CHAPTER II
LITERATURE REVIEW

2.1 Bacterial Cellulose

2.1.1 Introduction of Cellulose

Cellulose has been used daily for centuries in applications for making textiles, paper, plastic and food additives. It can be considered the oldest .The most abundant natural polymer. Cellulose consists of un-branched polymers of linked glucose residues arranged in linear chains where every other glucose residue is rotated 180 degrees. The glucose molecules are joined by a $C_1 - C_4$ glucosidic oxygen linkage. Cellulose is a natural polymer of cellubiose. Celluboise is the structural repeating unit of the glucose chains in cellulose. Both adopt a stable C_4 chair conformation and hydrogen bonding between adjacent oxygen and hydrogen atoms forces a linear arrangement. Cellulose is a β -1, 4-glucan chain as shown in Figure 2.1.

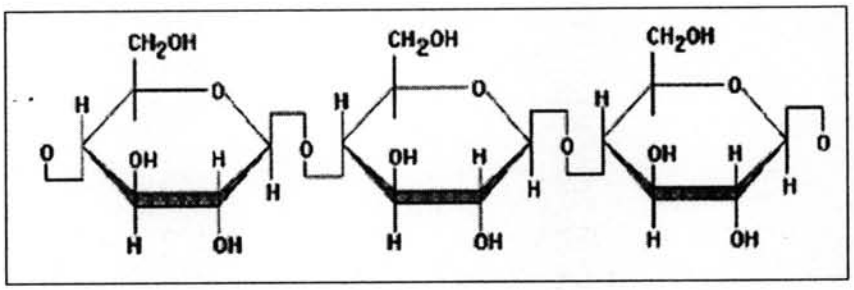

Figure 2.1 Chemical structure of cellulose.

When the cellulose molecule is extended, it is a flat ribbon like structure that is further stiffened by Van der Waals forces and also by the hydroxyl groups protruding laterally which are able to form intra- and inter-molecule hydrogen bonds. The surface of the ribbon consists mainly of hydrogen atoms linked to carbon. The essential feature of cellulose is the primary and secondary alcohol groups in each monomer unit and the glucosidic unit bonds. The glucosidic bonds are not easily

broken and therefore cellulose is very stable. The primary and secondary alcohol units in cellulose react in a similar manner as in simple substances of similar chemical constitution. They may be readily oxidized, esterified and converted into ethers. The cohesive energy density of cellulose is high with the result that cellulose is insoluble in water. Although water vapor cm be absorbed strongly on fibrillar surfaces and in less ordered regions.

2.1.2 Principal Pathways to Cellulose

Up to now there are four different pathways to from the biopolymer cellulose as shown in Figure 2.2.

