin and BMP-2 (Karageorgiou V, 2004). These signaling cues required by cells to develop into tissues would be useful to incorporate into biomaterial designs. Silk fibroin sponges were used to form immobilized gradients of morphogens (Vepari CP, 2006). Immobilized gradients provide new opportunities for biomaterial scaffolds for the generation of more complex cell and tissue outcomes in vitro and in vivo. Organic–inorganic composites can also be generated through covalent linkage. Nano-particles of hydroxyapatite, less than 200 nm in diameter, were chemically bonded to silk fibroin (Furuzono T, 2004). Vinyl groups were introduced on silk fibroin by treatment with 2-methacryloxyethyl isocyanate. Subsequently, the modified silk fibroin surface was grafted with poly g-methacryloxypropyl trimethoxysilane (polyMPTS). Hydroxyapatite particles were reacted with alkoxysilyl group of MPTS to form a siloxane bond. The crystallinity of silk fibroin remained constant during the coupling procedure and resulted in a silk–hydroxyapatite composite with properties of both components (Furuzono T, 2004).

2.2 Wound Healing

The amount of knowledge and understanding about the wound healing process and dressing practices has expanded and changed dramatically over the past three decades. Prior to this time, the healing process was considered to be a passive process with respect to the physician. This theory was summed up by Ambroise Pare, who said "I dressed the wound, God heals it". For the development of dressing product, the wound definition and healing process have to well understand firstly.

2.2.1 Wound

A wound is a type of injury that can be described as a defect or a break in the skin that can be happened from physical, chemical, or thermal damage or as a result of surgical or medical procedure. Based on the nature of the repair process, wounds can be classified as acute or chronic wounds. Acute wounds are usually tissue injuries that can heal completely as expected with minimal scarring. The primary causes of acute wounds include mechanical injuries due to external factors such as abrasions and tears which are caused by frictional contact between the skin and hard surfaces and also include mechanical injuries that caused by that penetration of knives and gun shots as well as in surgical process. Another category of acute wounds include burns and chemical injuries which caused from various sources such as radiation, electricity, corrosive chemicals and thermal. The temperature and contact time influence the degree of a thermal burn that each state required specialist care because of the associated trauma.

Chronic wounds are defined as wounds that do not follow the well-defined stepwise process of physiologic healing and often reoccurs. Such wounds fail to heal due to repeated tissue insults or underlying physiological conditions such as diabetes and malignancies, persistent infections, poor primary treatment and other patient related factors. These result in a disruption of the wound healing process. Chronic wounds include decubitis ulcers (bedsores or pressure sores) and leg ulcers (venous, ischemic or of traumatic origin).

2.2.2 Stage of Wound Healing

After an injury or surgical procedure, the healing process begins. This process consists of three phases and is found in all normal wound healing (James R. 1997). These phases are not distinct, but form a continuum of the wound healing process. The inflammatory or substrate phase begins immediately after wounding and lasts approximately 4 days. The initial goal of this phase is hemostasis. This is carried out through smooth muscle contraction and subsequent occlusion of the larger damaged blood vessels. The activation and aggregation of platelets and release of clotting factors at the vessel wall injury site starts the coagulation process that helps to thrombose the smaller damaged vessels. Hemostasis therefore, plays a protective role as well as contributing to successful wound healing.

A second goal of the inflammatory phase is the removal of bacteria, foreign debris, and other contaminants. The inflammatory response increases vascular permeability, resulting in migration of neutrophils and monocytes into the surrounding tissue. Neutrophils, also known as polymorphonuclear leukocytes (PMNs), migrate from the surrounding microvasculature and serve to accomplish this. Although they appear first, PMNs are short-lived lasting about 6 hours. In the late inflammatory phase, monocytes converted in the tissue to macrophages that appear at the wound site approximately 48 hours after injury. These cells, in addition to aggressively removing necrotic or foreign debris and phagocytizing bacteria, initiate two important aspects of healing angiogenesis and fibroplasias. These are mediated by various proteins or cytokines released by activated macrophages. Angiogenesis and the formation of capillary buds start at 3 days post-wounding and are essential for providing the metabolic needs of the healing process.

