CHAPTER II
THEORETICAL BACKGROUND AND LITERATURE REVIEW

2.1 Alginate (Pawar *et al.*, 2012)

2.1.1 Source

Alginate is a natural polymer that extracted from three species of brown algae, Laminaria hyperborean, Ascophyllum nodosum, and Macrocystis pyrifera. Generally, a normal alginate form is a salt complex. Alginate can complexes with other cations that found in seawater for examples Mg^{2+}, Sr^{2+}, Ba^{2+},and Na^{+} .

2.1.2 Chemical Structures

Alginate is a liner polysaccharides that consisting of blocks of 1–4 linked α-L-guluronic(G) and β-D- mannuronic acid residues(M). The structures of mannuronic acid, M block, and guluronic units, G block, shows in Fig.2.1a. The geometries of the G-block and M-block show in Fig.2.1b. There are three different geometries of alginate, G-block regions, M-block regions and G-M block regions.

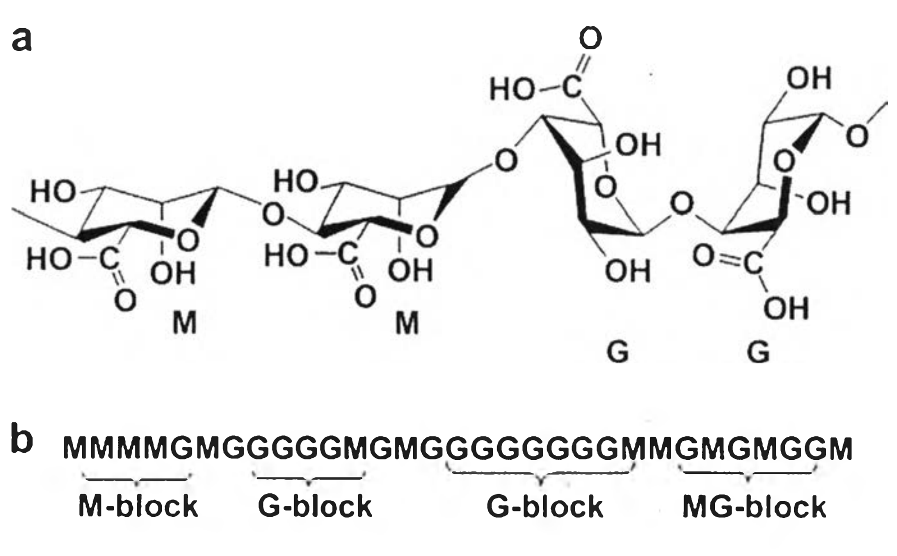

Figure 2.1 Representative alginate structure: (a) chain conformation and (b) block distribution (Pawar *et al.*, 2012).

2.1.3 Ionic Crosslinking

Alginate can form gel structure by reaction that an extremely mild environment and uses non-toxic reactants. The most important gelation is a reaction between divalent cations and alginate. Most of divalent cations such as Ca^{2+} Sr^{2+} and Ba^{2+} ion can form gelation with alginate but monovalent cation and Mg^{2+} ion do not induce the gelation. Alginates that have different amount of G and M block are effect on gel's properties. Normally the gelation is occurred between divalent cation and G-block of alginate. Thus the alginates that contenting of high G unit are higher stiffness gel. Fig. 2 shows egg-box structure, the crosslinked structure between cations and alginates.

Each alginate chain creates many junctions with other chains, the result is formed gel networks. Calcium ion reacts with alginate. It induced dimeric association of the G-block regions. These gels which are similar to solids in retaining their shape and resisting stress, are 99–99.5% water with rest being alginate. Amount of calcium ion is effect on their properties. Alginate with low levels of calcium, the interaction is temporary associations that giving rise to highly viscous, thixotropic solutions. Alginate with higher calcium levels, the gelation of alginate is permanently formed. Many studies have report about factor of gelation and their properties. the chemical structure, molecular size and the gel forming kinetics of cations have effected on including porosity, swelling behaviour, stability, biodegradability, gel strength and the gel's immunological characteristics and biocompatibility.