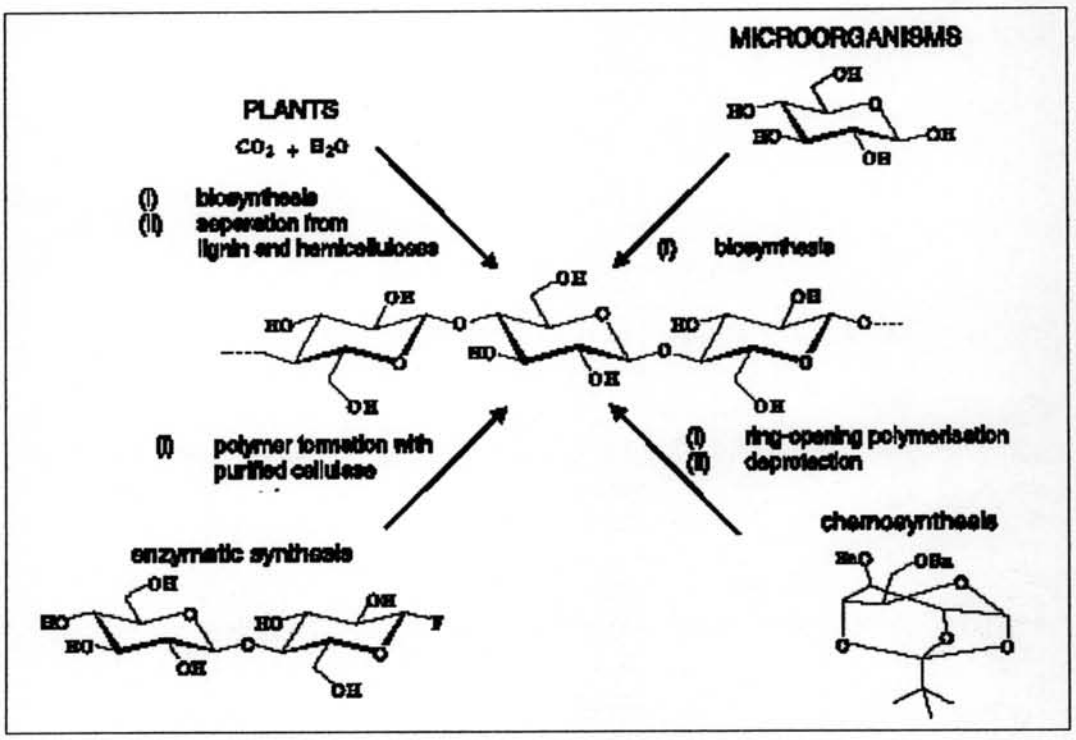

Figure 2.2 Pathways to the cellulose.

The first one is the most popular and industrial important isolation of cellulose from plants including separation processes to remove lignin and hemicelluloses (Tarchevsky *et al.,* 1991). The second way consists in the biosynthesis of cellulose by different types of microorganisms. From the scientific point of view the first enzymatic in vitro synthesis starting from cellobiosyl fluoride

and the first chemosynthesis from glucose by ring-opening polymerization of benzylated and pivaloylated derivatives (Nakatsub *et al.*, 1996) are of importance. These principle pathways are described schematically in Figure 2.2.

Several bacteria are in strains from the genera *Acetobacter, Agrobacterium, Pseudomonas, Rhizobium* etc. But not all these bacterial species are able to secrete the synthesized cellulose as fibers. Table 2.1 gives the overview. Special attention was given to strain from *Acetobacter xylinum* (Rainer & Farah, 1997).

Table 2.1 Bacterial cellulose producers and feature of their product

Genus	Cellulose structure
Acetobacter	Extracellular pellicle
Achromobacter	Fibrils
Aerobacter	Fibrils
Agrobacterium	Short fibrils
Alcaligenes	Fibrils
Pseudomonas	No distinct fibrils
Rhizobium	Short fibrils
Sarcina	Amorphous cellulose
Zoogloea	Not well defined

2.1.3 Cellulose Synthesis Using *Acetobacter Xylinum*

2.1.3.1 Acetobacter Xylinum

Acetobacter Xylinum is a member of Family IV., Acetobacteraceae. This family is well known in the vinegar industry. The bacteria of this family convert ethanol to acetic acid. These are Gram-negative, rod-like shaped bacteria. They are strict aerobes. The optimum temperature range for growth is 25-30°C and the optimum pH range is *5-6. Acetobacter Xylinum* produces cellulose for two reasons; as a by-product of its metabolism and as an environmental defense mechanism. This defense mechanism is used to allow the bacteria to float at the air-

liquid interface so it can access oxygen and the media as well as protecting it from the harmful UV rays of the sun.

2.1.3.2 Biosynthesis Pathway of Bacterial Cellulose

As known from the literature (Wulf *et al.,* 1996) the cellulose formation includes five fundamental enzyme mediated steps; the transformation of glucose to UDP-glucose via glucose-6-phosphate and glucose-1-phosphate and finally the addition of UDP-glucose to the end of a growing polymer chain by cellulose synthase (Figure 2.3). Cellulose synthase (UDP-glucose: 1,4-b-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.