Fibroplasia and collagen synthesis start by the third to fifth day post-injury. The proliferative or fibroblastic phase begins toward the end of the inflammatory phase and lasts as long as 3 weeks (approximately 3 to 21 days post-injury). Activated macrophages already present in the wound site release angiogenesis factor (AGF) and fibroblast stimulating factor as noted previously. AGF initiates the development of capillary buds and neovasculature. Fibroblast-stimulating factor cause the activation of fibroblasts at approximately 1 week after injury. Fibroblasts, thus, become the key cells of the proliferative phase. Collagen and proteoglycans are produced by the fibroblast. The neovasculature, along with collagen and the proteoglycan ground substance, form granulation tissue. Granulation tissue fills in wound defects during the proliferative phase. The production of collagen causes an increase in wound tensile strength between days 5 and 15 post-injury. Contraction of the wound helps to reduce the size of the defect and brings the wound edges closer together. This process occurs between the first and second week after injury and is mediated by special fibroblasts with contractile properties called myofibroblasts. The process of re-epithelialization occurs in the proliferative phase. This involves the proliferation and migration of epithelial cells over the granulation tissue to bridge the defect. Reepithelialization is essential to wound healing as the new epithelial cells provide a barrier to microbial invasion and

help to prevent fluid loss. This step occurs rapidly in a wound closed by primary intention, but requires a longer time in wounds having a larger defect. Because the epithelial cells must migrate over the wound surface, the rapidity of the migration is greatly affected by the type of surface. A moist wound surface facilitates epithelial migration, whereas a dry or eschar covered surface provides an impediment to migration. As will be discussed later, the goal of many new wound dressing products is to provide a moist environment to enhance re-epithelialization.

The final phase of wound healing is called the maturation or remodeling phase. This begins at approximately 21 days post-injury and continues until 1 to 2 years after the injury. Early in this phase, fibroblasts continue to produce collagen.

Remodeling is carried out by collagenases that are secreted during this phase. The collagen bundles synthesized during the proliferative phase and randomly laid are partially lysed by these enzymes. This allows for a debulking of the collagen as well as a reorganization of the bundles into a more parallel arrangement. Wound contraction and a gradual increase in tensile strength are seen as this phase progresses. The ultimate strength of the healed wound is determined by the amount of collagen synthesis and the extent to which cross-linking has occurred between collagen bundles. Maximal tensile strength is achieved at the end of the maturation phase and is usually about 80% of the original, uninjured tissue's strength. There is some loss of elasticity seen in tissue that has undergone normal healing, in some instances, and this can cause adhesions or excessive contractures. Wound can be simply classified by the appearance that showed in Table 2.1.

Table 2.1 Classification of Wounds Based on the Appearance (JOSHUA S, 2007)

Wound Type	Appearance	Stage of Wound Healing Affected
Necrotic	Often black or olive green due to dead devitalised tissue, that is dry, thick and leathery to touch. Common with pressure sores	Under favourable conditions, dead tissue in a wound such as a pressure sore will usually separate spontaneously from the healthy tissue beneath. This occurs as a result of autolysis and presumably involves macrophage activity and the action of proteolytic enzymes which act at the interface of the necrotic and healthy tissue A dry environment prevents the autolytic and proteolytic actions of macrophages and enzymes
Sloughy	Fluid, moist, loose and stringy rehydrated necrotic tissue that is typically yellow in colour	Associated with excess exudates during inflammatory phase. Slough leads to wounds getting stuck in the late inflammatory stage leading resulting in delayed wound healing
Granulating	Significant quantities of granulation tissue, generally red or deep pink in colour. May produce excess exudate	Proliferative phase
Epithelialising	Pink in colour with formation of new epidermis	Involves both migratory and proliferative phases. Final stages of wound healing

Wound Type	Appearance	Stage of Wound Healing Affected
Infected and malodorous	Red, hot inflamed tissue, pus present. Infection with anaerobic bacteria causes unpleasant odour	Inflammatory response, collagen synthesis, epithelisation. Infection prolongs the inflammatory process which delays wound healing

Each wound type represents the phases that a single wound may go through as it heals.

2.3 Update on Wound Dressing

2.3.1 Ideas of Wound Dressings

"No single dressing is suitable for all types of wounds" that is the classical sentence for topical wound dressing since today. Mankind has been covering wounds with a variety of materials since the earliest written records. Sumerian cuneiform tablets before 2000 BCE describe the application of poultices formed of mud, milk, and plants to wounds, and Egyptian papyruses from 1550 and 1650 BCE provide specific details of how to wash the wound, prepare and apply plasters of honey, plant fibers, and animal fat, and then bandage the wound (Liza G, 2007). Nowadays, perhaps one of the most significant ideas can change the nature of wound dressing materials that has been concept in universal wound healing.

Turner in 1979 outlined the performance parameters of an "ideal wound dressing" and they included the ability to: (1) absorb exudates and toxic components from the wounds surface; (2) maintain a high humidity at the wound/dressing interface; (3) allow gaseous exchange; (4) provide thermal insulation; (5) protect the wound from bacterial penetration; (6) be nontoxic; and (7) be removed easily without trauma to the wound. Criteria that were added later included that the material should: (1) have acceptable handling qualities (resistance to tear and disintegration when wet or dry), and (2) be comformable and be sterilizable. With a clearer understanding of the cellular and molecular biology of healing, other criteria were added which have primarily been directed toward the

functional characteristic of the material and the microenvironment created which would optimize wound healing.

