RESEARCH ARTICLE

Chitin and Chitosan: Production and Application of Versatile Biomedical Nanomaterials.

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Manuscript Info

Abstract

Chitin is the most abundant aminopolysaccharide polymer occurring in nature, and is the building material that gives strength to the exoskeletons of crustaceans, insects, and the cell walls of fungi. Through enzymatic or chemical deacetylation, chitin can be converted to its most well-known derivative, chitosan. The main natural sources of chitin are shrimp and crab shells, which are an abundant byproduct of the food-processing industry, that provides large quantities of this biopolymer to be used in biomedical applications. In living chitin-synthesizing organisms, the synthesis and degradation of chitin require strict enzymatic control to maintain homeostasis. Chitin synthase, the pivotal enzyme in the chitin synthesis pathway, uses UDP-N-acetylglucosamine (UDPGlcNAc), produce the chitin polymer, whereas, chitinase enzymes degrade chitin. Bacteria are considered as the major mediators of chitin degradation in nature. Chitin and chitosan, owing to their unique biochemical properties such as biocompatibility, biodegradability, non-toxicity, ability to form films, etc, have found many promising biomedical applications. Nanotechnology has also increasingly applied chitin and chitosan-based materials in its most recent achievements. Chitin and chitosan have been widely employed to fabricate polymer scaffolds. Moreover, the use of chitosan to produce designed-nanocarriers and to enable microencapsulation techniques is under increasing investigation for the delivery of drugs, biologics and vaccines. Each application is likely to require uniquely designed chitosan-based nano/micro-particles with specific dimensions and cargo-release characteristics. The ability to reproducibly manufacture chitosan nano/microparticles that can encapsulate protein cargos with high loading efficiencies remains a challenge. Chitosan can be successfully used in solution, as hydrogels and/or nano/microparticles, and (with different degrees of deacetylation) an endless array of derivatives with customized biochemical properties can be prepared. As a result, chitosan is one of the most well-studied biomaterials. The purpose of this review is to survey the biosynthesis and isolation, and summarize nanotechnology applications of chitin and chitosan ranging from tissue engineering, wound dressings, antimicrobial agents, antiaging cosmetics, and vaccine adjuvants.

Introduction:-

Chitin (β-(1-4)-poly-N-acetyl-D-glucosamine) is widely distributed in nature and is the second most abundant polysaccharide after cellulose. Chitin, which occurs in nature as ordered macrofibrils, is the major structural component in the exoskeletons of the crustaceans, crabs and shrimps, as well as the cell walls of fungi. For biomedical applications chitin is usually converted to its deacetylated derivative, chitosan (1). Chitin and chitosan are both biocompatible, biodegradable, and non-toxic biopolymers. They are also antimicrobial and hydrating agents (2). Depending on the source of chitin, it occurs as two allomorphs, namely the α and β forms, that can be characterized by infrared and solid-state NMR spectroscopy, together with X-ray diffraction. γ-Chitin is a third allomorph that has also been described (3). The allomorphs differ in the orientation of the micro-fibrils. Chitin biosynthesis is catalyzed by a widely conserved enzyme in nature, called chitin synthase. This enzyme exists in every chitin-synthesizing organism. Chitin synthase remains bound to the growing polymer chain through many polymerization steps that sequentially adds single GlcNAc units to the non-reducing end of the extending chain (4, 5). The linear polymers of chitin that are first obtained, then spontaneously assemble into microfibrils of varying diameter and length. After polymer synthesis, the completed fibrils are transported to the extracellular space(4). Glycosyl hydrolases (GH) are a broad class of enzymes that degrade polysaccharides; they are currently classified into 130 families based on similarities in their amino acid sequences (6, 7). Chitinases are mostly classified in the GH-18 and GH-19 families, and have been found to be present in a wide range of organisms, including bacteria, fungi, insects, plants, and animals (8-11). Chitin and chitosan are used in a wide range of biomedical applications such as tissue engineering, drug and gene delivery, wound healing, and stem cell technology (12). These biopolymers can be easily processed into various products, including hydrogels (1), membranes (13-16), nanofibers (17-19), beads (20), micro/nanoparticles (1, 21), scaffolds (22, 23) and sponges (1). Tissue engineering is one of the most-studied fields in which chitin and chitosan have been successfully used to fabricate polymeric scaffolds for purposes of tissue repair and regeneration.