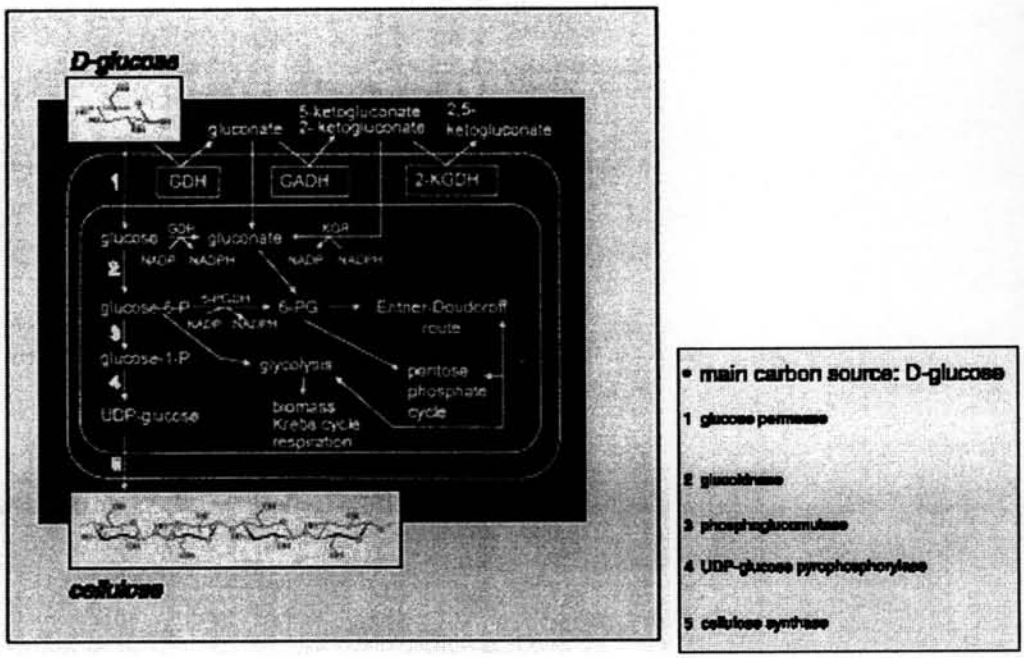

Figure 2.3 Pathways of carbon metabolism in *Acetobacter Xylinum.*

Acetobacter 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 (Figure 2.4). The matrix of the interwoven ribbons constitutes the bacterial cellulose membrane or pellicle. Figure 2.5a shows bacterial cellulose ribbon produced by one bacterial cell and Figure 2.5b demonstrates that *Acetobacter xylinum* cells are distributed throughout the network of the cellulose ribbons (Tokoh *et al.*, 1998).

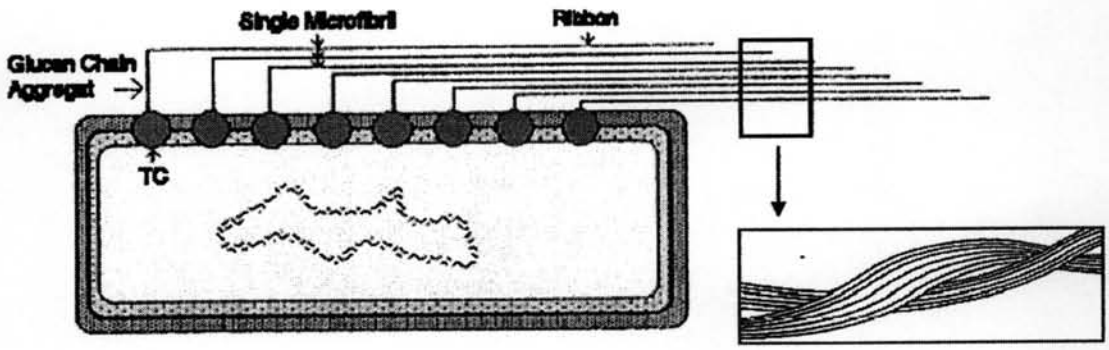

Figure 2.4 Formation of bacterial cellulose ribbon.

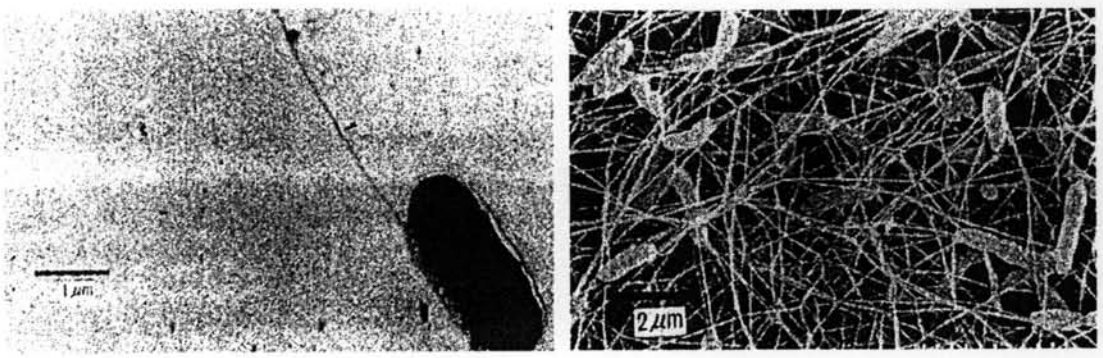

Figure 2.5 SEM image of (a) the bacterial cellulose ribbon produced by a bacterial cell and (b) the bacterial cellulose network including the bacterial cells.