Furthermore, Wound dressing can be classified in 3 types. First are passive products that is traditional dressings provided cover and conceal the wound, e.g. gauze and tulle dressings. Then, the advance dressings were developed , which known as interactive products, in form of polymeric films and forms which are mostly transparent, permeable to water vapor and oxygen, non-permeable to bacteria, e.g. hyaluronic acid, hydrogels, foam dressings. Recently, dressings have been developed that do more than simply manage moisture or exudate levels. A new function of advanced dressing is to interact with the biochemical environment of the wound. These dressing can be considered as active products, e.g. hydrocolloids, alginates, collagens, chitosan. Dressing type and characteristics along with commercially available products are described in Table 2.2.

Table 2.2 Topical wound dressing type, important characteristics and commercially available products (Christopher E. Attinger, MD, with permission) (Alexander S, 2008)

Dressing	Characteristics	Available commercial products
Hydrogels	• Cross-linked polymer gels or sheets • Available with adhesive borders as well as silver ion-impregnated formulations • Generally waterproof, which may prevent bacterial and environmental contamination; their high water content inhibits absorption of exudates, but they are also hydrophilic in nature, which may allow evaporation of exudates	• AquaGauze (DeRoyal, Powell, Tenn.) • Aquasite (Dumex Medical, Toronto, Canada) • CarraDres (Carrington Laboratories, Irvin, Texas) • Curasol (Healthpoint, Fort Worth, Texas) • Intrasite (Smith & Nephew, Largo, Fla.) • Skintegrity (Medline, Mundelein, Ill.)

Dressing	Characteristics	Available commercial products
	• Change daily to every 7 days	
Film/transparent	• Adhesive, semi-permeable, polyurethane membrane dressings that vary in thickness and size	• Bioclusive Transparent (Johnson & Johnson Wound Management, Somerville, N.J.)
	• Waterproof and impermeable to bacteria and contaminants	• Mefilm (Molnlycke, Newtown, Pa.)
	• Allows for observation of the wound bed	• OpSite (Smith & Nephew)
	• Should not be used on fragile skin or with wounds that have moderate to heavy exudates	• Tegaderm (3M Healthcare, St. Paul, Minn.)
Foams	• Hydrophilic polyurethane/polymer or gel-coated	• Allevyn (Smith & Nephew)
	• Support autolytic debridement	• Curafoam (Tyco Healthcare/Kendall, Mansfield, Mass.)
	• Minimal to moderate absorption capability	• Lyofoam (Convatec, Skillman, N.J.)
	• Maintain a moist wound environment	• Mepilex Border (Molnlycke, Newtown, Pa.)
	• Non-adherent	• Optifoam (Medline)
	• Not to be used over dry eschars	• Polymem (Ferris Manufacturing Corp., Burr Ridge, Ill.)
	• Do not prevent peri-wound maceration in heavily exudating wound	• Tielle (Johnson & Johnson Wound Management)
	• Available as pads, sheets, and cavity dressings	
	• Change daily to every 7 days	

Dressing	Characteristics	Available commercial products
Alginates	• Made of brown seaweed • Absorbs up to 20 times its weight • Wicks fluid away from wound bed • For use in moderate to heavily exudating wounds • Subset of Hydrofiber dressings absorbs 30% more exudates (Aquacel); not as effective as a wick • Ideal to fill dead space within the wound	• Algicell (Dumex Medical) • Algisite (Smith & Nephew) • Aquacel (Convatec) • Carrasorb (Carrington Laboratories) • Curasorb (Tyco Healthcare/Kendall) • Kalginate (DeRoyal) • Kaltostat (Convatec) • Maxorb (Medline) • SeaSorb (Coloplast, Marietta, Ga.) • Silvercel (Johnson & Johnson Wound Management) • Sorbsan (Bertek Pharmaceuticals, Research Triangle Park, N.C.)
Hydrocolloids	• Occlusive or semi-occlusive dressings • Autolytically débrides necrotic tissue • Impermeable to bacteria • Not to be used in heavily exudative wounds or over sinus tracts • Manufactured in various shapes, sizes, adhesive properties, and forms, including wafers, pastes, powders	• Comfeel (Coloplast) • DuoDERM (Convatec) • Exuderm (Medline) • MPM Excel (MPM Medical, Irving, Texas) • Primacol (Dumex Medical) • Restore (Hollister, Libertyville, Ill.) • 3M Tegasorb (3M Healthcare)

