CHAPTER II
LITERATURE REVIEW

2.1 wound

The definition of wound is a circumscribed injury which is caused by an external force and it can involve any tissue or organ ; surgical, traumatic It can be mild, severe, or even lethal. In case of would classification have many ways such as acute wound and chronic wound.

Figure 2.1 Different types of wound.

2.1.1 Wound Healing Process

Wound healing is continuous and complex process, involving the interaction of biological system an immunological with coordinated interaction. It is divided into four phases : (1) Homeostasis, (2) Inflammatory, (3) Proliferation and (4) Remodeling (Velnar T. *et al.*, 2009)

2.1.1.1 *Homeostasis*

This phase occur immediately after injury. The aim of this phase is to limit blood loss by platelet aggregation and clot formation. The blood clot formation consists of fibronectin, fibrin, vitronectin and thrombospondin. The important of clot formation is also in term of providing as matrix for cell migration in the subsequent phases of the homeostasis and inflammatory phases

2.1.1.2 *Inflammatory*

This inflammatory phase, produced immune barrier against invading of microorganisms. Neutrophil infiltrate in the wound site to prevent

infection by phagocytosis. The function of phagocytosis activity is to destroy and remove bacteria, foreign particles and damaged tissue by releasing proteolytic enzymes and oxygen derived from free radicals species. The excess of ROS cause the harmful on cells and tissue from oxidative damage by involving NADPH mechanism which generate from neutrophil accumulation in wound area Moreover, the inflammatory response regulate cells and provide an abundant reservoir of potent tissue growth factors, particularly TGF-β, as well as other mediators (TGF-α, heparin binding epidermal growth factor, fibroblast growth factor (FGF), collagenase), activating keratonocytes, fibroblasts and endothelial cells (Velnar T. *et al.*, 2009)

2.1.1.3 *Proliferation*

The proliferative phase involves the fibroblast migration and deposition of newly synthesized extracellular matrix to replace the loss tissue. First, the formation of granulation occurs in this stage. Fibroblast synthesized collagen, act as foundation for intracellular matrix formation in the wound and many new capillaries are formed. After that, the open wound was closed by wound contracture. Myofibroblasts composed of actin and myosin which generate contractile force resulting in smaller wound size (Stojadinovic A. *et al.*, 2008). Next, migration of epithelial cells from wound edge, cells meet resulting in migration stops and the basement membrane starts to form (Velnar T. *et al.*, 2009)

2.1.1.4 *Remodeling*

This phase is final step of wound healing process. This phase is responsible for controlling the equilibrium of synthesis and breakdown collagen. The tensile strength of wound derives from collagen collection. Collagen fibers contribute to the approximately 80 % of the original strength compared with unwound tissue (Velnar T. *et al.*, 2009)

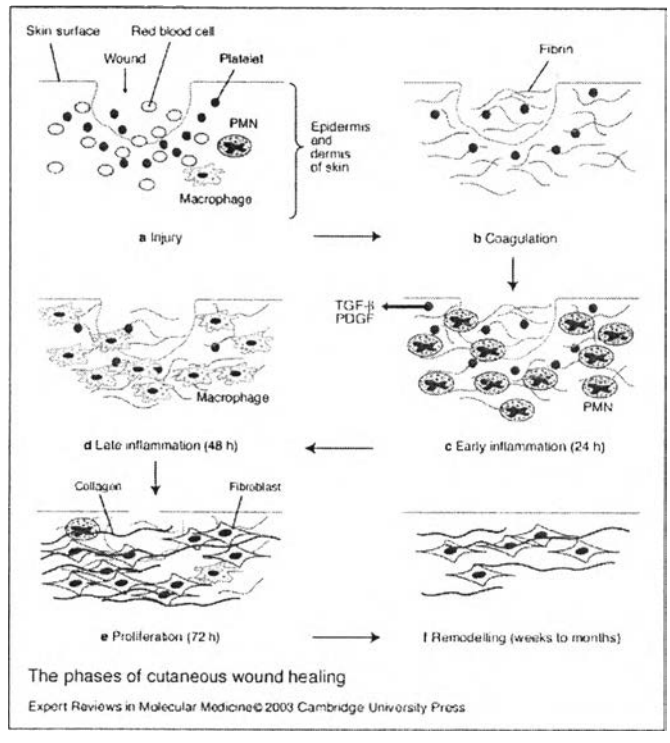
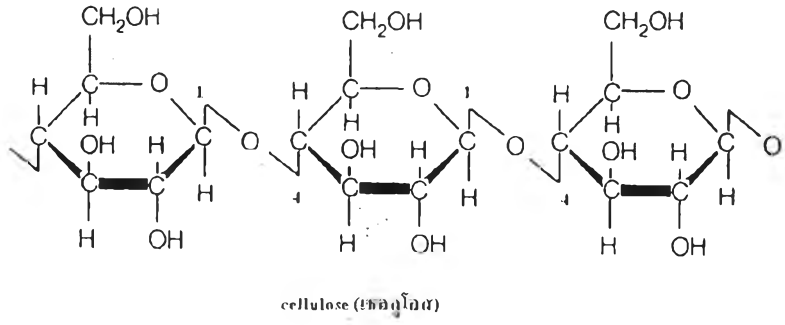


Figure 2.2 The phases of continuous wound healing.

2.2 Bacterial Cellulose

2.2.1 Cellulose

Cellulose is an organic compound with the formula $(C_6H_{10}O_5)_n$, a polysaccharide consisting of a linear chain of several hundred to over ten thousand β(1→4) linked D-glucose units, as shown in Figure 2.1. Cellulose is the most abundant biopolymer in the earth with an estimated output of over 10^{11} tons per year. Most of its biosynthesis takes place in the cellular walls of plants, but four sources are known, animal, bacterial, chemical and enzymatic.



cellulose (เซลลูโลส)

Figure 2.3 The structural of cellulose.

2.2.2 Principal Pathways to Cellulose

There are four different pathways to synthesize the biopolymer cellulose that are described schematically in Figure 2.2. The first on is the most popular and industrial important isolation of cellulose of plants including separation processes to remove lignin and hemicelluloses. The second way is biosynthesize of cellulose by different types of microorganisms such as algae (Vallonia), fungi (Saprolegnia, Dictystelium, Discoideum) and bacteria(Acetobacrter, Achromobacter, Aerobacter, Rhizobium). The third way is enzymatic in vitro synthesis starting from cellobiosyl fluoride. The last way is the first chemosynthesis from glucose by ring-opening polymerization of benzylated and pivaloylated derivatives (Klemm D. *et al.*, 2001).

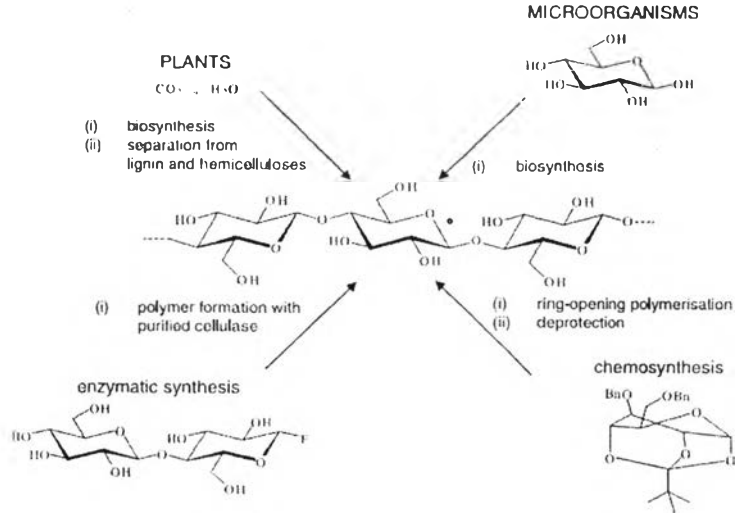


Figure 2.4 Pathways to the cellulose

In this research, we investigated on cellulose that can be synthesized by bacteria, it was called bacterial cellulose (BC). Bacteria which used in this research is *Acetobacter xylinum* genus because it is the most efficient producer of cellulose. There are many bacterial cellulose producers that can be used for biosynthesis of bacterial cellulose but the structures of bacterial cellulose are different depend on the type of bacterial cellulose producers. All of these bacterial cellulose producers shown in Table 1.1. (Jonas & Farah, 1998).

Table 2.1 Bacterial cellulose producers (Jonas & Farah, 1998)

Genus	Cellulose Structure
Acetobacter	extracellular pellicle composed of ribbons
Achromobacter	fibrils
Aerobacter	Fibrils
Agrobacterium	short fibrils
Alcaligenes	Fibrils
Pseudomonas	no distinct fibrils

Rhizobium	short fibrils
Sarcina	amorphous cellulose

2.2.3 Bacterial Cellulose Synthesis using Acetobacter Xylinum

Figure 2.5 Characteristic of Acetobacter Xylinum

For the first time, the bacterium *A. xylinum* was decribed in 1886 by Brown. He identified a gelatinous mat formed in the course of vinegar fermentation on the surface of the broth as chemically equivalent to cell-wall cellulose.

Acetobacter xylinum is a gram negative, rod-shaped, non-pathogenic and aerobic bacterium which is interested for many studies due to the large quantity of cellulose product. The cellulose synthesized by *A. xylinum* is identical to that made by plants in the respect to molecular structure. However, the secreted polysaccharide is free of lignin, pectin and hemicelluloses as well as other biogenic products, which are associated with plant cellulose. Additional, extracellularly synthesized microbial cellulose differs from plant cellulose with respect to its high crystallinity, high water absorption capacity and mechanical strength in the wet state, ultrafine network structure, mold ability in situ and in an initial wet state. (Dieter K. *et al.*, 2001). Figure 2.3., SEM images shown difference between the structure of bacterial cellulose and the structure of plant cellulose.

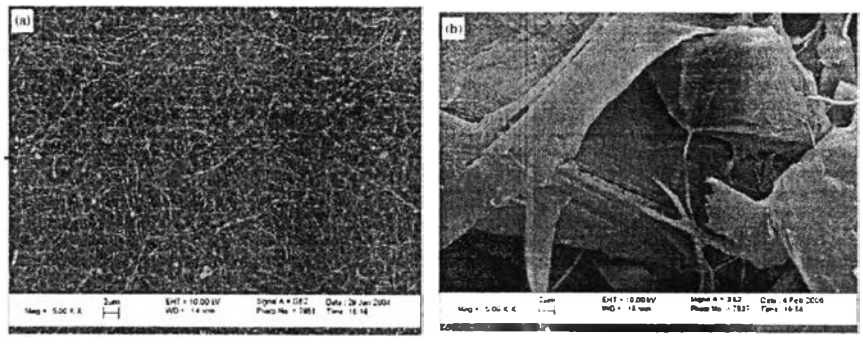


Figure 2.6 SEM image (a) plant cellulose and (b) bacteria cellulose (Czaja M. *et al.*, 2006).

The cellulose formation includes five fundamental enzyme mediated steps: the transformation of glucose to UDP-glucose-6-phosphate and glucose-1-phosphate and finally the addition of UPD-glucose to the end of growing polymer chain by cellulose synthase a shown in Figure 2.4. Cellulose synthase (UPD-glucose: 1,4-β-D-glycosyltransferase) is regarded as the essential enzyme in the synthesis process. It is subjected to a complicated regulation mechanism, which controls activation and inactivation of the enzyme (Klemm D. *et al.*, 2001)

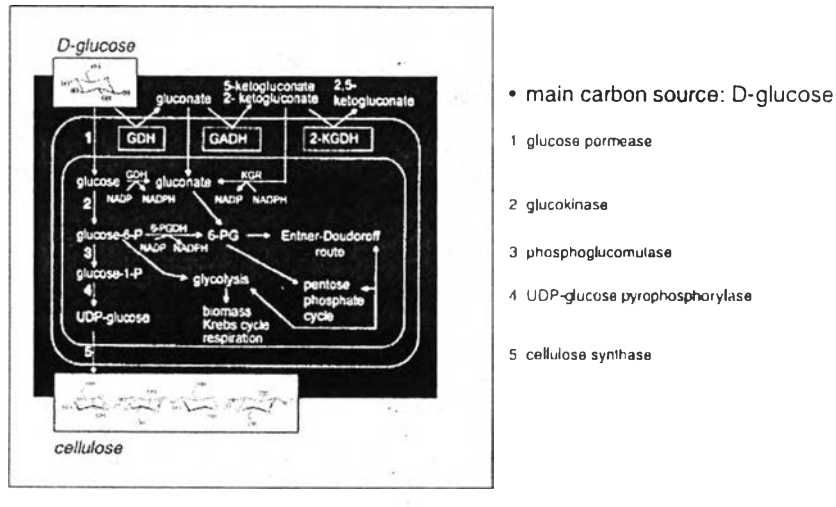


Figure 2.7 Pathways of carbon metabolism in *Acetobacter xylinum*.(Klemm D. *et al.*, 2001).