2.1.3.3 Structure Features and Properties of Bacterial Cellulose

Bacterial cellulose revealed that it is chemically identical to plant cellulose, but its macromolecular structure differs from the latter. Bacterial cellulose microfibril is much smaller than that of plant cellulose. Bacterial cellulose is also distinguished from its plant counterpart by a high crystallinity index (above 60%) and different degree of polymerization, usually between 2000 and 6000 (Jonas & Farah, 1998), but in some cases reaching even 16000 or 20000, whereas the average DP of plant polymer varies from 13000 to 14000. Macroscopic morphology of bacterial cellulose strictly depends on culture conditions (Watanabe *et al.,* 1998; Yamanaka *et al.,* 2000). In static conditions, bacterial cellulose was produced in a form of leather-like pellicle on the surface of nutrient broth (Figure 2.6a). The subfibrils of cellulose are continuously extruded from linearly ordered pores at the surface of the bacterial cell, crystallized into microfibrils, and forced deeper into the growth medium. Therefore, the leather-like pellicle, supporting the population of *Acetobacter xylinum* cells, consists of overlapping and intertwisted cellulose ribbons, forming parallel but disorganized planes (Jonas & Farah, 1998). In agitated conditions, bacterial cellulose was produced in a form of irregular granules, well-dispersed in a culture broth (Figure 2.6b) (Vandamme *et al.,* 1998).

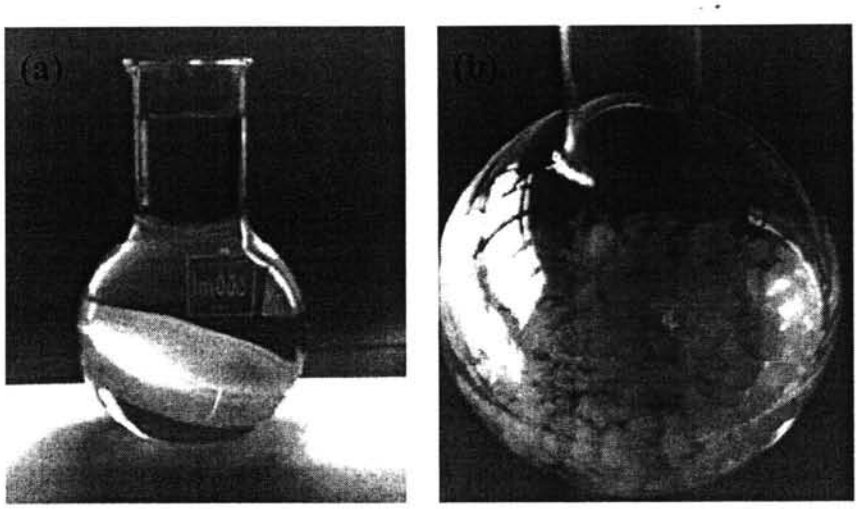

Figure 2.6 Bacterial cellulose in form of (a) pellicle and (b) irregular granules.

The agitated culture method seems to be suitable for industrial production of bacterial cellulose and serves commercial applications in various fields. The static culture method has been adopted mainly for the investigation of cellulose production, because cellulose deficient mutants sometimes appear in agitated cultures; these mutants were believed to interfere with the production of bacterial cellulose. There is some difference in the structure of cellulose crystal and molecular chain between the two types of bacterial cellulose produced in the different culture conditions. In an agitated culture, the crsytallinity and cellulose Iα content of cellulose were found to be lower than that in a static culture as shown in Table 2.2; in addition, the degree of polymerization of cellulose molecule was lower. This structural disorder of bacterial cellulose from an agitated culture seems to arise from the agitation and the cellulose excreted from *Acetobacter xylinum*. These finding suggest that the smaller particle size of agitated bacterial cellulose leads to a higher water holding capacity than static bacterial cellulose. Cross polarization-magic angle spinning ^{13}C nuclear magnetic resonance (CP/MAS ^{13}C-NMR) analysis revealed that the cellulose Iα content of the cellulose produced in an agitated culture lower than that of the cellulose produced in a static culture. Therefore, it has a lower Yong's modulus of sheet, a higher water-holding capacity, and a higher suspension viscosity in the disintegrated form than produced in a static culture.