Dressing	Characteristics	Available commercial products
Enzymatic débridement	• Remove devitalized tissue • Can be used to epithelialize areas of skin grafts that did not take (Panafil, Gladase) • Target tissue as a proteolytic, collagenase, or fibrinolytic	• Accuzyme (Healthpoint) • Collagenase Santyl (Ross Laboratories, Ethezyme, Ethex Corp., St. Louis, Mo.) • Gladase-C (Smith & Nephew) • Panafil (Healthpoint)
Antimicrobials	• For use in critically colonized or infected wound beds • Choice to be determined by quantitative biopsy or culture • Most have broad-spectrum antimicrobial coverage • Combinations of ointments, creams, and silver-based dressings can be used	• Cadexomer iodine (Iodosorb/Iodoflex; Smith & Nephew) • Gentamicin (Garamycin 1%) • Mafenide acetate (Sulfamylon 5%; Bertek Pharmaceuticals, Inc., Morgantown, W.Va.) • Metronidazole (Flagyl/Metrogel 0.8%; Gladerma, Fort Worth, Texas) • Mupirocin (Bactroban 2%; GlaxoSmithKline, Research Triangle Park, N.C.) • Silver sulfadiazine (Silvadene 1%; King Pharmaceuticals, Inc., Bristol, Tenn.)

Dressing	Characteristics	Available commercial products
Silver ion-impregnated dressings	• Ideal as an antimicrobial barrier to prevent critical colonization of the wound beds • Instantly kill a broad spectrum of bacteria • Can deliver silver instantly to the wound bed (0.5% silver nitrate) or slowly in a sustained release form (up to 7 days) • Resistance to silver is not a problem • Silver has been shown to affect keratinocytes; use only on colonized or infected wounds	• Acticoat Absorbent, Burn, 7 days, Moisture Control (Smith & Nephew) • Actisorb (Johnson & Johnson Wound Management) • Arglaes (Medline) • Aquacel Ag (Convatec) • Contreet (Coloplast) • Silvasorb (Medline) • Silverlon Pad, Tubular Stretch, Wound and Burn Contact Dressings, and Packing Strip (Argentum Medical, Willowbrook, Ill.)
Antiseptics	• Unselective in their effect • Destroy both bacteria and local tissue • Can be used as a rinse	• Acetic Acid 0.25% • Chlorhexidine • Dakin's Solution 0.5% • Gentian Violet • Iodine
Impregnated gauze	• Woven or non-woven materials in which substances have been incorporated into the dressing material • Maintain moisture in the wound bed • Act as non-adherent primary dressings • Can prevent sutures from "snagging" on gauze	• Adaptic nonadhering dressing (Johnson & Johnson Wound Management) • Scarlet Red Ointment Dressing (Tyco Healthcare/Kendall) • Xeroform (Tyco Healthcare/Kendall)

Dressing	Characteristics	Available commercial products
Other dressings	• Topical Platelet-Derived Growth Factor (PDGF)	• Becaplermin (Regranex 0.01% gel; Johnson & Johnson
Other dressings (cont.)	• Silicone non-adherent mesh Wound Management) • Matrix Metalloproteinase (MMP) binder • MMP binder with silver • Capillary bed stimulant that also improves Wound Management) epithelialization and promotes healing	• Mepitel (Molnlycke) • ORC/Collagen (Promogran; Johnson & Johnson • ORC/Collagen with silver (Prisma; Johnson & Johnson Wound Management) • Xenaderm (Healthpoint)

2.3.2 Desirable Properties of a Wound Dressing Material

The most suitable wound dressing must be applied depend on a wound type and its healing. Usually for a rapid wound healing, different types of wound dressing materials have to be used. For example, the unique properties of nanofibrous bandages such as high surface area to volume ratio of the nanofibers, the applications of these materials on various types of wounds are abundant with respect to other modern wound dressing materials, such as hydrocolloids, hydrogels, and so forth. The following properties are generally considered for all modern wound dressing materials: Maintain the most suitable environment of the wound/ dressing interface, absorb excess exudates without leakage to the surface of a dressing, provide thermal insulation, mechanical and bacterial protections, allow gaseous and fluid exchanges, absorb wound odor, be non-adherent to the wound and easily removable without trauma, provide some debridement action (remove dead tissue and foreign particles) and be nontoxic, non-allergic, non-sensitizing (to both patient and medical staff), sterile and non-scaring.

2.4 Biomimetic Morphology Design (Kesong L, 2011)

In the billions years of evolution, creatures in nature come up with almost perfect structures that can exhibit the amazing functions. Multi-scale structures ranging from nano, micro to macro are characteristic for biological materials, which play an important role in achieving structural and functional integrity.