The most important characteristics of these biopolymers which have made them ideal candidates to fabricate polymeric tissue scaffolds are: high porosity; biodegradability; predictable degradation rate; structural integrity; non-toxicity to cells; and biocompatibility. Loading chitin-based nanoparticles with various drugs such as lamivudine and 5-fluorouracil, are just two examples of successful applications of chitin and chitosan in drug delivery. More recently, semiconductor nanocrystals (quantum dots) have been coated with chitosan for bioimaging applications in cancer diagnosis (24). The enhancement of immune responses against pathogenic microorganisms by using chitosan derivatives as vaccine adjuvants has been reported (25). Other applications of chitin and chitosan will be discussed in the following sections.

Chitin and chitosan: general characterization:-

Chitin, poly (β-(1-4)-N-acetyl-D-glucosamine), is a natural polysaccharide of major importance (3). The name 'chitin' is derived from the Greek word `chiton', meaning a coat of mail (26). The use of chitin was first described by the French chemist, Henri Braconnot in 1811. The structure of chitin $(C_8H_{13}O_5N)_n$ is similar to that of cellulose, but with 2-acetamido-2-deoxy-β-D-glucose (NAG) monomer units, which are attached to each other via β(1→4) linkages. Chitosan is the deacetylated form of chitin (that can have varying degrees of deacetylation), and it is soluble in acidic solutions (sometimes with difficulty) (27, 28). The conversion of chitin to chitosan is possible either by enzymatic preparations, or chemical hydrolysis (29-32). The worldwide natural production rate of chitin has been estimated to be approximately 10^{11} tons annually (9, 33). The material form of chitin is usually a white and hard nitrogenous polysaccharide which is inelastic. It has also been considered to be the major source of beach pollution in coastal areas (34). Chitin occurs as crystalline microfibrils in nature, that form structural elements of the exoskeletons of arthropods, and in fungal cell walls. For greater reinforcement and strength protection properties, chitin is also produced by a number of other living organisms in the lower plant and animal kingdoms (3, 35). (Fig.1)
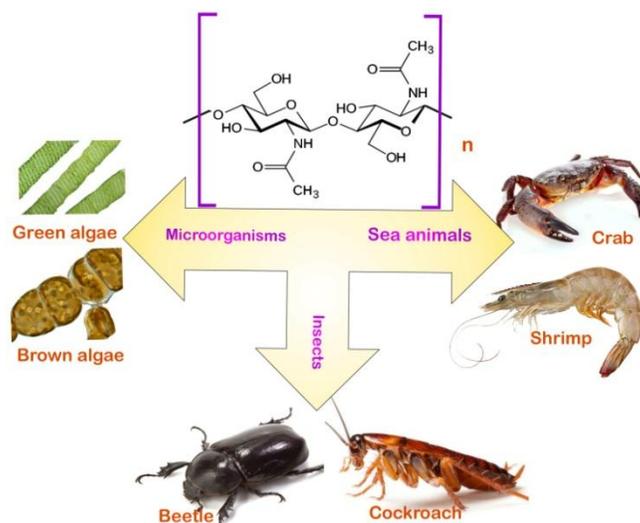
(Fig.1) These organisms are united by the presence of chitin as an integral structural component; In some organisms (for example the snail) chitin is found in the mandible, as well as a supply of the chitin degrading enzyme, chitinase is present in the gut (36). The wide occurrence of chitin and chitosan in these creatures make them good natural sources of these biopolymers(3).

There have been no reports of quantitatively-significant long-term accumulation of chitin in nature, which implies that its production, degradation and turnover must be efficiently balanced. Alongside the abundance and ubiquity of chitin, chitin-degrading enzymes have also been detected in many organisms (37). Chitin owes its biodegradability to the action of chitinase enzymes that are widely distributed in nature (38).

The widespread occurrence of chitin in the biosphere and its insolubility, led to the idea that chitin should survive in fossils (37). There have indeed been reports of fossilized chitinous materials, e.g., in pogonophora (37, 39), and in insect wings preserved inside amber (37). The immunogenicity of chitin (in spite of presence of nitrogen in its structure) is exceptionally low. Chitin is a highly insoluble material that resembles cellulose in its low solubility and chemical non-reactivity. It may be regarded as a cellulose structure where the hydroxyl group at position C-2 has been replaced by an acetamido group. (Fig.2)
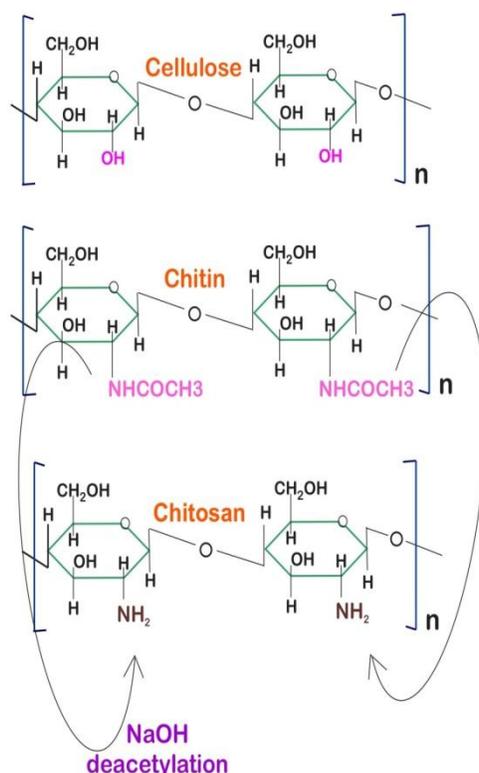
(Fig.2) Structures of chitin, chitosan and cellulose.