A. xylinum forms the cellulose between the outer and the cytoplasma membrane. The cellulose-synthesizing complexes or terminal complexes (TC) are linearly arranged, and in association with pores at the surface of the bacterium. In the first step of cellulose formation glucan chain aggregates consisting of approximately 6-8 glucan chains are elongated from the complex. These subelementary fibrils are assembled in the second step to form microfibrils followed by their tight assembly to form a ribbon as the third step that presented in Figure 2.5. The Matrix of the interwoven ribbons constitutes the bacterial cellulose membrane or pellicle. Bacteria cellulose ribbon produced by one bacterial cell as shown in Figure 2.6 and Figure 2.7 demonstrate that *A. xylinum* cells are distributed throughout the network of the cellulose ribbons.

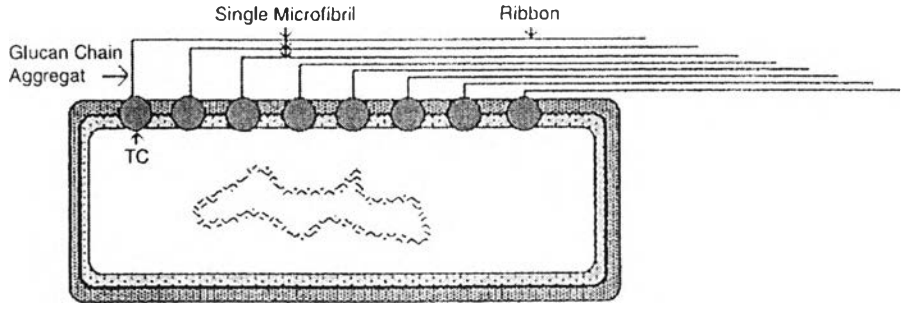


Figure 2.8 Formation of bacterial cellulose. (Klemm D. *et al.*, 2001).

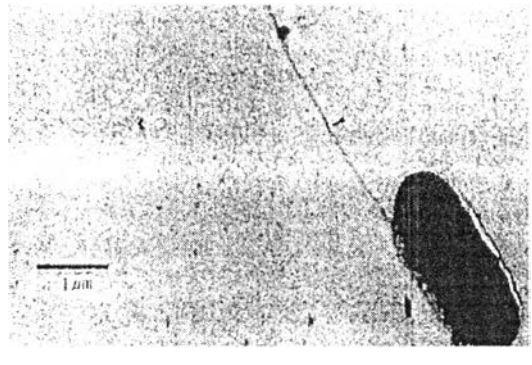


Figure 2.9 TEM image of bacterial cellulose ribbon produced by a bacterial cell.(Klemm D. *et al.*, 2001).

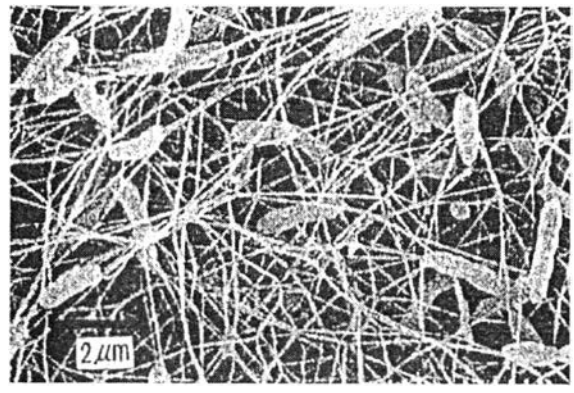


Figure 2.10 SEM image of a bacterial cellulose network including the bacterial cells. (Klemm D. *et al.*, 2001).

2.2.4 Structure of Bacterial Cellulose

Cellulose is an unbranched polymer of β(1→4) linked glucopyranose residues. Extensive research on BC revealed that it is chemically identical to plant cellulose (PC), but its macromolecular structure and properties differ from the latter (Figure 2.8). In table 2.2 shown the difference between bacterial cellulose and plant cellulose.

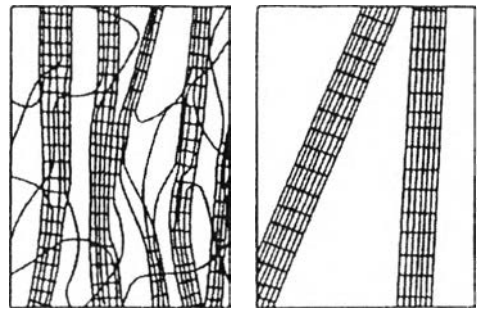


Figure 2.11 Schematic model of BC microfibrils (right) drawn in comparison with the 'fringed micelles'; of PC fibrils (left) (Iguchi M. *et al.*, 2000).

Table 2.2 Comparison between bacterial cellulose (BC) and plant cellulose (PC) (Edison P. *et al.*, 2008)

Properties	PC	BC
Fibre width	$1.4–4.0 \times 10^{-2}$ mm	70–80 nm
Crystallinity	56–65%	65–79%
Degree of polymerization	13 000–14 000	2 000–6 000
Young's modulus	5.5–12.6 GPa	15–30 GPa
Water content	60%	98.5%

In the bacterial cellulose biosynthesis, the cellulose chains (formed by glucose units linked through β(1→4)glucosidics bonds) interact through hydrogen bonds, assuming a parallel orientation among them. The structure and rigidity of bacterial celluloses is provided by the OH intra and intermolecular hydrogen bonds as shown in Figure 2.9.

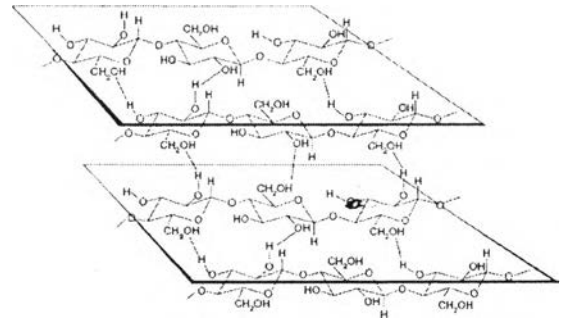


Figure 2.12 Outline of intra and intermolecular hydrogen bonds among cellulose chains. (Edison P. *et al.*, 2008).

The physical and mechanical properties of bacterial cellulose arise from the unique 3-D ultrafine network structure. Preliminary study has measured the Young's modulus of bacterial cellulose as high as (>15 GPa) , in any direction across the plane of sheet. It is considered that the high mechanical strength arise from the high density of interfibrillar hydrogen-bonds, due to the very fine fibrils and large

contact area. In addition, there is no significant effect of varying cultivation time and amount of cellulose content on mechanical properties. Furthermore, the pulp derived from bacterial cellulose can enhance reinforcement to the ordinary cotton lint pulp (Yamanaka S. *et al.*, 1989).

This unique structure can also enhance to absorb a large amount of water (up to 200 times of its dry mass) because of the large surface area. Moreover, bacterial cellulose in wet state show great elasticity, high wet strength, high conformability and transparency (Klemm D. *et al.*, 2001; Czaja W. *et al.*,2006).

The production of bacterial cellulose has quite successfully in static culture that resulted in pellicle formed on the surface of static culture as shown in Figure 2.10. But there are low productivity and labor intensive. In case of, bacterial cellulose in agitated culture produced in well-dispersed slurry as irregular mass as shown in Figure 2.10. (Hestrin&Schram, 1954). The agitated culture has not been successful in bacterial cellulose production due to its low yield (Byrom D. 1991). Another problem for agitated culture is associated with the culture instability that resulted in loss of cellulose producing cells because of non-producing mutants (Valla &Kjosbakken, 1982). However, some researchers suggested that the agitated culture might be suitable for economical scale production (Yoshinaga F. *et al.*, 1997).

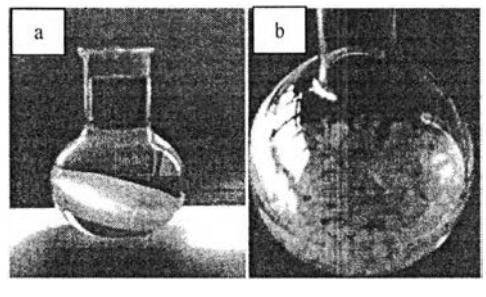


Figure 2.13 BC pellicle formed (a) in static culture and (b) in agitated culture (Bielecki *et al.*,2002).

2.2.5 *Bacterial Cellulose in Wound Dressing Applications*

The physical and mechanical propertied of bacterial cellulose membranes arise from their unique structure, which differs significantly from the structure. Basically, well-separated nano and micro fibrils of bacterial cellulose create an extensive surface area which allows it to hold a large amount of water while maintaining a high degree of conformability. The hydrogen bonds between these fibrillar units stabilize the whole structure and give it a great deal of mechanical strength. Even though plant cellulose is composed of microfibrils which are similar to those found within bacterial cellulose, the plant cellulose microfibrils are part of a larger aggregation of the cell wall. Thus, bacterial cellulose can absorb much higher volumes of liquid than plant cellulose. BC can be considered an ideal material for high quality wound dressing. Table 2.3 summarizes most of the physical and mechanical properties of bacterial cellulose which characterize it as an ideal wound dressing material. Wound repair is a dynamic process that associates with a complex interaction of various cell types, extracellular matrix (ECM) molecules, soluble compounds. Typically, the process of wound healing has been divided into four phases: homeostasis, inflammation, granulation tissue and remodeling (Eming S.A. *et al.*, 2002).

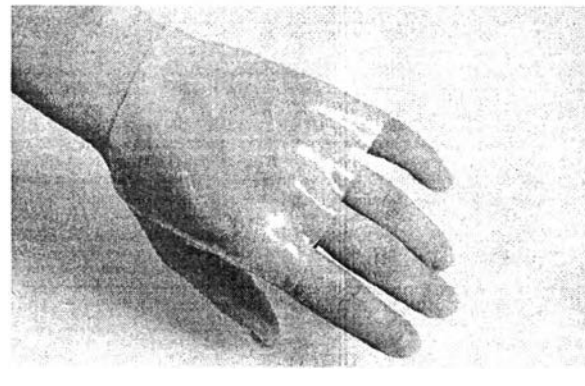


Figure 2.14 Wound dressing from bacterial cellulose.

Wound dressings can be classified into traditional and advance wound dressings (moist wound dressings such as hydrocolloid, alginate and hydrogel). Advance wound dressings have been developed because it provid moist environment which facilitate for wound healing process (Boateng J.S. *et al.*, 2008). In 1962, George Winter found that the re-epithelization was accelerated if the wound was kept moist. Since then, almost effective wound dressing are designed to maintain a moist environment within the affected region. Moist dressing are permeable to water, and this property has advantages for wound healing. For example, high water vapor permeable dressing show enhanced healing, probably due to an increased concentration of growth-promoting factors within the exudates and to the creation of a more extensive ECM of fibrinogen and fibronectin. The highly water vapor permeable wound dressing (PEU) can promote a high amount of fibrinogen and fibronectin which associated with accelerated epithilization during wound healing process (Jonkman M.F. *et al.*, 1990). In addition, the moist wound environment can enhance eshar and clot removal, re-epithelialization and collagen synthesis which promote proteolytic environment and the growth factor over the dry wound(Chen W.Y.J. *et al.*, 1992). Thus, moist wound dressings have been developed as an improvement on the traditional wound dressings.

Due to its unique properties, bacterial cellulose has shown great potential for using as wound dressing material as shown in table 2.3. Bacterial cellulose actually performed better than conventional wound dressings in 1.conforming to the wound surface (excellent molding to all facial contours and a high degree of adherence even to the contoured parts such as nose, mouth, etc., 2. Maintaining a moist environment within the wound, 3. Significantly reducing pain, 4.Accelerating re-epithelialization and the formation of granulation tissue, and 5.Reducing scar formation (Czaja W. *et. al.*, 2007). These BC membranes can be created in any shape and size, which is beneficial for the treatment of large and difficult to cover areas of the body.