Nature has always inspired human ideas and has led to effective materials, structures, tools, mechanisms, processes, algorithms, methods, systems, and many other benefits. In the last few decades, a great number of natural materials have been investigated by scientists and engineers from multidisciplinary fields. It was found that the complex unique structure of biological materials is not possessing only one function. Some representative biological materials with multi-scale structures for function integration are listed in Table 2.3. Creating multifunctional materials is an eternal goal of mankind. Nature is a school for scientists and engineers. There are numerous examples of biomimetic successes that involve making simple copies, such as the use of fins for swimming.

In recent years, a great deal of work has been devoted to fabricating multi-scale structures for function integration through the biomimetic or bio-inspired approach. In this review article, we summarize recent research progress in some typical biological materials with function integration, such as lotus leaves, rice leaves, butterfly wings, water strider legs, insect compound eyes, fish scales, red rose petals, brittlestars, spider silks, nacre, glass sponges, gecko feet, mussels, and others. The corresponding biomimetic materials with multifunctional properties are also presented. This may be helpful to stimulate interdisciplinary collaboration of material science, chemistry, physics, biology, engineering, etc., which is essential for the further discovery of additional function of biological materials and rational design and the reproducible construction of bio-inspired multifunctional materials. In the final section, we discuss challenges and perspectives for bio-inspired design of multi-scale structures for function integration in the future.

2.5 Biocompatible Polymers and Surface Functionalization for Enhance Biological Activity

The field of biomedical application often requires an interdisciplinary approach combining life science and medicine, materials science, and engineering. For a successful application, the material must be biocompatible, meaning that the ability of a material to perform with an appropriate host response in a specific application. Due to the complicate interaction between materials and biological system, there is no precise definition or accurate measurement of biocompatibility. Nevertheless, whether the materials will be accepted by a living body should be the criterion to evaluate the biocompatibility of materials. The requirements of this response will vary from application to application; however, toxicity as well as inflammatory and possibly immune responses should typically be minimized. Initial studies in biocompatibility materials relied upon the potential use of bioinert substrates which attempted to reduce host specific interactions. While processes such as protein adsorption and the inflammatory response will occur to some degree with any implant, a bioinert material will form no or very little specific interactions with the surrounding environment, including the extracellular fluid or surrounding tissues. Since the interaction of materials with biological environment such protein, proteoglycan receptors on cell surfaces and biological molecules normally present in the extracellular matrix (ECM), through their interfaces are largely dependent on the surface chemistry and topography of the materials. Protein adsorption on the material surface is believed to be the initial state when a material contact with a biological environment. These mechanisms will influence the subsequent biological reactions including cell adhesion and proliferation. However, polymeric materials with different surface properties such as hydrophilicity or hydrophobicity, smooth or roughness surfaces and random or alignment direction may provide the different of cell response in vitro and in vivo. Therefore, understanding the influence of surface properties is critical, and control of protein–surface interactions continues to be an important factor for consideration in the design of biocompatible surfaces.

Recently, current research has been investigating the incorporation of bioactive materials which can intentionally interact with the biological environment

and influence such things as cell function. These interactions are often accomplished through surface modifications and functionalization with bioactive molecules such as extracellular proteins such as laminin fibronectin etc. In addition, the materials must exhibit suitable physical and mechanical properties closely matching the desired requirements such as modulus of elasticity, strength, structural integrity etc. need to match that of the neighboring tissue. The material selection plays a key role in the biomedical application. Synthetic polymers provide many advantages over natural polymers because they can be tailored to give a wider range of properties with predictable lot-to-lot uniformity and reliable source of raw materials. However, naturally occurring polymers normally exhibit better biocompatibility and low immunogenicity. Within the subdivision of synthetic materials, they can further divided into biodegradable and non-degradable materials. Biodegradable materials have been the more popular choice due to the elimination of a second surgery to remove the implanted scaffold. It is imperative that the rate of degradation coincide with the rate of new tissue formation. If the rate of degradation is too slow then new tissue formation will be impeded; however, if the rate of degradation is too fast then the mechanical stability of the scaffold and developing tissue will be compromised. The rate of degradation can be controlled to some extent by altering parameters such as polymer blends, and ratio of amorphous to crystalline segments. In order to more accurately mimic the natural ECM, research has also examined the electrospinning of natural materials such as: collagen, chitosan, gelatin, fibrinogen, chitin, and hyaluronic acid. However, these materials often lack the desired physical properties or are difficult to electrospin on their own, which has led to the development of hybrid materials, which consist of a blend of synthetic and natural materials.