Table 2.2 Cellulose I_α and I_β content (%) and percent crystallinity (%) of bacterial cellulose from different culture conditions

Cellulose sample	I_α	I_β	Percent crystallinity (%)
Stationary	76	24	89
Agitated	71	29	84

Moreover, the properties of bacterial cellulose are also different form properties of cellulose produced from plant. The distinguish properties of bacterial cellulose are concluded in Table 2.3

Table 2.3 Distinguishing features of microbial cellulose

Property	Description
Purity	– Cellulose is the only biopolymer synthesized – Absence of lignin or hemicelluloses – Completely biodegradable and recyclable, a renewable resource
Great mechanical strength	– High strength crystalline cellulose I – Consistent dimensional stability – High tensile strength – Light weight – Remarkable durability
Extraordinary absorbency in the hydrated state	– Remarkable capacity to hold water – Selective porosity – High wet strength – High surface-to-volume carrier capacity
Direct membrane assembly during biosynthesis	– Intermediate steps of paper formation from pulp unnecessary – Intermediate steps of textile assembly from yarn unnecessary – Extremely thin, submicron, optical clear membranes can be assembled
Cellulose orientation during Synthesis	– Dynamic fiber-forming capabilities – Uniaxially strengthened membranes
Direct modification of cellulose during assembly	– Delayed crystallization by introduction of dyes into culture medium – Control of physical properties of the cellulose during assembly (molecular weight and crystallinity)

2.1.3.4 Bacterial Cellulose as a Wound Dressing

Healing of skin wounds is a complex process which requires the involvement of many different tissues, cell types and matrix components (Balasubramani *et al.,* 2001). There are three major directions in which wound-healing research is aimed presently: (a) improvement of wound healing by elements which may potentially accelerate healing and reduce scarring, (b) development of novel skin substitutes as equivalents of autograft skin, and (c) identification of signals that trigger the process of healing by regeneration rather than repair (scar formation). The present status of modern wound healing systems generally requires that materials used for the wound cover should create an optimal environment for epidermal regeneration by providing a barrier against wound infection and fluid loss. Many different biological and synthetic wound dressings have been developed in order to treat surgical and non-surgical lesions (Walker *et al.,* 2003; Innes *et al.,* 2001). Some of these have been quite successful in wound closure, however a search for the ideal wound dressing material is still continuing. According to modern approaches in the field of wound healing, an ideal wound dressing system must display similarity to autograft skin, both structurally and functionally (Vloemans et *al.,* 2001). Table 2.4 shows the set of requirements to be fulfilled by a modern, successful wound care dressing material.

Table 2.4 Characteristics of the modern wound care dressing material

Characteristics
Non-toxic, non-pyrogenic, biocompatible
Able to provide barrier against infection
Able to control fluid loss
Able to reduce pain during treatment
Able to create and maintain a moist environment in the wound
Enable introduction or transfer of medicines into the wound
Able to absorb exudates during inflammatory phase
Display high mechanical strength, elasticity and conformability
Allow for easy and painless release from the wound

There have been several publications and reports on the successful use of bacterial cellulose as a medical product. In 1990, Fontana *et al.* reported the application of cellulose pellicles of varying thickness, produced by *Acetobacter*, as temporary skin substitutes. The product, called Biofill®, has been used for several skin injury 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 skin grafts. In 2004, Alvarez *et al.* reported the use of bacterial cellulose in the form of a hydrated membrane (Xcells, Xylos Co.) in the treatment of chronic venous ulcers. The applied, hydrated membrane of bacterial cellulose allowed both; (a) maintenance of a proper moist environment around the wound, and (b) due to its highly nano-porous structure, absorbance of the wound's exudates. Another interesting and important advantage of the bacterial cellulose dressing includes its transparency, which allows for continuous clinical observation of the healing progress. Generally, the studies showed that bacterial cellulose membranes significantly facilitated the process of necrotic debris removal (autolytic debridement), improved the development of granulation tissue, and accelerated the entire process of re-epithelialization, in comparison with the control group of patients. A significant decrease in daily wound care needs, degree of pain, and the overall time of healing were observed in the treatments with bacterial cellulose dressings in comparison with the control procedures.

2.2 Silver Nanoparticle

2.2.1 Preparation of Silver Nanoparticle

The most common methods used for the preparation of colloidal suspensions of metals (silver including) are the reduction of corresponding metal cation. In addition to the inorganic or organic reduction agents, the ultrasound, the UV radiation and gamma radiation can be used to initiate the reduction.