Many studies have reported on the successful of bacterial cellulose as wound dressing. The product called Biofill has been used for temporary skin substitutes. It can help to promote healing of many skin injuries treatments such as

basal cell carcinoma, skin graft, severe body burns, facial peeling, sutures, dermabrasions, skin lesions, chronic ulcers and both donor and receptor sites in graft (Fontana J.D. *et al.*, 1990). Farah, *et al.*,(1990) described many advantages of Biofill product on the lesion region such as close adhesion to body location, enhancing the absorption of exudates, reduced pain (isolated nerve ending), reducing scar formation, no allergic reaction and easily stored.

Another bacterial cellulose product is Xcell. Unlike other wound dressing products in the market, Xcell product has ability to manage the moisture balance by absorbing excess exudates and donating moisture in wound area. Alvarez O.M. *et al.* (2004) reported that Xcell success with the chronic venous ulceration treatment. The combination of bacterial cellulose wound dressing and compression bandage resulted in less wound pain, improved autolytic debridement and developed of granulation tissue as compared with standard wound care. Moreover, Heasley D. *et. al.* (2003) proved that Xcell can be effectively used to treat on the diabetic foot ulcers.

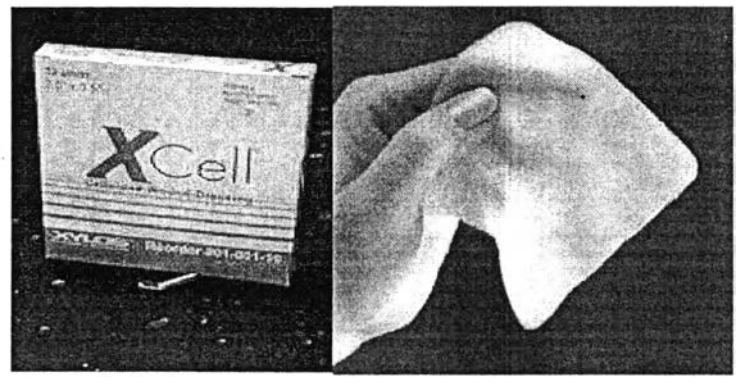


Figure 2.13 The product of bacterial cellulose under Xcell brand.

Another interesting and important advantage of the bacterial cellulose dressing includes its transparency, which facilitate for observation in the healing progress.

Table 2.3 Properties of bacterial cellulose and how they relate to the properties of an ideal wound dressing material. (Czaja W. *et. al.*, 2007)

Properties of ideal wound care dressing	Properties of bacterial cellulose
Maintain a moist environment at the wound/dressing surface	High water holding capacity (typical membrane can hold up to 200 g of its dry mass in water); high water vapor transmission rate
Provide physical barrier against bacterial infections	Nanoporous structure does not allow any external bacteria to penetrate into wound bed
Highly absorbable	Partially dehydrated membrane is able to absorb fluid up to its original capacity
Sterile, easy to use, and inexpensive	Membranes are easy to sterilize (by steam or γ-radiation) and package. The cost of production of 1 cm^2 is \$0.02
Properties of ideal wound care dressing	Properties of bacterial cellulose
Significantly reduce pain during treatment	The unique BC nanomorphology of never-dried membrane promotes specific interaction with nerve endings
Provide porosity for gaseous and fluid exchange	Highly porous material with pore sizes ranging from several nanometers to micrometers
Nontoxic, nonpyrogenic, and biocompatible	Biocompatible, nonpyrogenic, nontoxic
Provide high conformability and elasticity	High elasticity and conformability

Available in various shapes and sizes	Ability to be molded in situ
Provide easy and close wound coverage,but allow easy and painless removal	High elasticity and conformability

2.2.6 Literature Review bacterial cellulose in biomedical application.

Chiaoprakobkij N. *et al., 2004* develop to studied bacterial cellulose/alginate composite sponges used in medical application. They found the bacterial cellulose sponge with 30% alginate had a good tear resistance for sewing. The bacterial cellulose composite sponge is a promising material for use as a non-adherent hydrogel dressing due to many advantages in terms of skin tissue compatibility, excellent water uptake ability, and high mechanical strength and stability in both water and PBS buffer.

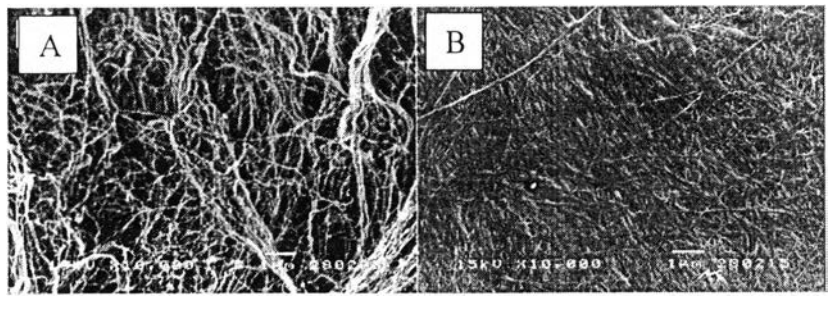


Figure2.16 (A) cellulosic fibers after homogenization (B) surface morphology of the BC/alginate sponge.

Maneerung T. *et al., 2007* develop to studied bacterial cellulose/silver nanoparticles composite foe used in wound dressing. Bacterial cellulose a better wound healing but bacterial cellulose itself has no antimicrobial activity to prevent wound infection. In this work used A recent study showed that impregnation, instead of coating the wound dressing with silver nanoparticle or nanocrystal improved the

antimicrobial activity of the wound dressing and lowered possibility of the normal human tissue damage.

Saibuatong O. *et al., 2009* develop to studied bacterial cellulose/ Novo aloe vera composite. They adding aloe vera gel in the culture medium during biosynthesis using Acetobacter xylinum in static cultivation. a fibre-reinforced bio-polymer film displayed significantly improved properties in mechanical strength, crystallinity, water absorption capacity and water vapor permeability in comparison to those of the unmodified BC film. The average pore size of the modified film either in the dry or re-swollen form was approximately reduced to 1/5 of those of the unmodified BC films with a narrow pore size distribution.

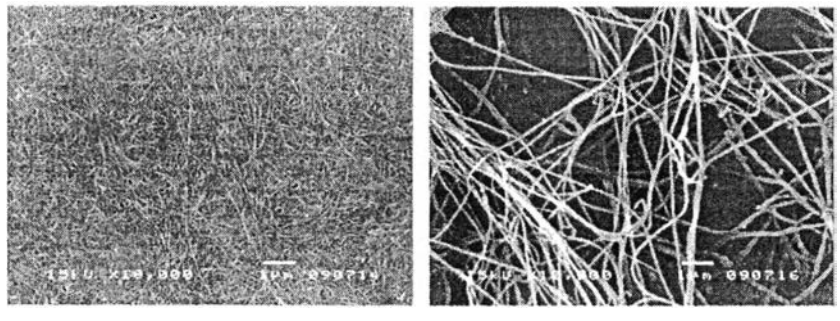


Figure2.17 SEM images of surface morphology of BCA-30% aloe vera film in dry form (left) and re-swollen form (right).

Meftahi A. *et al., 2009* develop to studied bacterial cellulose/ cotton gauze composite. microbial cellulose formed coated on cotton gauze samples during its biosynthesis in a static medium (Hestrin & Scharm) for 6 days by Acetobacter Xylinium. cotton gauze coating with microbial cellulose increases water absorbency and wicking ability over 30%, and reduces drying time about 33%. It can be concluded that covering of cotton gauze with microbial cellulose can promote some important characteristics of it specially for wound dressings.

Bin W. *et al., 2010* develop to studied bacterial cellulose (freeze dry) after that immersed in a benzalkonium chloride solution. results showed that the drug-loading capacity of the BC dry film was about 0.116mg/cm2 when soaked in 0.102% benzalkonium chloride solution. High water absorbing capacity, an

important quality for wound dressings was also achieved with a swelling ratio of 26.2 in deionized water and of 37.3 in saline solution. With respect to the antimicrobial effect, a stable and prolonged antimicrobial activity for least 24 h was obtained especially against Staphylococcus aureus and Bacillus subtilis, which were general Gram-positive bacteria that found on the contaminated wound.

Mazhar U.I. *et al., 2012* develop to studied bacterial cellulose/montmorillonite (MMT) composite. For potent antibacterial activity and potential therapeutic value in wound healing and tissue regeneration. These modified MMT, Na-MMT, Ca-MMT and Cu-MMT. BC/MMT sheets as wound dressings and regeneration materials with antibacterial properties for therapeutic applications without any side effects.

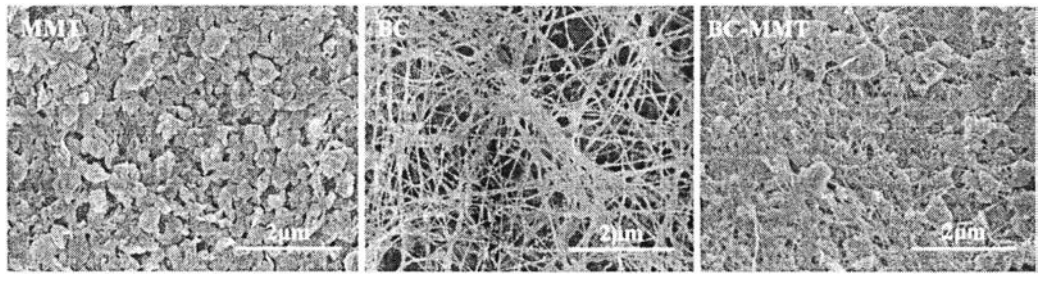


Figure 2.18 FE-SEM morphology of MMT, BC and BC-2 %MMT composites.

Wen C.L. *et al., 2013* develop to studied bacterial cellulose/ chitosan membranes composite. prepared by immersing BC in chitosan followed by freeze-drying. Results indicated that membranes maintained proper moisture contents for an extensive period without dehydration. The tensile strength and elongation at break for composite were slightly lower while the Young's modulus was higher. Cell culture studies demonstrated that had no cytotoxicity. In the antibacterial test, the addition of chitosan in BC showed significant growth inhibition against *Escherichia coli* and *Staphylococcus aureus*.

2.3 Plasma Technology

2.3.1 Basic Principle

Plasma, a quasi-neutral gas, is considered to be the fourth state of matter, following the more familiar states of solid, liquid & gas and constitutes more than 99% matter of the universe. It is more or less an electrified gas with a chemically reactive media that consists of a large number of different species such as electrons, positive and negative ions, free radicals, gas atoms and molecules in the ground or any higher state of any form of excited species (figure 2.11). It can exist over an extremely wide range of temperature and pressure. It can be produced at low-pressure or atmospheric pressure by coupling energy to a gaseous medium by several means such as mechanical, thermal, chemical, radiant, nuclear, or by applying a voltage, or by injecting electromagnetic waves and also by a combination of these to dissociate the gaseous component molecules into a collection of ions, electrons, charge-neutral gas molecules, and other species. It is thus an energetic chemical environment that combines particles and radiations of a diverse nature, an incredibly diverse source of chemistry that is normally not available in other states of matter. Parallel to the generation of plasma species, loss processes also take place in the plasma. In fact, all energy ends up as heat with a small fraction invested in surface chemistry.

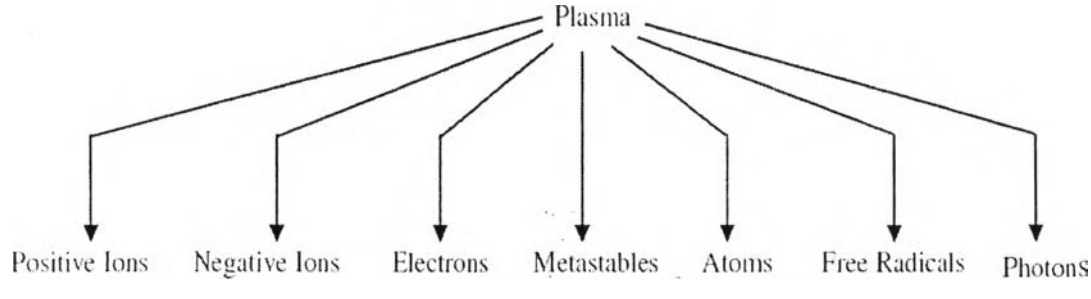


Figure 2.19 Constituents of plasma.