Table 2.3 Typical biological materials with function integration (Kesong L, 2011)

Biological materials	Functions
Butterfly wing	Superhydrophobicity, directional adhesion, structural color, self-cleaning, chemical sensing capability, fluorescence emission functions
Brittlestar	Mechanical and optical functions
Cicada wing	Anti-reflection, superhydrophobicity
Fish scale	Drag reduction, superoleophilicity in air, superoleophobicity in water
Gecko foot	Reversible adhesive, superhydrophobicity, self-cleaning
Lotus leaf	Superhydrophobicity, low adhesion, self-cleaning
Mosquito compound eye	Superhydrophobicity, anti-reflection, anti-fogging
Nacre	Mechanical property, structural color
Peacock feather	Structural color, superhydrophobicity
Polar bear fur	Optical property, thermal insulation
Rice leaf	Superhydrophobicity, anisotropic wettability
Rose petal	Superhydrophobicity, structural color, high adhesion
Shark skin	Drag reduction, anti-biofouling
Spicule	Mechanical and fibre-optical properties
Spider capture silk	Water collection ability, mechanical property, elasticity, stickiness
Spider dragline silk	Mechanical property, supercontraction,torsional shape memory
Water strider leg	Durable and robust superhydrophobicity

2.6 Wound Dressing Material Based on Electrospinning of Nanofibers

2.6.1 Electrospinning Process (Payam Z, 2009)

In 1934, Formhals patented his first invention about an instrument for production of synthetic fibers using electrical power called "Electrospinning". This fibers fabrication technique is a unique approach using electrostatic forces to produce nanofibers which are very fine size pores and high surface area. Figure 2.4 (a) shows schematic setup of an electrospinning process that mainly composed of syringe, needle, high voltage generator and collector. At first, when a sufficient voltage is applied to the polymer solution, the storage of the charge will generate electrostatic-repulsive force to stretches the polymer droplet which is encountered by surface tension. At the critical point that the electrostatic force is dominant, the Taylor cone is formed before an eruption of the polymer jet as also shown in Figure 2.4 (b). Then Fluid jet is solidified by solvent evaporation and stretched by whipping process resulting from electrostatic repulsion to yield the ultra-thin fibers, if a molecular cohesion is sufficient to maintain a continuous fluid jet, and form into nonwoven nanofibrous webs on the collector surface. The important advantages of electrospinning technique are the production of very thin fibers to the order of few nanometers with large surface areas, ease of functionalization for various purposes, superior mechanical properties and ease of process as suggested by many experts in this field.

2.6.2 Process Parameters

Despite the electrospinning technique is relative ease of use; there are a number of process parameters that can greatly affect fiber formation and structure. Grouped in order of relative impact to the electrospinning process involves the applied voltage, polymer flow rate, and capillary-collector distance. All three parameters can influence on the formation of bead defect nanofibers.

2.6.2.1 Applied Voltage

The fiber diameters can be controlled by the applied voltage which the achieved results vary strongly with the polymer system. The strength of the applied electric field controls formation of fibers from several microns in diameter to tens of nanometers. Suboptimal field strength could lead to bead defects

in the electrospun fibers or even failure in jet formation. Based on the various previous works, it is evident that there is an optimal range of electric field strengths for a certain polymer/solvent system, as either too weak or too strong a field will lead to the formation of beaded fibers.

2.6.2.2 Polymer Flow Rate

Polymer flow rate also has an impact on fiber size, and additionally can influence fiber porosity as well as fiber shape. Additionally, at high flow rates significant amounts of bead defects were noticeable, due to the inability of fibers to dry completely before reaching the collector. Incomplete fiber drying also leads to the formation of ribbon-like (or flattened) fibers as compared to fibers with a circular cross section.

2.6.2.3 Capillary-collector Distance

While playing a much smaller role, the distance between capillary tip and collector can also influence fiber size. The fiber diameter decreased with increasing distances from the Taylor cone to the collector. Additionally, morphological changes can occur upon decreasing the distance between the syringe needle and the substrate by forming beaded on as-spun fibers, which can be attributed to inadequate drying of the polymer fiber prior to reaching the collector.

2.6.3 System Parameters

In addition to the process parameters a number of system parameters play an important role in fiber formation and structure. System parameters involve molecular weight, molecular weight distribution, architecture of polymer and solution properties. Especially, the solution properties play an important role on the electrospinning process. In relative order of their impact on the electrospinning process these include polymer concentration, solvent volatility and solution conductivity.