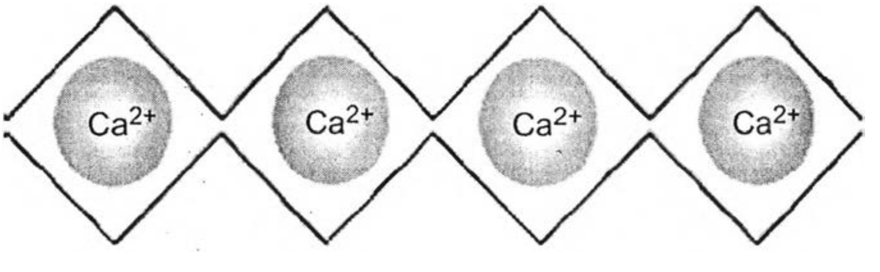

Figure 2.2 Possible junction points in alginates (Pawar *et al.*, 2012).

2.1.4 Mild Gelation Conditions

The gelation of alginate, between alginate and divalent cations, can occur under an extremely mild environment with non-toxic catalysts. Most of divalent cations such as Ca^{2+} Sr^{2+} and Ba^{2+} ion induced ionic crosslink. The network structure of alginate and cations can entrap proteins.

There are two different methods to crosslink alginate with cations. The first method is a "diffusion" method. The method is occurred by crosslinking ions diffusing into the alginate solution from an outside reservoir. Another method is "internal setting" method. In this method, ion-source locates in alginate solution. Generally, the ion-source is insoluble calcium salts such as $CaCO_3$. A change in the pH caused the release of Ca^{2+} ions internally and leads to gel formation. Hydrolyzing lactone such as D-glucono-d-lactone (GDL) triggers is use to slowly changing pH of the solution. The result in the concentration on Ca^{2+} ion is uniform.

2.2. Wounds

A wound is one type of injury. It is occurred by physical, chemical mechanical, and/or thermal damages on skin.

2.2.1 Type of Wounds (Zahedi *et al.*, 2010)

2.2.1.1 Acute Wounds

The acute wounds are usually caused by mechanical damage, including in sheer, blunting, and/or stabbing action of hard objects. The mechanical damage include injured from gun and knives. It is also formed by exposure to extreme heat, irradiation, electrical shock, and/or irritated with corrosive chemicals but the acute wound can normally heal in 8 to 12 weeks. The four steps of healing process are hemostasis, inflammation, proliferation, and remodeling.

2.2.1.2 Chronic Wounds

The chronic wound is an open wound that at least the second layer of the skin, called the dermi, is damaged. Fig. 4 shows layers of skin. Normally, the chronic wound is occurred by of specific diseases for examples diabetes, malignancies, tumors, and severe physiological contaminations. The healing time of chronic wound takes more than 12 weeks and recurrence of the wounds is not uncommon.

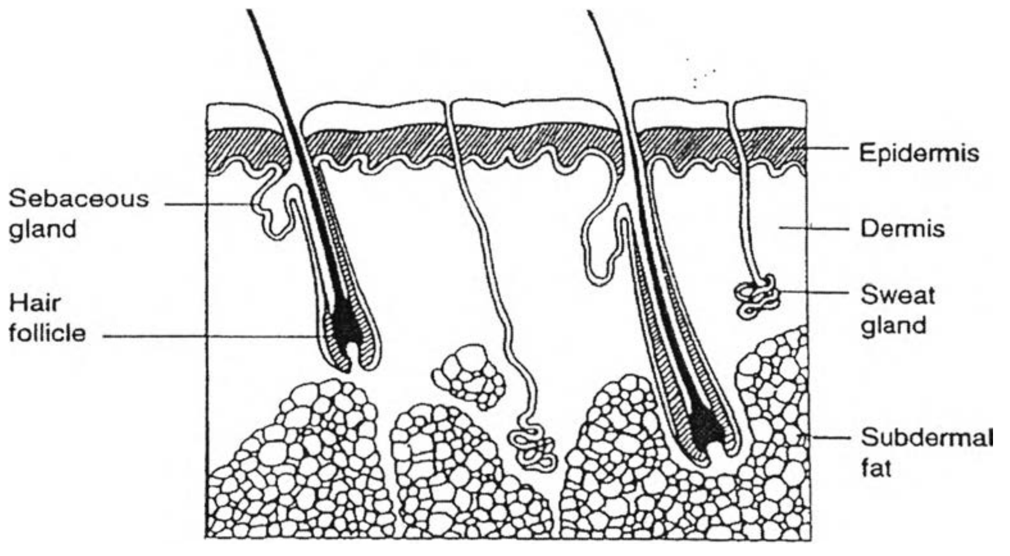

Figure 2.3 Layer of the skins.

2.3 Wound Healing

The wound healing process is a general phenomenon involved in growth and tissue regeneration. This process helps both replacing structure and function of damaged or injured tissue. The wound healing process was reported by Schults; these can be summarized into five steps (Fig.2.4) as hemostasis including hemostasis, inflammation, migration, proliferation, and maturation.