Plasma principle is a mixture of gases consisted of charged particles with a roughly zero net electrical charge. When apply a high energy source, like electric field, to a high voltage (HV) electrode, an ionization process occurs, thus generating a number of ionized species. The plasma has been used to modify the surfaces of both organic and inorganic substrates. This technique is a dry process and is operated under a wide range of pressure. Moreover, the surface modification by the plasma treatment occurs at the outermost surface, so it does not change the bulk properties of materials. Due to its several advantages over the conventional chemistry methods, the plasma becomes more interested.

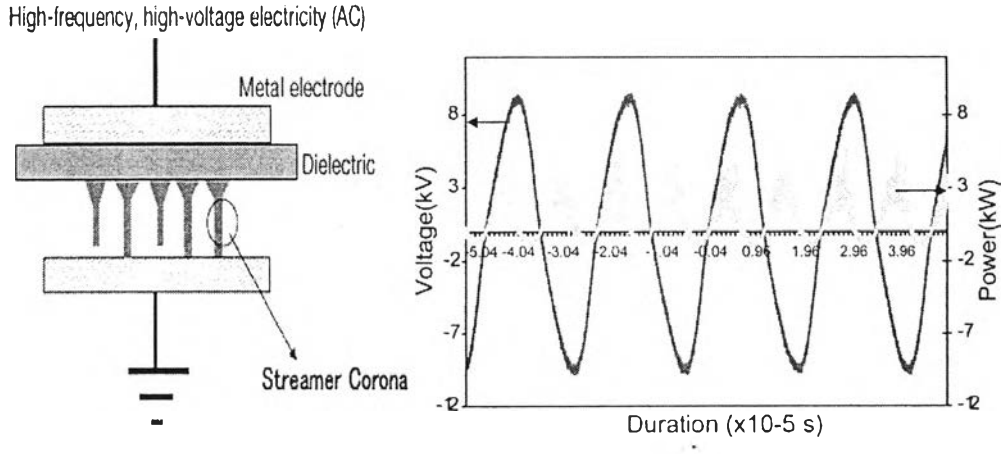


Figure 2.20 Basic of generate plasma and Typical voltage and power waveform outputs for DBD plasma system.

2.3.2 Classification of Plasma

Plasmas can be distinguished into two main groups i.e., the high temperature or fusion plasmas and the so called low temperatures or gas discharges. High temperature plasma implies that all species (electrons, ions and neutral species) are in a thermal equilibrium state. Low temperature plasma is further subdivided into thermal plasma, also called quasi-equilibrium plasma, which is in a local thermal equilibrium (LTE) state, and non-thermal plasma (NTP), also called non equilibrium plasma or cold plasma.

Thermal plasmas (TP) are characterized by an equilibrium or near equality between electrons, ions and neutrals. Commonly employed thermal plasma generating devices are those produced by plasma torches, and microwave devices. These sources produce a high flux of heat and are mainly used in areas such as in plasma material processing and plasma treatment of waste materials. High temperature of TPs can process even the most recalcitrant wastes including municipal solids, toxic, medical, biohazard, industrial and nuclear waste into elemental form, ultimately reducing environmental pollution caused due to them. But for several technological applications, the high temperature characteristic of TPs is neither required nor desired, and in some cases it even becomes prohibitive. In such application areas, cold plasmas become more suited.

Cold plasmas refer to the plasmas where most of the coupled electrical energy is primarily channeled to the electron component of the plasma, thereby producing energetic electrons instead of heating the entire gas stream; while the plasma ions and neutral components remain at or near room temperature. Because the ions and the neutrals remain relatively cold, this characteristic provides the possibility of using cold plasmas for low temperature plasma chemistry and for the treatment of heat sensitive materials including polymers and biological tissues. The remarkable characteristic features of cold plasma that include a strong thermodynamic non- equilibrium nature, low gas temperature, presence of reactive chemical species and high selectivity offer a tremendous potential to utilize these cold plasma sources in a wide range of applications.

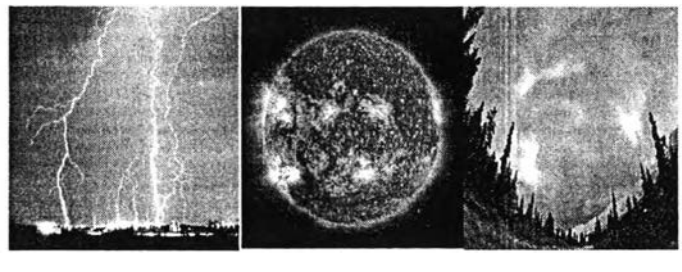


Figure 2.21 Different types of plasma.

2.3.3 Dielectric Barrier Discharge (DBD) Plasma

Dielectric barrier discharge, also referred to as barrier discharge or silent discharge is a specific type of AC discharge, which provides a strong thermodynamic, non-equilibrium plasma at atmospheric pressure, and at moderate gas temperature. It is produced in an arrangement consisting of two electrodes, at least one of which is covered with a dielectric layer placed in their current path between the metal electrodes. The presence of one or more insulating layer on/or between the two powered electrodes is one of the easiest ways to form non-equilibrium atmospheric pressure discharge. Due to the presence of capacitive coupling, time varying voltages are needed to drive the DBD. One of the major difference between the classical and a DBD discharge is that in a classical discharge, the electrodes are directly in contact with the discharge gas and plasmas, and therefore during the discharge process, electrode etching and corrosion occurs. On the contrary, in DBDs the electrode and discharge are separated by a dielectric barrier, which eliminates electrode etching and corrosion. Another fundamental difference is that the DBDs cannot be operated with DC voltage because the capacitive coupling of dielectric requires an alternating voltage to drive a displacement current. An AC voltage with amplitude of 1-100 kV and a frequency from line frequency to several megahertz is applied to DBD configurations. DBD cold plasma can be produced in various working mediums through ionization by high frequency and high voltage electric discharge. The DBDs unique combination of non-equilibrium and quasi-continuous behavior has motivated a wide range of applications and fundamental studies.

The DBD plasma was invent by Siemens in 1857. The discharged plasma are generated by a number of individual filaments. The breakdown channel (micro-discharge) is controlled and the DBD process is optimized for appropriate application. The DBD plasma system is composed of DBD reactor, energy source, metallic electrode (such as aluminum, copper, etc.), and dielectric material (such as glass, quartz, ceramic, etc.).

Generally, dielectric material is cover either at one side of electrode or both of them. To initiate and sustain the generated electron in the DBD system, the energy sources are important. The energy source in DBD system can be alternative

current (AC), DC, RF, and MW. After applying the energy to HV electrode, the electrons from metallic electrode strike the dielectric material before emitting the electron in the system. These electron are subsequently at the bond of gas molecules, so the high energetic species are generated. These species are used to modify the substrate surfaces. The lower electrode is ground electrode which is connected to the resister.

Dielectric barrier discharge (DBD) plasma is widely used to modify the surface properties of polymer in many application such as improving the adhesion of coating to polymers, printing and biomedical application. The interactions of plasma with polymer surface are physical bombardment and chemical reaction. Leading to increase surface roughness and hydrophilicity by etching polymer surface and incorporated new polar functional groups, respectively. The major advantages of DBD plasma treatment over other techniques include modification on the top layer of substrates, minimization of thermal degradation, rapid treatment time and DBD plasma treatment does not require a vacuum system and can be operate at atmospheric pressure.

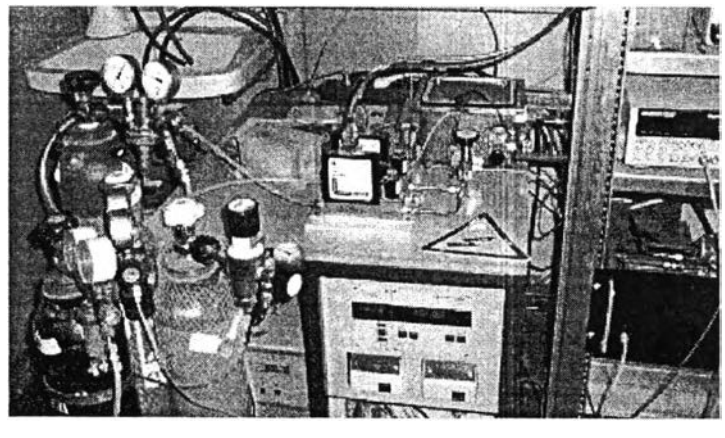


Figure 2.22 The plasma instrument.

2.3.4 DBD Structure

The discharge burning between two electrodes, at least one electrode insulated with a dielectric layer can be operated in a wide range of geometrical configurations such as the classical volume discharge, surface discharge, and

coplanar discharge.

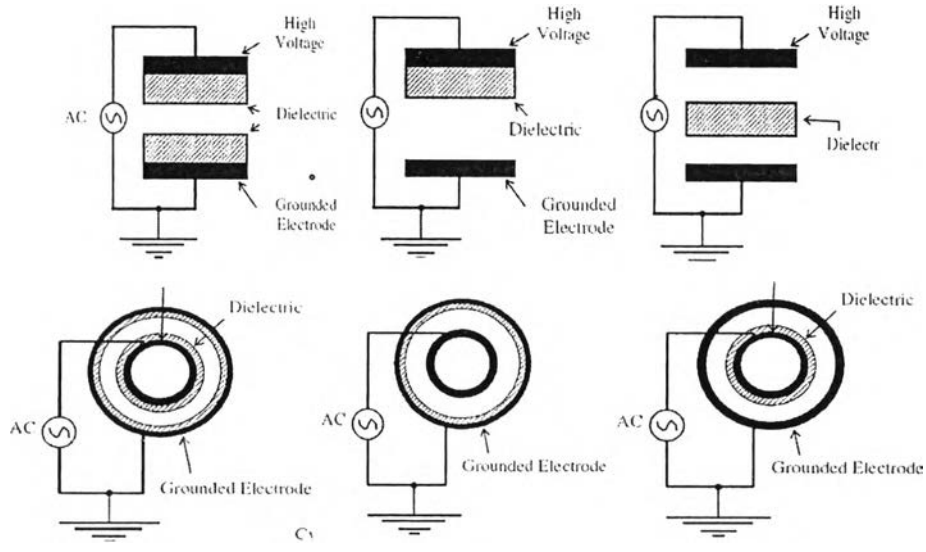


Figure 2..23 Typical DBD electrode arrangements.

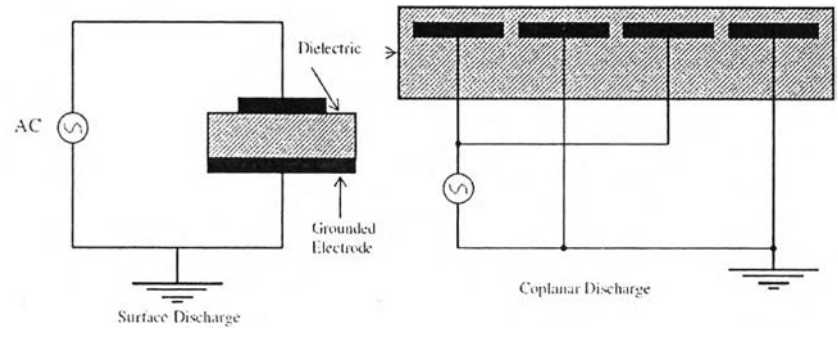


Figure 2.24 Typical DBD electrode arrangements.

Volume discharges can also have either planar or coaxial arrangements. In planar electrode arrangements, the two electrodes are parallel to each other, and one or two dielectric barriers are always located either (1) on the powered or the ground electrode, or (2) on both the electrodes, or (3) in between the two metal electrodes. The electrodes in DBD can also be arranged in a coaxial manner having one electrode inside the other with at least one or two dielectric barriers located either (1) on the outer side of the inner electrode/on the inner side of the outer electrode, or (2) on both the electrodes facing each other, or (3) in between

the two cylindrical electrodes. Besides the volume discharges, other designs also exist that use either surface or coplanar discharge geometry. Surface discharge device have a thin and long electrode on a dielectric surface and an extended counter-electrode on the reverse side of the dielectric. In this configuration, the discharge gap is not clearly defined and so the discharge propagates along the dielectric surface. There also exist combinations of both volume and surface discharge configuration such as the coplanar arrangement used in plasma display panel. The coplanar discharge device is characterized by pairs of long parallel electrodes with opposite polarity, which are embedded within a dielectric bulk nearby a surface. In addition to these configurations, other variants of DBD are also used in various applications. The typical arrangements of DBD are shown in figure 2.12. DBD can exhibit two major discharge modes either filamentary mode, which is the common form of discharge composed of many micro discharges that are randomly distributed over the electrode surface; or homogenous glow discharge mode, also known as atmospheric pressure glow discharge mode due to similarity with dc glow discharges.