2.6.3.1 Polymer Concentration

The polymer concentration influences both the viscosity and the surface tension of the solution. The formation of nanofibers through electrospinning is based on the uniaxial stretching of a viscoelastic solution. For instance, the polymer solution must have a concentration high enough to cause polymer entanglements. However, the viscosity dose not so high that prevents

polymer motion induced by the electric field and not too be low to prevent the charged jet from collapsing into droplets before the solvent has evaporated. Further investigations on polymer concentration and viscosity are observed that fibers become more uniform and assume a cylindrical shape with increasing polymer concentration in solution and fiber diameters also increase significantly with increasing polymer concentration. At lower concentrations, increasingly thinner fibers are formed with additional beads along the fiber axis. However, at very high dilution, the fiber formation no longer takes place. It is not possible to make a general recommendation for particular concentrations and the resulting viscosities, because the ideal values of these parameters vary considerably with the polymer–solvent system.

2.6.3.2 Solvent Volatility

Choice of solvent is also critical as to whether fibers are capable of forming, as well as determining surface topography. If the fluid jet is collected prior to complete solvent evaporation, the deformable fibers may either flatten upon impact with the surface of a collector or adhere to other fibers. If the arriving jet or fiber lands on previously collected fibers the still fluid material can merge and coalesce at crossing point to create interconnected network which appear to be useful in some situations where a well established network is desirable.

2.6.3.3 Solution Conductivity

Solution conductivity can influence fiber size within 1 to 2 orders of magnitude. Solutions with high conductivity will have a greater charge carrying capacity than solutions with low conductivity. Therefore, the fiber jet of highly conductive solutions will be subjected to a greater tensile force in the presence of an electric field than will a fiber jet from a solution with a low conductivity. The highly conductive solutions were extremely unstable in the presence of strong electric fields, which led to a dramatic bending instability as well as a broad diameter distribution. However, semi-conducting and insulating liquids such as paraffinic oil produced relatively stable fibers. By adding a small amount of salt or ion, the electric force exerted on the jet also increase and attributed to decrease in the mean fiber diameter. Generally, the radius of the fiber jet is inversely related to the cube root of the solution conductivity.

The above description of the process suggests that many parameters can influence on the electrospinning apparatus. By appropriately varying all or some of these parameters, fibers are successfully electrospun.

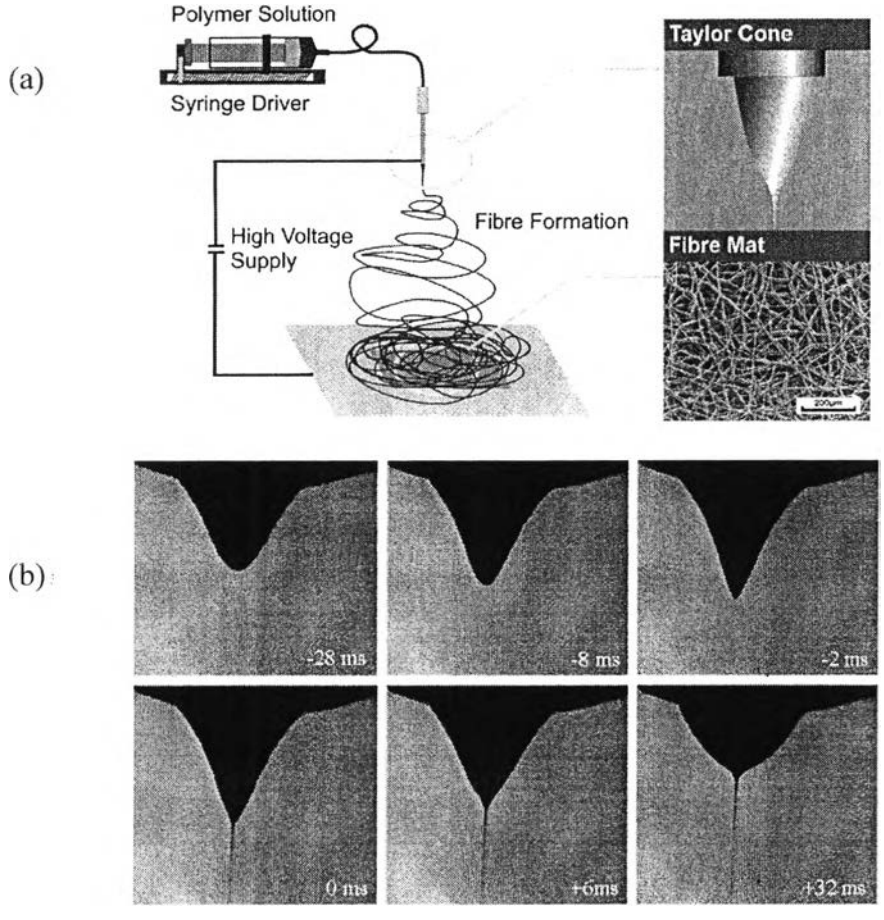


Figure 2.3 (a) Schematic of electrospinning process (b) Shape evolution of poly(ethylene oxide) solution in the electrospinning process which time zero is when the first eruption appears. (Reneker and Yarin 2008)