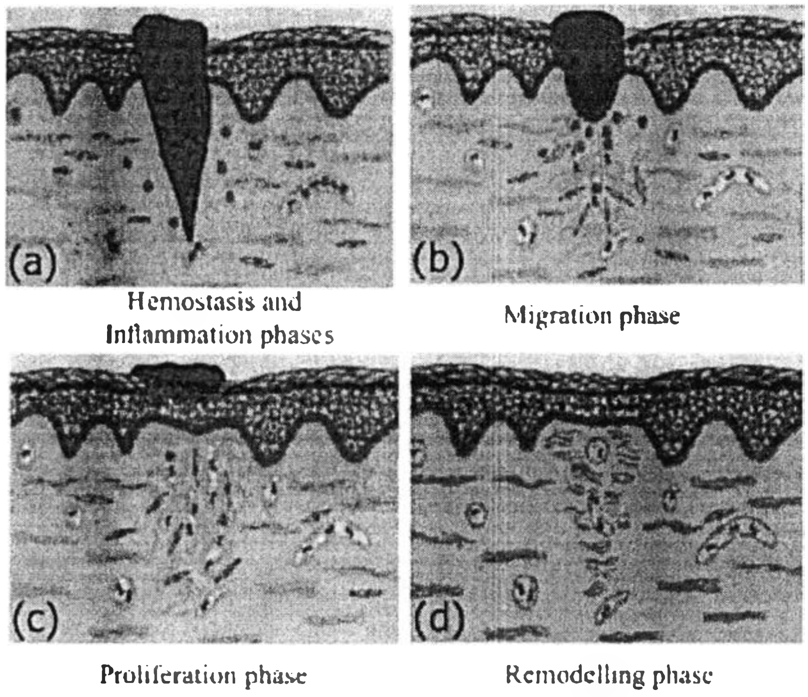

Figure 2.4 Schematic representation of different phases of wound healing (Zahedi *et al.*, 2010).

2.3.1 Hemostasis and Inflammation

When the wound and breeding occured, the initial response is hemostasis, in the first step. This step involves in clotting and platelets. Platelets come into the wound surface to block bleeding. Clotting factors are activated and fibrinogen form to fibrin structure. The fibrin network induced clotting in wound area and cause bleeding to stop. A scab is form by clot dries and it provides strength and protection the injured wound.

The inflammation step occurs almost at the same time with hemostasis. This step relates with cellular and vascular responses. The protein-rich exudate is released into the wound area and causes vasodilation. Free-platelets from damaged blood vessels become activated and come into contact with exposed collagen. These platelets form aggregates as an essential part in clotting mechanism.

2.3.2 Migration

The migration step involves in epithelial cells' movement and formed fibrin onto wound area. That step use to replace damaged tissue. New cells are regenerate and rapidly growing under the dried clot.

2.3.3 Proliferation

The proliferative step occurs contemporary or just after the migration step. In this step, granulation tissue is formed and collagen is created by fibroblasts. The result of this step is generating strength skin. In the same time, blood vessels decrease.

2.3.4 Maturation

The maturation step is also known "remodeling step". This step involves in new tissue and epithelium regeneration which determines the nature of the final scar. Maturation continues even after mothers of closed wound.

2.4 Wound Dressing

Wound dressing is a material which to cover wound area. It has developed over the years for many applications. Wound dressing should minimize infection and pain, create moist environment, promote proliferation, protection the wound from environmental irritants, biocompatible and antimicrobial.

2.4.1 Classification of Wound Dressing

The wound dressings are classified in a number of ways such as theirs antibacterial function, types of material and the physical form of wound dressing. The simplest classification of wound dressing is diving into two types; Traditional dressing and Modern dressing.

2.4.1.1 Traditional Dressing

The traditional dressing is a gauze made from cotton, wool, natural or synthetic woven. These wound dressings are dry and do not create moist environment.

Gauze dressing is one of important traditional dressing, which made from woven and non-woven fibers include in cotton and rayon polyester. Gauze dressing is used for open wound to absorb wound's exudates. Gauze dressings need to improve the prevention of maceration of the healthy underlying tissue and have been reported to be less cost effective compared with the more modern dressings.