Chitin sometimes is considered to be a cellulose derivative, however it does not occur in cellulose producing organisms (40).There is no generally accepted nomenclature with respect to the degree of N-deacetylation of chitin and its derivatives. Chitin and chitosan have a high percentage of nitrogen (6.89%) compared to synthetically substituted cellulose derivatives that can only be prepared with a lower nitrogen content (1.25%). Most of the naturally occurring polysaccharides, e.g. cellulose, dextran, pectin, alginic acid, agar, agarose and carrageenan are neutral or acidic in nature, whereas chitosan is an examples of a highly basic polysaccharide. Other unique properties of chitin and chitosan include formation of polyoxysalts, ability to form films, biocompatibility, biodegradability, non-toxicity, molecular adsorption properties, etc. In spite of several reports describing the preparation of functionalized chitosan derivatives by chemical modification of the amino groups, very few of these have acceptable solubility in general organic solvents, or binary solvent systems. Some chemically modified chitin and chitosan derivatives possessing improved solubility in general organic solvents have been reported (41).

Chemical structure and properties:-
Chitin is poly-(1→4)-β-linked N-acetyl-D-glucosamine (42). The individual sugar units in its structure are rotated 180°with respect to each other, and each pair forms the disaccharide N,N'-diacetylchitobiose [(GlcNAc)$_2$ (33, 43, 44). The individual polymer chains can be described as helices, in which each sugar unit is inverted with respect to its neighbors. Such a structure leads to high stability as the rigid ribbons are connected by 03-H→05 and 06-H→07 hydrogen bonds.
Chitin also has three different crystalline allomorphs: the α-, β- and γ-forms (32). These differ in the orientation of the micro-fibrils. The commonest form of chitin is α-chitin. Its unit cell is composed of two N,N'-diacetylchitobiose units forming two chains in an antiparallel arrangement. Therefore, adjacent polymer chains run in opposite directions, held together by 06-H→06 hydrogen bonds, and the chains are held in sheets by 07→H-N hydrogen bonds(45-47). This gives a statistical mixture of -CH$_2$OH orientations, equivalent to half the oxygen atoms on each residue, being able to form inter- and intramolecular hydrogen bonds. This results in two different types of amide group; all are involved in forming the interchain C=O→H-N bonds, while half of the amide groups also serve as acceptors for 06-H→O=C intramolecular hydrogen bonds. Formation of these intermolecular hydrogen bonds leads to quite a stable structure. The polymer chains eventually give rise to microfibrils by self-assembly if they are allowed to crystallize (37).

β-chitin is a less common form of chitin, in which the unit cell is a N,N'-diacetylchitobiose unit, giving a polymer stabilized as a rigid ribbon, the same as α-chitin, by 03→05 intramolecular H-bonds (47, 48). The chains in this structure are held together in sheets by C=O→H-N H-bonds between the amide groups and by the -CH$_2$OH side chains, which leads to formation of intersheet H-bonds to the carbonyl oxygens on the adjacent chains (06-H→07). This gives a structure of parallel poly-N-acetylglucoseamine chains with no intersheet H-bonds. The parallel arrangement of polymer chains in β-chitin allows for more flexibility than the antiparallel arrangement found in α-chitin, but the resultant polymer still has immense strength (49). γ-Chitin is the third allomorph, possessing mixed parallel and antiparallel orientations. It has been reported to occur in mushrooms (50, 51). Chitin is always found cross-linked to other structural components with the exception of the β-chitin found in diatoms. Chitin is found covalently bonded to glucans in fungal cell walls, either directly, as in *Candida albicans* (1) or via peptide bridges. Moreover, in insects and other invertebrates, chitin is always associated with specific proteins, with both covalent and noncovalent bonding. This association means it produces the observed ordered structures. There are also varying degrees of mineralization, such as calcification, and sclerotization, involving interactions with phenolic and lipid molecules (45). In organisms like fungi and invertebrates, there have been reported varying degrees of deacetylation, giving a continuum of structure between chitin (fully acetylated) and chitosan (fully deacetylated) (52). Although either acids or alkalis can be used to deacetylate chitin, the fact that glycosidic bonds are more susceptible to acid which would destroy the chain, the alkali deacetylation process is used more frequently (32, 53).