2.6.4 Potential Use of Electrospinning Process in Wound Dressing Application

The wound dressing materials produced by electrospinning technique have unique properties as compared to the topical dressings produced by conventional methods. These properties are as follows (Payam Z, 2009):

Hemostasis: Nanofibrous wound dressings with their small porous and high effective surface area to volume ration can promote hemostasis phase. Normally, this stage could be achieved by hemostatic agent. But by electrospinning technique, the enhancement of this stage is only due to nanofibrous structure of the dressing material.

Absorbability: According to the high surface area to volume ratio of the nanofibers, they can exhibit more water absorption than typical film dressings that only show a few of water absorption. Therefore, the nanofibrous dressings can be able to apply on the wound which have a lot of exudates more efficiently than the topical film dressings, if hydrophilic polymers are employed.

Semi-permeability: The porous structure of an electrospun dressing is excellent for the respiration of cells which does not lead to wound desiccation. This dressing is more suitable to deal with the moisture management for the wound. In addition, the small pore size of electrospun can effectively prevent bacteria from the wound to against infection. High gas permeation can also possess on electrospun nanofibrous membrane wound dressings which have the opportunity to provide effective protection with environmental control of wound

Conformability (3 D-dressing): Conformability or the ability to conform to the contour of wound is one of the parameters that need to be clinically assessed for the flexibility and resiliency of the medical dressings. In the field of textile, it is widely known that the conformability of a fabric is closely related to the fiber fineness. Finer fiber fabrics are easier to fit to complicated 3-D contours. Therefore, wound dressing that was produced from ultra fine fibers can provide excellent conformability which has better coverage and protection of the wounds from infection.

Functional ability: By electrospinning technique, Multifunctional bioactivity can be achieved easily by incorporating therapeutic compounds into the nanofibers via a co-spinning process. This special dressing are readily available because of the ease of production that depending on the stage of treatment and the intended functionality of the drugs, active components including pharmaceutical compounds such as antiseptics, antifungal, vasodilators (e.g. minoxidil used to promote wound epithelialization and neovascularization), growth factors (e.g. FGF,

EGF, and TGF), and even cells (e.g. keratinocytes) can be integrated into the same nanofibrous substrate. Another property of electrospinning is that, unlike other commercial dressings, which was facbicated into multilayer configuration to attain desired objectives and functions such as control drug delivery, medication, growth factors, and so forth, such functionalities can be achieved by electrospinning of blended materials into one layer to obtain multifunctional wound dressing. These properties introduce an extra benefit of reduced frequency in changing dressings of the wound which may interfere regenerating of neotissue.

Scar-free: Furthermore, electrospun nanofibers can be applied on healing wounds without leaving scars. Many researchers and clinicians tried to study how to heal a wound with as little scar as possible although this is difficult to achieve. Electrospinning technique was used to fabricate nanofibrous dressings which would promote normal skin to grow immediately instead of scarring of wound because the biodegradable fibrous scaffolds would provide the skin cells a better road map for self-repair. From a tissue-engineering point of view, nanofibrous structure has excellent cell conductivity and improves biocompatibility, which will facilitate wound healing and skin regeneration due to the biomimically of the electrospun structure. The polymers used for production of wound dressing, which can be spun by electrospinning process, can be selected in relation to the required properties such as preparation of nanofibrous membranes, the identification of their morphology, thermal and mechanical properties, chemical treatment or surface modification for improvement of their compatibility and cellular activities.

2.7 The Future of Wound Dressings

There are many topical wound dressings available in the market that almost fit to types of wound. But dressing have to continue to advance in terms of the functions and benefits for enhancing the wound healing process to heal as fast as possible or for addressing impediments to wound healing such as bacteria or chemical imbalances for non-healing wound. Many modern dressings offer promise as delivery vehicles for active ingredients or even for allogeneic cells, which may provide a specific benefit, with the dressing itself acting to maintain a suitable

environment. The incorporation of active ingredients into topical dressings poses the questions of whether the dressing should be regulated as a medical device or a drug. Currently, this, the answer to this question, has hinged on whether the primary mode of action derives from the dressing material or from the ingredient. The aforementioned antimicrobial dressings are currently regulated as devices. Furthermore, in the coming future, the evolution of medical and material technology can be well synchronized enough to make the universal wound dressing and then the quote of "Only one of single dressing c for all types of wounds" can be possible.