2.4.1.2 Modern Dressing

The modern dressings have been developed from traditional dressing to improve their properties. The important modern dressing's characteristic is to maintain and create a moist environment around wound area. The moist environment helps to facilitate healing process. There are many types of modern dressing i.e. hydrocolloids, alginates and hydrogels, semi-permeable adhesive film dressings, foam dressing and biological dressings.

2.5 The Clotting Process (Hemostasis) (Urbana, 2009)

The process by which the body prevents blood loss is referred to as coagulation. Coagulation involves the formation of a blood clot (thrombus) that prevents further blood loss from damaged tissues, blood vessels or organs. This is a complicated process with a cellular system comprised of cells called platelets that circulate in the blood and serve to form a platelet plug over damaged vessels and a second system based upon the actions of multiple proteins (called clotting factors) that act in concert to produce a fibrin clot. These two systems work in concert to form a clot; disorders in either system can yield disorders that cause either too much or too little clotting.

Platelets serve three primary functions:

1) Sticking to the injured blood vessel (called platelet adherence),

2) Attaching to other platelets to enlarge the forming plug (called platelet aggregation), and

3) Providing support (molecules on the surface of platelets are required for many of the reactions) for the processes of the coagulation cascade.

When a break in a blood vessel occurs, substances are exposed that normally are not in direct contact with the blood flow. These substances (primarily collagen and von Willebrand factor) allow the platelets to adhere to the broken surface. Once a platelet adheres to the surface, it releases chemicals that attract additional platelets to the damaged area, referred to as platelet aggregation. These two processes are the first responses to stop bleeding. The protein based system (the coagulation cascade) serves to stabilize the clot that has formed and further seal up the wound.

The diagram (Fig. 2.5) shows many of the reactions in the coagulation cascade that are necessary to form a clot. As noted above, the third role of the platelet is to support the coagulation cascade. This support is provided, in part, by one of the components of the outside of a platelet, called phospholipids (lipid=fat), which are required for many of the reactions in the clotting cascade.

The goal of the cascade is to form fibrin, which will form a mesh within the platelet aggregate to stabilize the clot. All of the factors have an inactive and an active form. Once activated, the factor will serve to activate the next factor in the sequence until fibrin is formed.

The coagulation cascade takes place at the site of a break in a blood vessel that has the platelet aggregate. Tissue factor and factor VIIa (the 'a' denotes the active form of the factor) activate factor X, forming factor Xa. Factor Xa is then able to activate prothrombin (also referred to as factor II) to form thrombin (factor IIa). Thrombin converts fibrinogen to fibrin (factors I and Ia respectively). Fibrin forms a mesh that, in concert with the platelets, plugs the break in the vessel wall. The fibrin mesh is further stabilized by factor XIII, which sews up the clot (much like forming an intricate network of cross-stitched strands of fibrin).