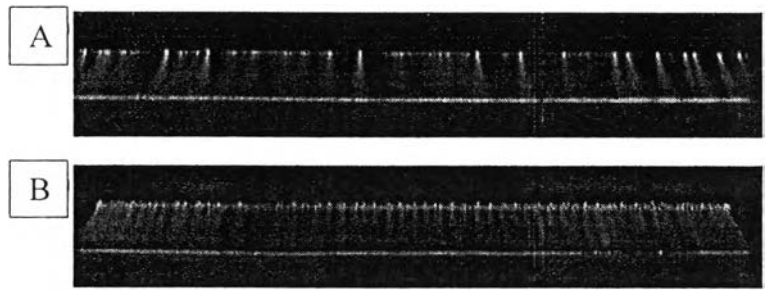


Figure 2.25 DBD obtained in the actuator with smooth (A) and serrated (B) active electrode.

2.3.5 Plasma-Substrate Interaction

In the plasma bulk, reactive species (positive and negative ions, atoms, neutrals, metastables and free radicals) are generated by ionization, fragmentation, and excitation. These species lead to chemical and physical interactions between plasma and the substrate surface depending on plasma

conditions such as gas, power, pressure, frequency, and exposure time. Plasma–substrate interaction can be classified as :

2.3.5.1 Plasma etching

Plasma etching is the key process for the removal of surface material from a given substrate. This process relies on the chemical combination of the solid surface being etched and the active gaseous species produced in the discharge. The resulting etched material will have a lower molecular weight and the topmost layer will be stripped. In previous methods, such as chemical wet processing, plasma has shown much more controllability and a much finer resolution. Due to the etching mechanism on polymer surfaces, morphological and topographical changes will occur. These changes are visible through atomic force microscopy (AFM) or scanning electron microscopy (SEM). Since most polymers are semi-crystalline, that is, they contain both crystalline and amorphous regions, they produce very distinctive morphology changes due to selective etching. Plasma etching leaded to increase surface roughness and surface degradation.

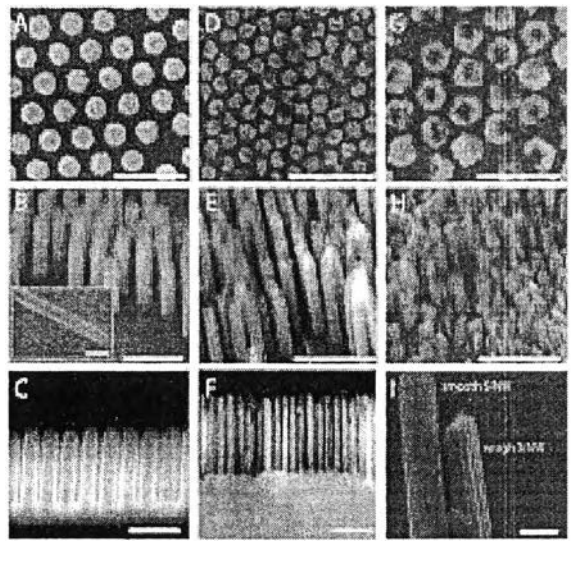


Figure 2.26 Different of plasma etching.

2.3.5.2 Chain-scission

Chain-scission is defined as any event that results in the breakage of one polymer molecule into two or more parts. This can occur through a

direct rearrangement of the backbone into two separate entities, or by the loss of side groups and consequent rearrangement, which inherently results in molecular division (Clough and Shalaby, 1996). Both processes can occur as a result of etching via plasma exposure. The first interaction involves ion bombardment, in which ion energy is transferred to the polymer molecules comprising the substrate. Bond scission and radical formation then occur, causing weight loss and a reduction in molecular weight (Inagaki, 1996). In addition to physical modifications, plasma exposure leads to changes in the elemental composition of the polymer surface. This includes the formation of free radicals. These radicals enable reactions such as cross-linking by activated species of inert gas, surface graft polymerization, as well as the incorporation of functional groups.

2.3.6 Literature Review on DBD Plasma Surface Treatment

Liu C. *et al., 2004* studied in case effects of DBD plasma operating parameter on the polymer surface modification. Used DBD plasma for treaded the surface of different types of polymer such as polytetrafluoroethylene (PTFE), polyimide (PI) and poly (lactic acid) (PLA). In terms of changes in surface wettability and surface chemistry. The purpose was to study the influence of the main operating parameters, i.e. plasma power, treatment period duration (treatment cycles) and electrode gap on the resultant surface properties. It was observed that the plasma parameters have a selective effect on the changes observed for the polymers processed. In particular, plasma processing time (treatment cycles), plays an important role in the treatment of PTFE and PI in this study, whereas the size of the electrode gap plays the dominant role in the treatment of PLA. Fast surface activation can be achieved in all cases after only a few seconds of treatment duration. The wettability improvement observed in all cases was attributed to changes in both surface chemistry and surface micro-structure.

Karahan H.A. *et al., 2004* study aims to investigate the viability of atmospheric plasma treatment over raw cotton fabric surfaces as an alternative method for superseding the wet textile pre-treatment processes. For this purpose, the fabric samples were treated with air plasma and argon atmospheric plasma. Thereafter, the hydrophilicity and the wickability of plasma treated samples

increased, and also the contact angles decreased significantly. Chemical changes and Morphological changes.

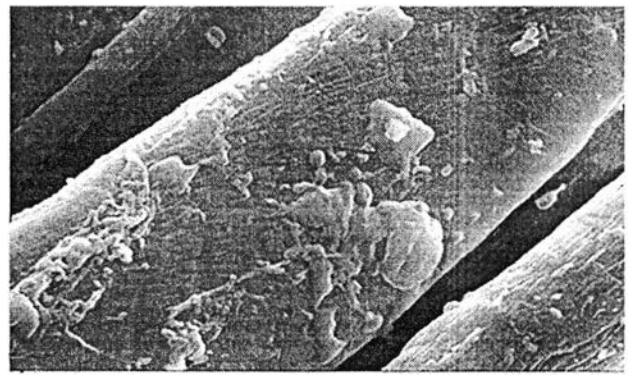


Figure 2.27 Cotton fabric after DBD plasma treatment

Onsuratoom S. *et al.*, 2009 developed dielectric barrier discharge (DBD) plasma to modify surface of woven PET incase for antimicrobial property. The woven PET surface was plasma-treated by dielectric barrier discharge (DBD) under various operating conditions (electrode gap distance, plasma treatment time, input voltage, and input frequency) and various gaseous environments (air, O_2, N_2, and Ar) in order to improve its hydrophilicity. They found that a decrease in electrode gap distance and an increase in input voltage increased the electric field strength, leading to higher hydrophilicity of the PET surface. The optimum conditions for a maximum hydrophilicity of the PET surface were an electrode gap distance of 4 mm, a plasma treatment time of 10 s, an output voltage of 15 kV, and a frequency of 350 Hz under air environment.

NASTUTA A. V. *et al., 2008* developed dielectric barrier discharge (DBD) plasma to modify polymer in different configurations. In the present paper the surface modifications of polyethylene terephthalate (PET) and polyethylene terephthalate with TiO2 additives (PET+TiO2) by atmospheric dielectric barrier discharge (DBD) treatment in different configurations. They was found that he shorter treatment durations, the asymmetric DBD configuration is more efficient than the others methods.

Wang C.X. *et al.*, 2008 developed dielectric barrier discharge (DBD) plasma to treat polyester fabric surface. Significant changes in morphology of fabric

surface were observed after plasma treatment. As shown in figure 2.13, untreated sample shown the smooth surface while for the plasma treated sample, micro pits were formed on the fabric surface. The formation of micro pits on the treated fabric surfaces were caused by the etching reactions, in which some degradation reaction occurred due to the bombardment of the ions and the electrons as well as the oxidative reactions with atomic oxygen. Therefore the surfaces of plasma treated polyester fabric were roughed resulting in change of the fabric surface properties.

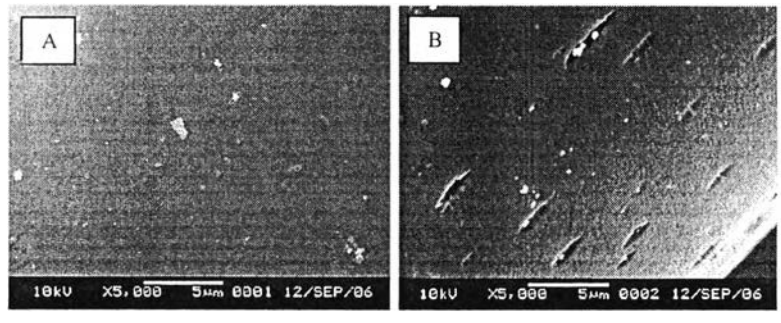


Figure 2.28 Surface morphology of polyester fabric (A) untreated and (B) treated DBD plasma 2 s.

Sophonvachiraporn P. *et al., 2010* developed dielectric barrier discharge (DBD) plasma to modify a woven PET with an antimicrobial activity was prepared by depositing chitosan on its surface. resulting in an enhanced hydrophilic property. The plasma-treated PET specimen was further deposited with chitosan by immersing in a chitosan acetate aqueous solution. The effects of temperature, chitosan concentration, and number of rinses on the amount of deposited chitosan on the PET surface were investigated. The disappearance of the above-mentioned polar groups from the PET surface was clearly observed after the chitosan deposition, indicating the involvement of these functional groups in interacting with the chitosan.

Fernando R. O. *et al., 2010* developed dielectric barrier discharge (DBD) plasma to modify Banana fibers. The influence of the plasma treatment applied on the banana fibers was performed considering the mechanical properties, wettability, chemical composition and surface morphology. The results of this study

showed considerable modifications in banana fibers when these are submitted to plasma treatment.

Yorsaeng S. *et al., 2012* developed dielectric barrier discharge (DBD) plasma to modify natural rubber latex (NRL) film. The results showed that surface hydrophilicity of the NRL film increased after the plasma treatment due to the presence of oxygen-containing polar groups on the plasma-treated surface. An increase in plasma treatment time increased the surface roughness of the NRL film, and eventually decreased the mechanical properties. From the obtained results, the optimum plasma treatment time of 20 s was chosen. After immersion in a chitosan solution, the amount of chitosan deposited on the plasma-treated NRL film increased with increasing chitosan concentrations. The chitosan coating smoothed the surface of the plasma-treated NRL film and also improved the mechanical properties.

Seyed A.M. *et al.*, 2012 found that hydrophilicity, wettability and roughness of polyhydroxylbutyrate (PHB) film was remarkably improve after plasma treatment. To study biocompatibility of the untreated and plasma-treated PHB samples cell proliferation method has been used. The attachment and growth of L929 fibroblast cells onto the untreated PHB surface was negligible, yet improved attachment and growth of L929 fibroblast cells were observed on plasma-treated PHB surfaces. It can be conclude that plasma treated PHB film will provide an environment for better cell attachment and growth.