2.2.1.1 Laser Ablation

Laser ablation of silver macroscopic material (e.g. silver foil) is a novel and promising physical method for the silver colloid particles preparation. The advantages of this method are namely an ease of the process, versatility with regard to metal identity or choice of solvent as well as the absence of additive chemical agent residues. Metal particles prepared by laser ablation are chemically pure.

2.2.1.2 The Reduction by the Action of Ultrasound

Except for the above-mentioned usage of ultrasound in a dispersion method of colloid particle preparation it can be also as a condensation method. The ultrasound is capable to decompose water into hydrogen and hydroxyl radicals. Subsequent reactions with suitable additives yield organic radicals which act as reducing agents. By sonification of aqueous silver salts solutions in the presence of surfactants the silver particles were prepared.

2.2.1.3 The Reduction by the Action of Gamma Radiation

For the preparation of submicroscopic silver particles a direct radiolysis of silver salt aqueous solutions can be used. The advantage of this preparation method is that minimum interfering chemical substances are introduced into the reduction mixture, which could possibly absorb onto particles and thus change their specific properties. During the irradiation of silver salt solution under hydrogen gas atmosphere hydrated electrons and hydrogen atoms are formed, which reduce the silver ions to form silver nanoparticles.

2.2.1.4 The Reduction by the Action of UV Radiation

Photochemical method of colloid particle preparation using UV radiation yields the particles with properties similar to the particles produced by

the above mentioned radiolytic method. Mercury discharge lamp is often used as the source of UV radiation. In addition to silver salt and evental stabilizers the reaction mixture contains suitable organic substance whose interaction with UV radiation generates radicals which reduce silver ions.

2.2.1.5 The Reduction by Chemical Agents

The most commonly used method for the preparation of silver sols is the reduction of silver salt by sodium borohydride ($NaBH_4$), which generated free electrons and reduce silver ions to form silver particle. By the standard methods of silver salt reduction by NaH_4 the particles with units of nanometers sizes and narrow size distribution are prepared.

2.2.2 Optical Properties of Silver Nanoparticle

Conduction electrons and ionic cores in metal form a plasma state. When external electric fields (electro-magnetic waves, electron beams etc.) are applied to a metal, electrons move so as to screen perturbed charge distribution, further move beyond the neutral states, and again return to the neutral states and so on. This collective motion of electrons is called a "Plasma Oscillation" as shown in Figure 2.7. The surface plasmon resonance is a collective excitation mode of the plasma localized near the surface. Electrons confined in a nanoparticle conform the surface plasmon mode. The resonance frequency of the surface plasmon is different from an ordinary plasma frequency. The surface plasmon mode arises from the electron confinement in the nanoparticle. Since the dielectric function tends to become continuous at the interface (surface), the oscillation mode shifts from the ordinary plasma resonance and exponentially decays along the depth from the surface.

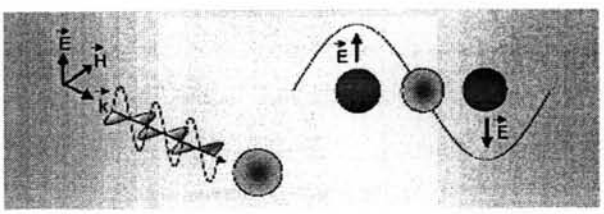

Figure 2.7 Plasmon oscillation of the free electron on the surface of metal nanoparticle.

2.2.3 Antimicrobial Properties of Silver Nanoparticle

Silver metal and silver compound have long been known to have strong inhibitory and bactericidal effects as well as a broad spectrum of antimicrobial activities. Several proposals have been developed to explain the inhibitor effects of silver ion/silver metal on the both negative and positive bacteria. Silver ions are known to react with nucleophilic amino acid residues in proteins, and attach to sulphydryl, amino, imidazole, phosphate and carboxyl groups of membrane or enzyme proteins that lead to protein denaturation. Silver is also known to inhibit a number of oxidative enzymes such as yeast alcohol dehydrogenase, the uptake of succinate by membrane vesicles and the respiratory chain of Escherichia coli, as well as causing metabolite efflux and interfering with DNA replication. Recent, microbiological and chemical experiments implied that interaction of silver ion with thiol groups played an essential role in bacterial inactivation. Also, it is revealed that bulk silver in an oxygen-charged aqueous media catalyzes the complete destructive oxidation of microorganisms. However, the antimicrobial effects of silver nanoparticles were not fully investigated. Metal nanoparticles (Me-NPs), which have a high specific surface area and a high fraction of surface atoms, have been studied extensively due to their unique physicochemical characteristics such as catalytic activity, optical properties, electronic properties, antimicrobial activity, and magnetic properties It can be expected that the high specific surface area and high fraction of surface atoms of Ag-nanoparticles will lead to high antimicrobial activity compared to bulk Ag metal (Kyung-Hwan Cho *et al.*,2005).