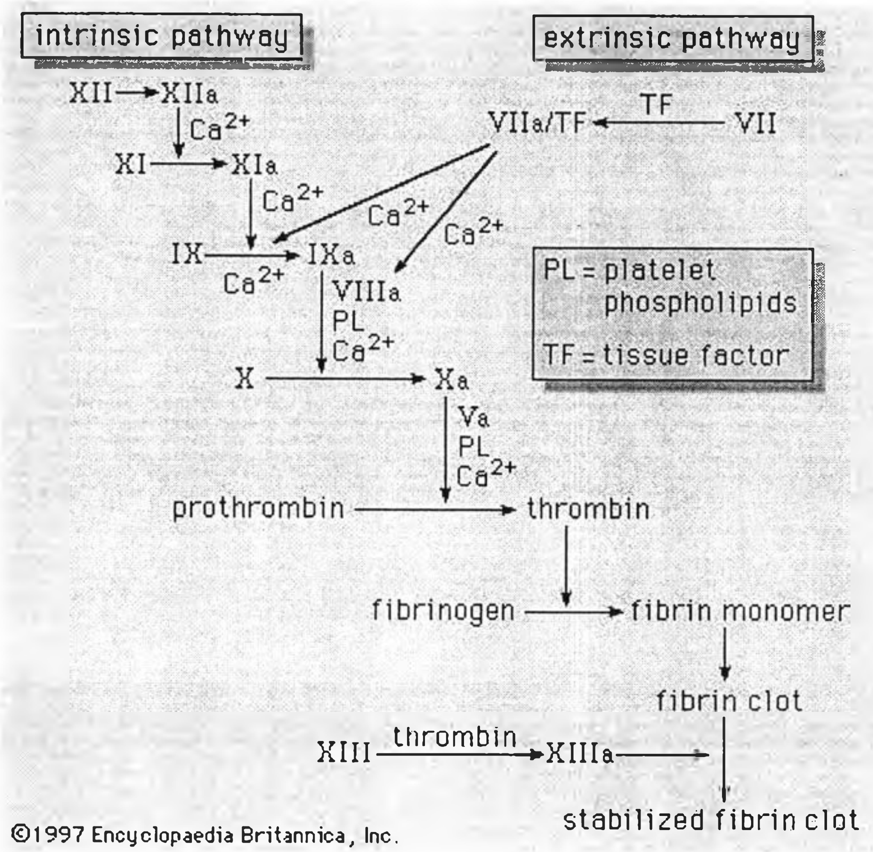

Figure 2.5 The blood coagulation pathways (Bruce, 2014).

LITERATURE REVIEWS

Rhim et al. (2004) modified sodium alginate films by adding $CaCl_2$ in two different methods. The first method was mixing method that directed addition of $CaCl_2$ into sodium alginate solution. The second method was immersion method that casted alginate film and then immersed film into $CaCl_2$ solution. Rhim found that the mixing method had limitation of concentration of $CaCl_2$ If adding $CaCl_2$ more than 0.12 g., the solution would form too strong of a gel to cast thin film and surface of alginate film was not smooth. Film that came from immersion method was slightly translucent and white-milk color. About mechanical properties, Rhim found that both mixing method and immersion method had tensile strength increased while modulus decreased with adding $CaCl_2$ but the degree of changing tensile strength and modulus in immersion method was higher than mixing method. In addition, Rhim reported the crosslinking the mixing method was immediately occurred thus crosslinking density was not homogenous.

Chan et al. (2012) studied effect of using different types and concentrations of cations in crosslinked alginate on their mechanical behaviors and stiffness. Chan found that the ion entrapment within the 'egg-box' structures related with release of structured water. The amount of water released was found to be related to the strength of the ion–uronate interaction, and the reduction of the bead diameter reflected the strength and extent of the cross-linking process. The alginate beads were found to be deformed plastically when they were compressed beyond 50% deformation with the exception of copper–alginate beads for which yield occured at a lower deformation.

Roger et al. (2006) prepared magnetic alginate film that treated surface by Ca^{2+} ion. Sodium-alginate films were prepared by casting sodium-alginate aqueous solution. The dried Sodium-alginate films were immersed in $CaCl_2$ aqueous solution, and washed by deionized water to remove residual Ca^{2+} ion. The result of amount of Ca^{2+} ion and Na^+ ion in the alginate film was as a function of the crosslinking time. Calcium ion rapidly diffused into the film and exchanged ion with Na^+ ion in the first 5 min. After 10 min. in $CaCl_2$ bath, the Ca^{2+} concentration was constant.

Straccia et al. (2014) studied effects of crosslinker on functional properties of alginate/N-succinylchitosan based hydrogels. The gel films were prepared by casting sodium-alginate/ N-succinylchitosan aqueous solution and then dipping film in Ca^{2+} or Zn^{2+} aqueous solution. The synersis is a phenomenon that volume of gels is reduced by release some water. Straccia found that 1% w/v Zn^{2+} crosslinker had higher synersis than 3% w/v Zn^{2+} crosslinker. Thus 1% w/v Zn^{2+} crosslinked gel films was more flexible than another. From a stability test, Straccia reported Zn^{2+} ion able to crosslink not only GG blocks, but also MG and MM Blocks too. On the other hand Ca^{2+} ion able to crosslink only GG blocks. In addition, Ca^{2+} ion crosslinked gel films had higher modulus and tensile strength than Zn^{2+} ion crosslinker.

Aslani et al. (1996) studied effect of calcium or zinc ion crosslinker in alginate gel on diffusion of Acetaminophen. To prepare alginate gel film, Aslani dissolve sodium-alginate into distilled water then pore solution into glass pipette to cast film. Next, a dry sodium-alginate was immersed into Ca^{2+} or Zn^{2+} ion solution to get gel film. Aslani reported that the crosslink density of Zn^{2+} ion was higher than Ca^{2+} ion because zinc was able to crosslink less selectively with sodium-alginate than calcium. Presumably, the more crosslink time and cation concentration, the more crosslinking structure was form. Thus the alginate gel film less able to swell and less water content.