Paisoonsin S. *et al.*, 2013 developed dielectric barrier discharge (DBD) plasma to modify polypropylene (PP) film and after that coading Zinc oxide (ZnO). the surface roughness of the DBD plasma-treated PP film gradually increased with increasing plasma treatment time, the DBD plasma treatment insignificantly affected the mechanical properties of the PP film. The DBD plasma treatment time was found to be optimized at 10 s. The DBD plasma-treated PP film was further immersed in an aqueous zinc nitrate ($Zn(NO3)_2$) solution at different concentrations before being converted to ZnO particles with the use of a 2.5 M sodium hydroxide (NaOH) solution, followed by sonication. The highest amount of ZnO deposited on the DBD plasma-treated PP surface was about 0.26 wt.% at the optimum $Zn(NO3)_2$ concentration of 0.5 M. The ZnO-deposited DBD plasma-treated PP film showed good antibacterial activities

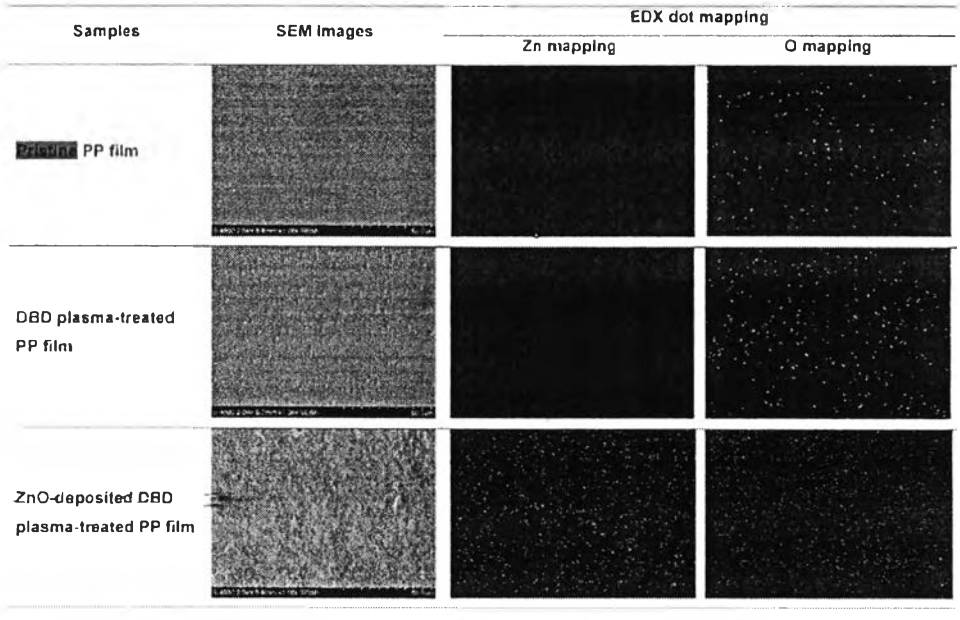


Figure 2.29 Representative SEM images and EDX dot mapping of Zn and O on surfaces of pristine PP film, DBD plasma-treated PP film, and ZnO-deposited DBD plasma-treated PP film.

2.4 Immobilization of Bacterial

Immobilization of microbial cells in biological processes can occur either as a natural phenomenon or through artificial process. While the attached cells in natural habitat exhibit significant growth, the artificially immobilized cells are allowed restricted growth. Since the time first reports of successful application of immobilized cells in industrial applications, several research groups worldover have attempted whole-cell immobilization as a viable alternative to conventional microbial fermentations. Various immobilization protocols and numerous carrier materials were tried. The cell immobilization process has also triggered our interest in bioreactor design. Using immobilized cells, different bioreactor configurations were reported with variable success. The study on the physiology of immobilized cells and development of noninvasive measuring techniques have remarkably improved our understanding on microbial metabolism under immobilized state.

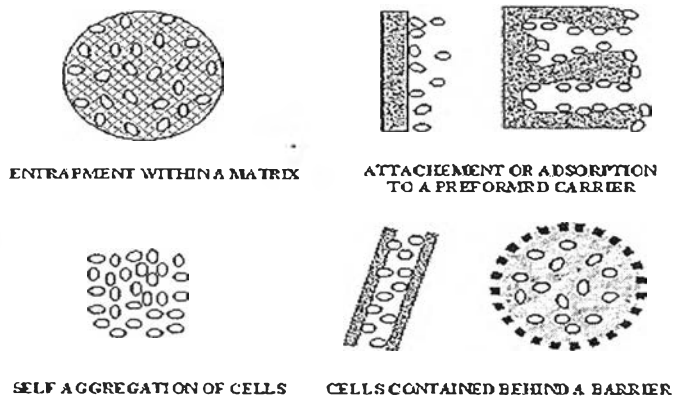


Figure 2.30 Different types of immobilization .

The industrial biotechnology processes using microorganisms are generally based on the exploitation of the cells in the fermentation medium during the process. The classical fermentations suffer from various constrains such as low cell density, nutritional limitations, and batch-mode operations with high down times. It has been well recognized that the microbial cell density is of prime importance to attain higher volumetric productivities. The continuous fermentations with free-cells and cell recycle options aim to enhance the cell population inside the fermenter. However, the free-cell systems cannot operate under chemostatic mode that decouples specific growth rate and dilution rates. During the last 20–25 years, the cell immobilization technology, with its origins in enzyme immobilization, has attracted the attention of several research groups. This novel process eliminates most of the constrains faced with the free-cell systems. The remarkable advantage of this new system is the freedom it has to determine the cell density prior to fermentation. It also facilitates operation of microbial fermentation on continuous mode without cell washout. The whole-cell immobilization process decouples microbial growth from cellular synthesis of favoured compounds. Since the early 70s, when Chibata's group announced successful operation of continuous fermentation of l-aspartic acid, numerous research groups have attempted various microbial fermentations with immobilized cells. During these years, over 2500 research papers on various aspects of whole-cell immobilization have been published. Several comprehensive reviews[1] as well as specialized reviews have been published on some important

aspects of this field. There are also specialized monograms and conference proceedings pertaining to cell immobilization technology, which have excited microbiologists and bioengineers.

Many processes have been practised traditionally, embodying the basic principle of microbial conversions offered by cells bound to surfaces. Waste treatment in trickling filters and ethanol oxidation to produce vinegar are but a few examples of such processes. Immobilization of cells is the attachment of cells or their inclusion in distinct solid phase that permits exchange of substrates, products, inhibitors, etc., but at the same time separates the catalytic cell biomass from the bulk phase containing substrates and products. Therefore it is expected that the microenvironment surrounding the immobilized cells is not necessarily the same experienced by their free-cell counterparts.

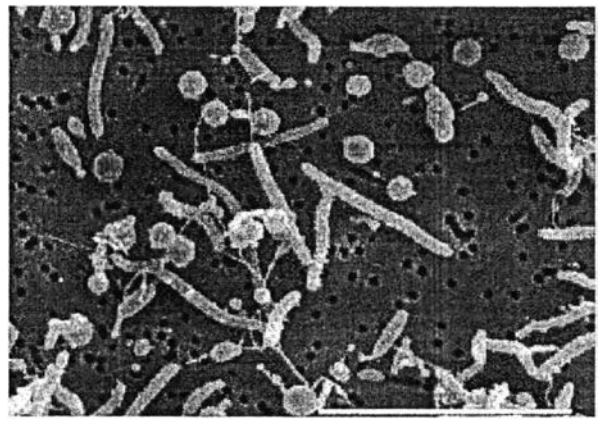


Figure 2.31 Microbial on the surface of substrate.

Immobilization commonly is accomplished using a high molecular hydrophilic polymeric gel such as alginate, carrageenan, agarose, etc. In these cases, the cells are immobilized by entrapment in the pertinent gel by a drop-forming procedure. When traditional fermentations are compared with the microbial conversions using immobilized cells, the productivity obtained in the latter is considerably higher, obviously partly due to high cell density and immobilization-induced cellular or genetic modifications. Nevertheless, a few critical parameters such as the cost of immobilization, mass transport limitations, applicability to a

specific end-product, etc. are to be carefully examined before choosing any particular methodology.

The use of immobilized whole microbial cells and/or organelles eliminates the often tedious, time consuming, and expensive steps involved in isolation and purification of intracellular enzymes. It also tends to enhance the stability of the enzyme by retaining its natural catalytic surroundings during immobilization and subsequent continuous operation. The ease of conversion of batch processes into a continuous mode and maintenance of high cell density without washout conditions even at very high dilution rates, are few of the many advantages of immobilized cell systems. The metabolically active cell immobilization is particularly preferred where co-factors are necessary for the catalytic reactions. Since co-factor regeneration machinery is an integral function of the cell, its external supply is uneconomical. There is considerable evidence to indicate that the bound-cell systems are far more tolerant to perturbations in the reaction environment and similarly less susceptible to toxic substances present in the liquid medium. The recent reports on higher retention of plasmid-bearing cells have further extended the scope of whole-cell immobilization to recombinant product formation. Another important advantage of immobilization, particularly in the case of plant cells, is the stimulation of secondary metabolite formation and elevated excretion of intracellular metabolites.

Table 2.4 Show history of develop microbial immobilization.

Phase	Years	Development
Early Phase	1916-1940	Glass [11]. Alumina[12]. Hydrophobic compound coated glass[13].
Underdeveloped Phase	1950	Non-specific physical adsorption of enzymes on solid carriers e.g amylase on activated carbon, bentonite or clay [14], chymotrypsin on kaolinite [15] AMP deaminase on silica [16],ribonuclease on the anionic exchanger Dowex-2 [17]
Developing Phase	1960	Entrapment of whole cells in synthetic gel [18]. Encapsulation in artificial cell [19]. Adsorption-cross-linking [20]. Active site titration [21]. Cross-linked enzyme (CLE) [22]. cross-linked enzyme crystals (CLEC). Immobilization or post-treatment by denaturant [23]
Developed Phase	1970	Many new method subgroups, for example affinity binding and coordination binding and many novel variations have been developed [24]. Increased enzyme loading in order to enhance the activity [25,26]
Post Developed Phase	1980	Encagement (double encagement) [27,28]. Covalent multilayer immobilized enzymes [29]. Organosoluble lipid-coated enzyme [30]. Introduction of genetically engineered tags [31]
Rational Design Phase	1990-till now	Stability and activity in organic solvents [32,33]. High enzyme loading and less diffusion limitation [34]. Development of single enzyme nanoparticle [35]

2.4.1 Classification of Immobilization Techniques

2.4.1.1 Adsorption

adsorption results from hydrophobic interactions and salt linkages where either the support is bathed in enzyme for physical adsorption or the enzyme is dried on electrode surfaces. Adsorbed enzymes are shielded from aggregation, proteolysis and interaction with hydrophobic interfaces (Spahn and Minteer 2008). Researchers have used eco-friendly supports like coconut fibers having good water-holding capacity and high cation exchange property; microcrystalline cellulose with irreversible binding capacity; kaolin with high enzyme retainability by chemical acetylation; and micro/mesoporous materials having thiol functionalized, large surface area ideally suited for reduction and oxidation reactions (Dey et al. 2002; Herna´ndez et al. 2007; Karagulyan et al. 2008; Bri´gida et al. 2010; Mitchell and Rami´rez 2011; Huang et al. 2011). Silanized molecular sieves have also been successfully used as supports for enzyme adsorption owing to the presence of silanols on pore walls that facilitate enzyme immobilization by hydrogen bonding (Diaz and Balkus 1996). Various chemical modifications of the currently used supports would definitely help in better immobilization. Water activity profiles of lipase adsorbed using polypropylenebased hydrophobic granules/Accurel EP-100 has been reported (Persson et al. 2000). It would be important to note that Accurel with smaller particle sizes increases reaction rates and enantiomeric ratios during biocatalyzation (Sabbani et al. 2006). For better process control and economic production, Yarrowia lipolytica lipase was immobilized on octyl-agarose and octadecyl-sepabeads supports by physical adsorption that resulted in higher yields and greater (tenfold) stability than that of free lipase. This was accounted by the hydrophobicity of octadecyl-sepabeads that enhances affinity between the enzyme and support (Cunha et al.2008). Candida rugosa lipase adsorbed on biodegradable poly (3-hydroxybutyrate-co-hydroxyvalerate) showed 94 % residual activity after 4 h at 50 degree C and reusability till 12 cycles (Cabrera-Padilla et al. 2011). These supports were preferred because they are less tough and crystalline than polyhydroxybutyrate. 1, 4-Butenediol diglycidyl ether-activated byssus threads have been suitable basement for urease that increased pH stability and retained 50 %

enzyme activity under dried conditions (Mishra et al.2011). Eco-friendly supports of biological origin not only prevent cropping up of ethical issues, but also cut down the production costs. Of late, biocompatible mesoporous silica nanoparticles (MSNs) supports have been used for biocatalysis in energy applications owing to their long-term durability and efficiency (Popat et al. 2011).

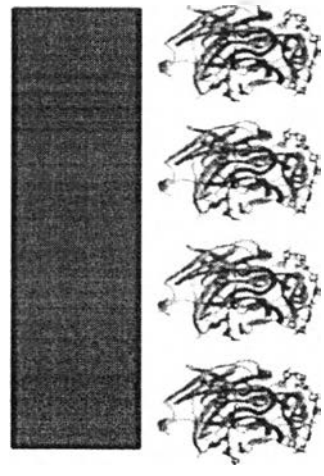


Figure 2.32 Adsorption Immobilization methods.