2.3 Antimicrobial Agent and Antimicrobial Wound Dressing

2.3.1 Silver Nitrate Solutions

A 0.5% solution was the lowest concentration that remained active against bacteria in vitro (on agar and broth) and in vivo on burns, but it had no toxic effect on growing epidermal cells. Grafts also took without trouble under a dressing moistened with 0.5% $AgNO_3$ solution. Great significance was attached to the way the silver nitrate was administered: thick cotton gauze dressing covering the wound had to be kept continuously moist (usually by wetting the dressing with $AgNO_3$ solution every 3±4 hrs. The dressings were changed once or twice a day. Cotton-wool and English lint were considered to be less suitable for the dressings. The wound should be free from fat, ointment or dead epidermis, so as not to hinder the effect of the $AgNO_3$. In fact, all dead tissue needed to be removed as quickly as possible, in order to ensure optimum action of the $AgNO_3$. Silver nitrate was active against Staphylococcus aureus, haemolytic streptococci and generally against Pseudomonas aeruginosa and Escherichia coli. The main complication occurring during the treatment was a drop in serum sodium and chlorine, due to ion exchange between Ag^+ and Cl^-, HCO_3^-, CO_3^- and protein anions and leading to the production of very slightly soluble or insoluble salt solutions. Another drawback of the $AgNO_3$ treatment was that all objects which came into contact with the $AgNO_3$ were blackened on exposure to light.

2.3.2 Silver Sulphadiazine

Silver sulphadiazine was introduced shortly after by Dr. Fox. Silver sulphadiazine was a combined formulation made from silver nitrate and sodium sulphadiazine by substituting a silver atom for a hydrogen atom in the sulphadiazine molecule. It was hoped that this would make it possible to combine the inhibitory action of the silver with the antibacterial effect of sulphadiazine. A possible explanation of this effectiveness could be the relatively strong bonding of silver sulphadiazine to DNA. This bonding differs from that of silver nitrate.

Silver sulphadiazine has a good antimicrobial activity like silver nitrate, although not demonstrating many of the negative aspects of silver nitrate, is

also somewhat problematic because of its propensity to form pseudoeschar that must be removed on a regular basis; a cause of considerable discomfort for some patients.

2.3.3 The Novel Silver-coated Dressing

The most common topical agents used in the treatment of burn wounds include mafenide acetate (MA), silver sulfadiazine (SSD), and silver nitrate that were explained above topic. Each of these agents, recognized as having good antimicrobial activity, has several potential drawbacks to its use. For example, MA is well known as being more painful than other treatments on application as well as having very limited biocidal effects against fungi or bacteria. Silver nitrate causes a significant amount of staining of virtually any surface with which it comes into contact, in addition to possibly causing tissue irritation SSD, although not demonstrating many of the negative aspects of silver nitrate, is also somewhat problematic because of its propensity to form pseudo-eschar that must be removed on a regular basis; a cause of considerable discomfort for some patients.

To overcome these problems, a novel silver-coated dressing recently has been developed. The dressing is coated with nano-crystalline silver, a form of silver that has been demonstrated to be effective at rapidly killing a broad spectrum (Wright *et al.,* 1998).

2.3.4 The Novel Silver-impregnated Dressing

Although a novel silver-coated dressing have the antimicrobial activity better then that of the topical silver agents but there are some the clinical experiment of the dressing is coated with nanocrystalline silver is fail (Lia *et al.*, 2006). The reason for clinical failure is mainly due to the obliteration of silver particles by proteinaceous materials from the human body (Furno *et al.,* 2004). A recent study showed that impregnation, instead of coating the medical device with nanoparticulate silver metal, improved the antimicrobial activity of the device. This is probably due to the slow and continual release of silver that prolonged the antimicrobial effect (Furno *et al.,* 2004).