Morch et al. (2006) researched the effect of Ca^{2+}, Ba^{2+} and Sr^{2+} on alginate microbeads. All beads were made by dripping alginate solution into solutions of divalent cations and using an electrostatic bead generator. The dimensional stability and gel strength increased for high-G alginate gels when crosslinked by Ca^{2+} with Ba^{2+} ions. About the Sequence-Ion-Binding relationship, Ca^{2+} was found to bind to GG- and MG-blocks, Ba^{2+} to GG- and MM-blocks, and Sr^{2+} to GG-blocks solely.

Goh et al. (2012) studied the effect of Ca^{2+}, Cu^{2+} and Zn^{2+} as a crosslinker in sodium-alginate on the mechanical properties and releasing cations. The crosslinked films showed higher tensile strength than uncrosslinked films. The highest tensile strength was observed for films crosslinked with Ca^{2+}. For the release of cations from crosslinked alginate film, the percentage release of Cu^{2+} was lowest. This could be attributed to the higher bining affinity of Cu^{2+} than Ca^{2+} and Zn^{2+}.

Grace et al. (2009) presented the synthesis of copper alginate-cotton cellulose composite (CACC) fibers. The CACC fiber was prepared by immersing cotton fiber in sodium-alginate aqueous solution. Thus there were ionic crosslinking between alginate chain and Cu^{2+} ion. The FTIR spectra showed some interaction between OH group of cotton fiber and carboxylate of sodium-alginate because of reducing of absorption band. When crosslinked CACC by copper, the FTIR spectra showed a broad peak was observed in the range 3400-3200 cm^{-1} that meant the formation of co-ordinate bond between the carboxylate moiety of sodium – alginate and Cu(II) ions. Grace studied of antibacterial activity of CACC fiber at 2 and 4 percent Cu^{2+} ion solution. It can also be observed that the area of inhibition zone increases with copper content within the fibers.

Li et al. (2007) studied released behavior of copper ion in crosslinked composite base on PVA. The released rate of copper ion was determined by soaking PVA that containing copper crosslink in simulated body fluid (SBF). The chemical composition of the fluid is as follows: Na^{+}: 142.0 mmol/L, Cl^{-}: 125:0 mmol/L, HCO_3^{-}: 27:0 mmol/L, K^{+}: 5.0 mmol/L, Mg_2^{+}: 1.5 mmol/L; Ca_2^{+}: 2:5 mmol/L, HPO_4^{2-}: 1:0 mmol/L, SO_4^{2-}: 0:5 mmol/L. The container was transferred to a constant temperature (37°C) bath. After soaking, the mass of copper released at any time was determined by measuring the copper solution concentration using atomic absorption spectrophotometer.

Mata et al. (2009) studied biosorption of cadmium, lead and copper with calcium alginate xerogels and immobilized Fucus vesiculosus. The FITR spectra confirmed that carboxyl groups were main groups involved in the metal uptake. Calcium in the gels was displaced by heavy metals from solution according to the “egg-box” model. The relationship between the amount of calcium released and amount of metal absorbed could be confirmed that calcium could be involved in the metal uptake through an ion-exchange mechanism. Nevertheless, none direct proportional relationship was found between the amounts of metal adsorbed and calcium released.

Ferrero et al. (2013) develop cotton gauzes, increase antibacterial activity, that were coated with chitosan by using UV-curing process. First ferrero prepared chitosan solution by dissolving chitosan and photoinitiator in glacial acetic acid aqueous solution and stirring 24 hours. Second modification of cotton gauze, the cotton gauze was immersed into a solution of NaOH and Tetgitol NP. Then 3-Chloro-2-hydroxypropopyl-trimethylammonium chloride, reagent for cotton gauze cationization, was added in solution. Next the modified cotton gauze was dried and spread chitosan and photoinitiator solution on modified cotton gauze. Then the coated cotton gauze was dried and exposed to UV radiation. Ferrero reported stiffness of coated cotton gauze increase compere to uncoated cotton and 100% reduction of E. coli, S. auteus and K.pheumoniae bacterial when coated cotton gauze with chitosan.

Shanmugasundaram *et al.* (2011) develop cotton gauzes by coating with chitosan and sodium alginate polymer or coating with calcium alginate and sodium alginate polymer. First Shanmugasundaram prepared calcium alginate solution by dispersion of calcium alginate in sodium carbonate solution and then added sodium alginate polymer into calcium alginate solution. The cotton gauzes were pedded twice in this solution and were dried. Shanmugasundaram reported about antibacterial activity by measuring zone of inhibition. The result showed coated cotton gauze had anti-bacterial activity that the zone of inhibition is greater than 4 cm. for all samples.