2.4.1.2 Covalent Binding

Covalent association of enzymes to supports occurs owing to their side chain amino acids like arginine, aspartic acid, histidine and degree of reactivity based on different functional groups like imidazole, indolyl, phenolic hydroxyl, etc. (D'Souza 1998; Singh 2009). Peptide-modified surfaces when used for enzyme linkage results in higher specific activity and stability with controlled protein orientation (Fu et al. 2011). Cyanogen bromide (CNBr)-agarose and CNBractivated-Sepharose containing carbohydrate moiety and glutaraldehyde as a spacer arm have imparted thermal stability to covalently bound enzymes (Hsieh et al. 2000; Cunha et al. 2008). Highly stable and hyperactive biocatalysts have been reported by covalent binding of enzymes to silica gel carriers modified by silanization with elimination of unreacted aldehyde groups and to SBA-15 supports containing cage-like pores lined by Si–F moieties (Lee et al. 2006; Szyman´ska et al. 2009). Increase in half-life and thermal stability of enzymes has been achieved by covalent coupling with

different supports like mesoporous silica, chitosan, etc. (Hsieh et al. 2000; Ispas et al. 2009). Crosslinking of enzymes to electrospun nanofibers has shown greater residual activity due to increased surface area and porosity. Use of such nanodiametric supports have brought a turning point in the field of biocatalyst immobilization (Wu et al. 2005; Kim et al. 2006; Ren et al. 2006; Li et al. 2007; Huang et al. 2008; Sakai et al. 2010). Covalent binding of alcohol dehydrogenase on attapulgite nanofibers (hydrated magnesium silicate) has been opted owing to its thermal endurance and variable nano sizes (Zhao et al. 2010). Biocatalytic membranes have been useful in unraveling effective covalent interactions with silicon-coated enzymes (Hilal et al. 2006). Cross-linked enzyme aggregates produced by precipitation of enzyme from aqueous solution by addition of organic solvents or ionic polymers have been reported (Sheldon 2011). Different orientations of immobilized enzyme on magnetic nanoclusters obtained by covalent binding have found their applications in pharmaceutical industries owing to their enhanced longevity, operational stability and reusability (Yusdy et al. 2009). Maintaining the structural and functional property of enzymes during immobilization is one of the major roles played by a cross-linking agent. One such agent is glutaraldehyde. popularly used as bifunctional cross-linker, because they are soluble in aqueous solvents and can form stable inter- and intra-subunit covalent bonds.

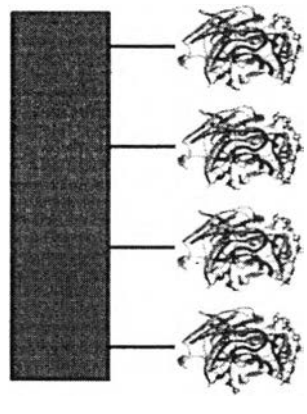


Figure 2.33 Covalent Binding Immobilization methods.

2.4.1.3 Affinity Immobilization

Affinity immobilization exploits specificity of enzyme to its support under different physiological conditions. It is achieved by two ways: either the matrix is precoupled to an affinity ligand for target enzyme or the enzyme is conjugated to an entity that develops affinity toward the matrix (Sardar et al. 2000). Affinity adsorbents have also beenused for simultaneous purification of enzymes (Ho et al. 2004). Complex affinity supports like alkali stable chitosan- coated porous silica beads and agarose-linked multilayered concanavalin A harbor higher amounts of enzymes which lead to increased stability and efficiency (Shi et al. 2003; Sardar and Gupta 2005). Bioaffinity layering is an improvisation of this technique that exponentially increases enzyme-binding capacity and reusability due to the presence of non-covalent forces such as coulombic, hydrogen bonding, van der Waals forces, etc. (Sardar and Gupta 2005; Haider and Husain 2008).

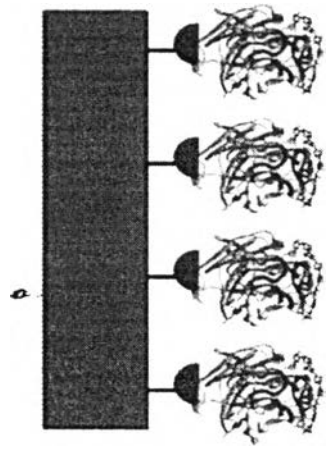


Figure 2.34 Affinity Binding Immobilization methods

2.4.1.4 Entrapment

Entrapment is caging of enzymes by covalent or noncovalent bonds within gels or fibers (Singh 2009). Efficient encapsulation has been achieved with alginate–gelatin–calcium hybrid carriers that prevented enzyme leakage and provided increased mechanical stability (Shen et al. 2011). Entrapment by nanostructured supports like electrospun nanofibers and pristine materials have

revolutionalized the world of enzyme immobilization with their wide-ranging applications in the field of fine chemistry, biomedicine biosensors and biofuels (Dai and Xia 2006; Kim et al. 2006; Wang et al. 2009; Wen et al. 2011). Prevention of friability and leaching and augmentation of entrapment efficiency and enzyme activity by Candida rugosa lipase entrapped in chitosan have been reported. This support has also been reported to be non-toxic, biocompatible and amenable to chemical modification and highly affinitive to protein due to its hydrophilic nature (Betigeri and Neau 2002). Entrapment by mesoporous silica is attributed to its high surface area, uniform pore distribution, tunable pore size and high adsorption capacity (Ispas et al. 2009). Simultaneous entrapment of lipase and magnetite nanoparticles with biomimetic silica enhanced its activity in varying silane additives (Chen et al. 2011a). Sol–gel matrices with supramolecular calixarene polymers have been used for entrapment of C. rugosa lipase keeping in view their selective binding and carrying capacities (Erdemir and Yilmaz 2011). Lipases entrapped j-carrageenan has been reported to be highly thermostable and organic solvent tolerant (Tu¨mtu¨rk et al. 2007; Jegannathan et al. 2010).

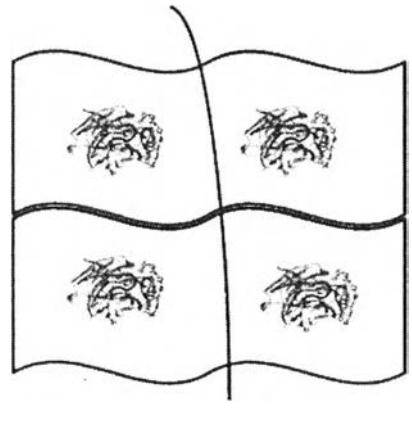


Figure 2.35 Entrapment Immobilization methods

2.4.1.5 Metal Linked Immobilization

In metal linked enzyme immobilization, the metal salts are precipitated over the surface of the carriers and it has the potential to bind with the nucleophilic groups on the matrix. The precipitation of the ion on the carrier can be

achieved by heating. This method is simple and the activity of the immobilized enzymes is relatively high (30-80%). The carrier and the enzyme can be separated by decreasing the pH, hence it is a reversible process [64].The matrix and the enzyme can be regenerated, by the process.

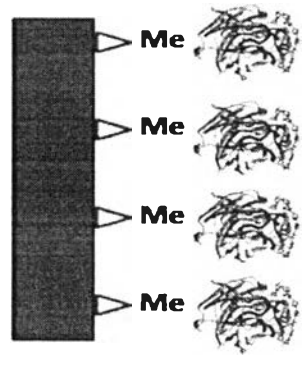


Figure 2.36 Metal ion Immobilization methods

2.4.2 Materials that Applying for Fabrication of Immobilization (supports)

2.4.2.1 Alginate

Alginate derived from cell walls of brown algae are calcium, magnesium and sodium salts of alginic acid and have been extensively used for immobilization as xanthan– alginate beads, alginate–polyacrylamide gels and calcium alginate beads with enhanced enzyme activity and reusability. Cross-linking of alginate with divalent ions (like Ca_2) and glutaraldehyde improves the stability of enzymes (Elc ,in 1995; Flores-Maltos et al. 2011).

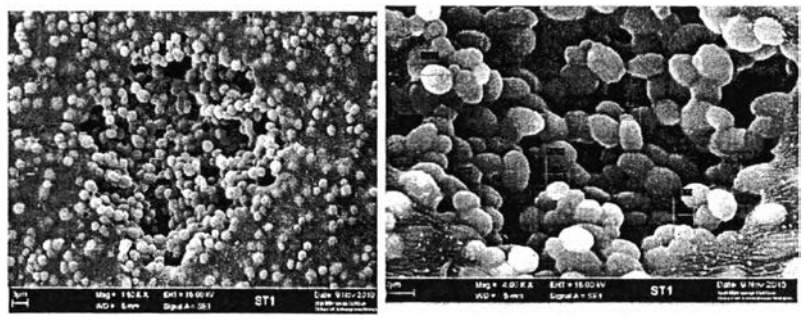


Figure 2.37 Immobilization microbial by alginate.

2.4.2.2 Chitosan and Chitin

Natural polymers like chitin and chitosan have been used as supports for immobilization (Vaillant et al. 2000; Kapoor and Kuhad 2007). The protein or carbohydrate moieties of enzymes are used for binding them to chitosan (Hsieh et al. 2000). Chitosan has been used in combination with alginate where chitosan-coated enzymes had less leaching effect compared to alginate owing to the physical and ionic interactions between the enzyme and support (Betigeri and Neau 2002). Similarly, a wet composite of chitosan and clay proved to be more reliable for enzyme trapping, because it has hydroxyl and amino groups, whicheasily link with enzymes, together with good hydrophilicity and high porosity. Chitosan in the form of beads can entrap twice as much of the enzymes (Chang and Juang 2007). According to Chern and Chao (2005), the chitin-binding domain of chitinase A1 from Bacillus circulans has a high affinity to chitin; so, this property has been exploited to retain D-hydantoinase.

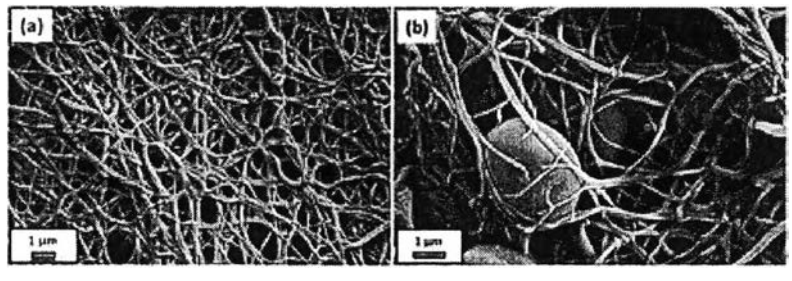


Figure 2.38 Immobilization microbial by chitosan.

2.4.2.3 Collagen

Being a natural polymer, collagen has been used for immobilization of tannase employing glutaraldehyde as cross-linking agent (Katwa et al. 1981). Fe-collagen fibers proved to be excellent supporting matrix for catalase immobilization by retaining significant activity even after 26 reuses (Chen et al. 2011b).

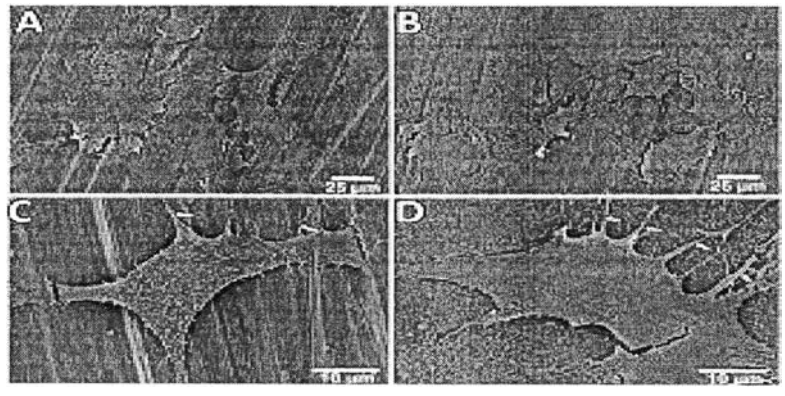


Figure 2.39 Immobilization microbial by collagen

2.4.2.4 Carrageenan

Carrageenan, a linear sulfated polysaccharide, has been consistently used for immobilizing a variety of enzymes, like lipase for improving stability (Tu¨mtu¨rk et al. 2007). This support is pseudoplastic in nature, which helps it to thin under shear stress and recover its viscosity once the stress is removed. Jegannathan et al. (2010) could achieve an encapsulation efficiency of 42.6 % by the co-extrusion method using the same support for biodiesel production. Carrageenan has been reported as a cheap and durable support with better entrapment for lactic acid and agalactosidase enzyme (Rao et al. 2008; Girigowda and Mulimani 2006).

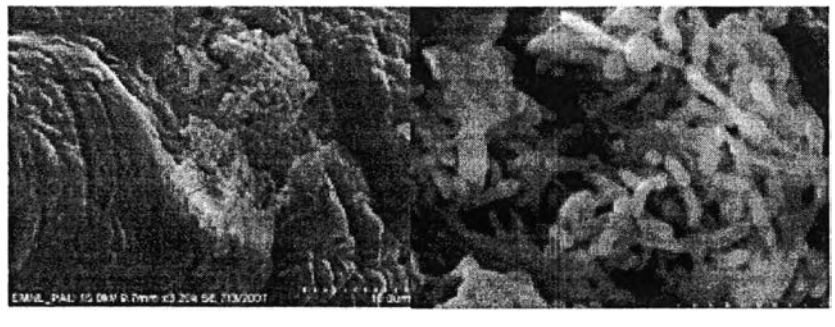


Figure 2.40 Immobilization microbial by carrageenan.

2.4.2.5 Gelatin

Gelatin is a hydrocolloid material, high in amino acids, and can adsorb up to ten times its weight in water. Its indefinite shelf life has attracted

attention for enzyme immobilization. Gelatin has been utilized in mixed carrier system with polyacrylamide where cross-linking with chromium (III) acetate proved better than chromium (III) sulfate and potassium chromium (III) sulfate (Emregul et al. 2006). Calcium alginate with gelatin forms a good template for calcium phosphate deposition for enzyme immobilization, and gelatin in combination with polyester films promoted 75 % loading efficiency, compared to previous studies which had 50 % loading efficiency (Shen et al. 2011; Ates¸ and Dog˘an 2010).

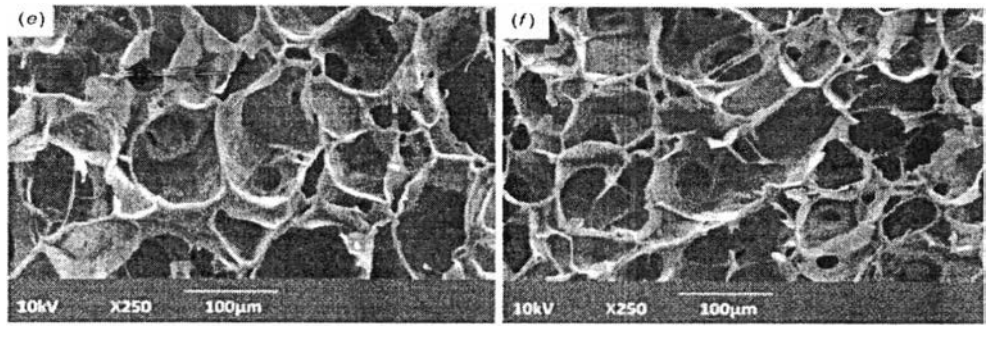


Figure 2.41 Immobilization microbial by alginate and gelatin.

2.4.2.6 Cellulose

This most abundant natural polymer has been widely used to immobilize fungi laccase, penicillin G acylase, glucoamylase, a-amylase, tyrosinase, lipase and b-galactosidase (Al-Adhami et al. 2002; Mislovicova´ et al. 2004; Bryjak et al. 2007; Namdeo and Bajpai 2009; Labus et al. 2011; Huang et al. 2011; Klein et al. 2011). Diethylaminoethyl (DEAE)-modified cellulosic supports have longer storage capacity (Al-Adhami et al. 2002). Cellulose-coated magnetite nanoparticles have been used for starch degradation where the attachment of a-amylase to cellulose dialdehydecoated magnetite nanoparticles resulted in the formation of a novel starch degrading system (Namdeo and Bajpai 2009). Immobilization with ionic liquid-cellulose film activated by glutaraldehyde gave better formability and flexibility (Klein et al. 2011).

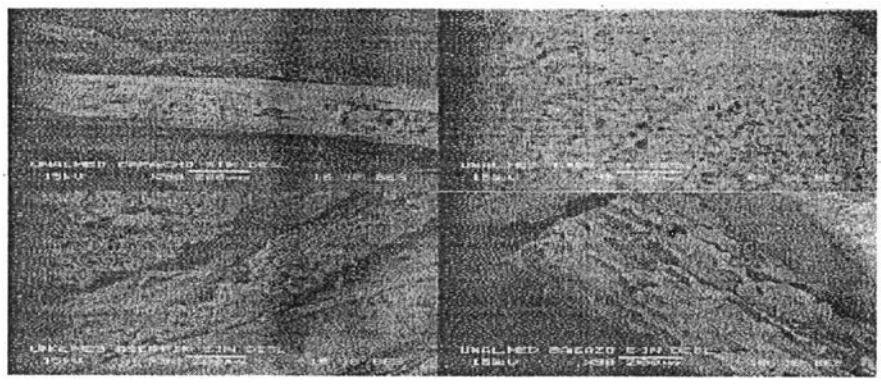


Figure 2.42 Immobilization microbial by cellulose.

2.4.2.7 Starch

Made of linear amylase and branched amylopectin units, starch has been used as enzyme immobilizer. Calcium alginate–starch hybrid supports were applied for surface immobilization and entrapment of bitter gourd peroxidase. Entrapped enzyme was more stable in the presence of denaturants like urea due to internal carbohydrate moieties, while surface-immobilized enzyme had superior activity (Matto and Husain 2009). Radiation grafting of substances like acrylamide and dimethylaminoethyl methacrylate onto starch are among the widely used industrial techniques for a high product yield (Dung et al. 1995; Raafat et al. 2011).

2.4.2.8 Pectin

This structural heteropolysaccharide along with 0.2–0.7 % glycerol acts as plasticizer to reduce brittleness of support and has been used to immobilize papain and for development of new materials for skin injury treatment (Ceniceros et al. 2003). Pectin–chitin and pectin–calcium alginate support have enhanced thermal and denaturant resistance and catalytic properties of entrapped enzymes due to the formation of high stable polyelectrolyte complexes between the enzyme and the pectin-coated support (Go´mez et al. 2006; Satar et al. 2008). CNBr-activated Sepharose-4B has been used to immobilize amylase and glucoamylase owing to its porosity and easy adsorption of macromolecules. Further matrix modifications like alkyl substituted Sepharose with multipoint attachment between

hydrophobic clusters of the enzyme and alkyl residues of the support play a major role in retaining the catalytic properties at extremes of pH, high salt concentrations and elevated temperatures (Hosseinkhani et al. 2003). Another example of modified Sepharose matrix is concanavalin A (Con A)–Sepharose 4B where biospecific interaction between the glycosyl chains of the enzyme and Con A plays a pivotal role in fabrication of various biosensors (Mirouliaei et al. 2007). Synthetic polymers as supports Ion exchange resins/polymers are insoluble supports with porous surface for enzyme trapping. Amberlite and DEAE cellulose, renewable matrices with large surface area, have been used for immobilization of a-amylase (Kumari and Kayastha 2011).Duringwhite radish peroxidase immobilization, glutaraldehyde and polyethylene glycol act as an additive and protective layer around the active center of the enzyme to prevent the attack of free radicals (Ashraf and Husain 2010). Some synthetic polymers used as enzyme supports are stated as follows: polyvinyl chloride that prevents enzyme, cyclodextrin glucosyltransferase from thermal inactivation; polyurethane microparticles derived from polyvinyl alcohol and hexamethyl diisocyanate in the ratio of 1:3 with high enzyme loading and efficiency; UV-curable methacrylated/fumaric acid-modified epoxy that is proposed to be useful for industrial applications; polyaniline in two different forms, viz. emeraldine salt and emeraldine base powder used for covalent binding of a- amylase; glutaraldehyde-activated nylon for immobilizing lipase and UV-activated polyethylene glycol having high porosity employed for wastewater treatment (Abdel-Naby 1999; Kahraman et al. 2007; Pahujani et al. 2008; Romaskevic et al. 2010;Xiangli et al. 2010; Ashly et al. 2011). Inorganic materials as support

2.4.2.9 Zeolites

Zeolites or 'molecular sieves' are microporous crystalline solids with well-defined structures and shape-selective properties and are widely used in molecular adsorption. Microporous zeolites were found to be a better support for a-chymotrypsin immobilization than microporous dealuminized ones because of the presence of more hydroxyl groups that form strong hydrogen bonds with the enzyme (Xing et al. 2000). Likewise, Na Y zeolite was used to immobilize lysozyme because it had higher activity compared to other supports as reported by Chang and Chu (2007). The heterogeneous surface of zeolites with multiple adsorption

sites are considered to be suitable for modulating the enzyme and support interactions (Serralha et al. 1998).

2.4.2.10 Ceramics

Immobilization of Candida antarctica lipase on ceramic membrane showed that this inert support could be exploited for carrying out hydrolytic and synthetic reactions by limiting feedback inhibition (Magnan et al. 2004). Ceramic foams containing both macro (77 nm) and micropores (45 lm) was found to be efficient in lowering diffusion rate and increasing the specific surface area (Huang and Cheng 2008). Another example of ceramics is toyonite whose variable pore structure can be modified using different organic coatings (Kamori et al. 2000).

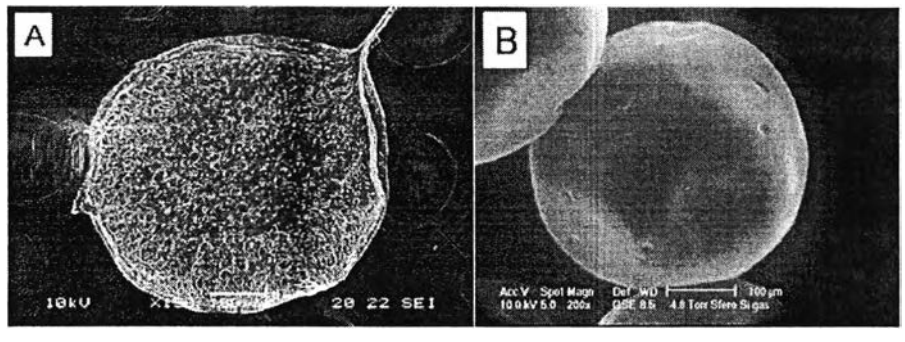


Figure 2.43 Immobilization microbial by ceramic.

2.4.2.11 Silica

Enzymes like lignin peroxidase and horseradish peroxidase (HRP) immobilized on activated silica have been effectively used for the removal of chlorolignins from eucalyptus kraft effluent (Dezott et al. 1995). a-Amylase immobilized on silica nanoparticles improves cleaning performance of detergents. They have been used because of their nano-sized structures with high surface area, ordered arrangement and high stability to chemical and mechanical forces (Soleimani et al. 2011). Surface modifications of silica by amination of hydroxyl and reactive siloxane groups and addition of methyl or polyvinyl alcohol groups strengthen enzyme and support bonds (Rao et al. 2000; Shioji et al. 2003; Pogorilyi et al. 2007).

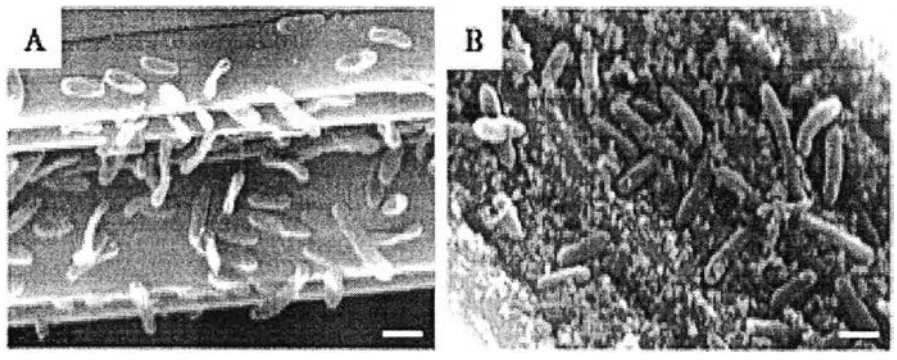


Figure 2.44 Immobilization microbial by silica.

2.4.2.12 Glass

Glass is a highly viscous liquid and has been employed in immobilizing a-amylase; phthaloyl chloride containing amino group functionalized glass beads was found to be robust and renewable for the process (Kahraman et al. 2007). Another enzyme nitrite reductase was immobilized on controlled pore glass beads, which served as a biosensing device for continuous monitoring (Rosa et al. 2002). Urease immobilized on glass pH-electrodes has provided a stable biosensor for monitoring as low as 52 lg/ml urea in blood samples (Sahney et al. 2005).