N-deacetylation of chitin can either by performed by heterogeneous or homogeneous reaction mixtures (32). The distinction between chitin and chitosan with different degrees of deactylation is not strict (25, 54). Putting a few exceptions aside, natural chitin occurs associated with other structural polymers like proteins or glucans, which often contribute more than 50% of the mass in chitin-containing tissue(55). Chitin can be N-deacetylated to such an extent that it becomes soluble in dilute acetic and formic acids. In chitin, the acetylated units prevail and the degree acetylation is typically 0.90, while chitosan is a fully or partially N-deacetylated derivative with a typical degree of deacetylation of more than 0.65. Many analytical tools have been used to determine this degree of deacetylation including IR spectroscopy, pyrolysis gas chromatography, gel permeation chromatography and UV-vis spectrophotometry, ^{1}H NMR spectroscopy, ^{13}C solid state NMR, thermal analysis, various titration schemes, acid hydrolysis, HPLC, separation spectrometry methods and more recently near-infrared spectroscopy (41).

Chitin biosynthesis:-
Chitin biosynthesis and cellular processing is a highly complex, multi-faceted and inter-connected series of events which starts intracellularly and ends in inclusion of chitin in exterior supra-macromolecular structures such as arthropod cuticles and fungi cell walls. (Fig.3)
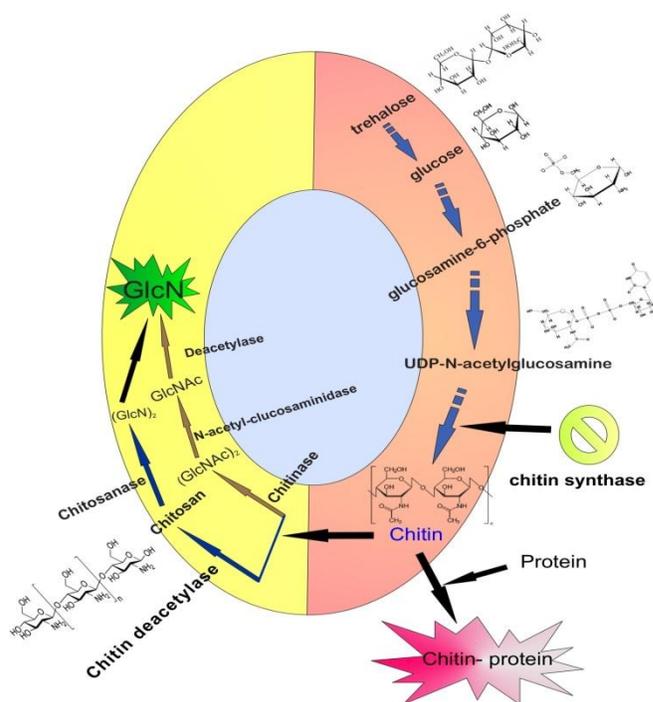
(Fig.3) Events associated with chitin formation and degradation.
The whole process encompasses several individual steps:

1. Sequential biotransformation of sugars (mainly trehalose or glucose). This step includes biochemical reactions like phosphorylation, amination and also formation of the enzymic substrate, UDP-N-acetylglucosamine.
2. Chitin synthase (CS) synthesizes the chains. The CS unit is an enzyme which is a part of a protein/carbohydrate cluster including closely topologically packed molecules. Such an arrangement ensures coalescence of nascent chitin polymers into a crystalline fibril.
3. The orientation of chitin molecules which have long chains.
4. Polymer translocation across the plasma membrane.
5. Crystallization and formation of microfibrils by inter-chain hydrogen bonding.
6. Association with arthropod cuticular proteins or with other carbohydrates in fungal cell walls.

Chitosomes are cytoplasmic microvesicles with CS activity. These microvesicles have been identified through electron microscopy studies using fungal systems (56-58). The abundance of chitosomes at the hyphal tip implies their crucial role in CS trafficking to pre-determined locations(59). Chitosomes originate from organelles such as endoplasmic reticulum and Golgi. Chitosome vesicles contain zymogenic CS clusters. After eventually fusion of the chitosomes with the plasma membrane, the CS units become activated through proteolytic reactions(60). CS insertion into plasma membranes, involves the mediation of targeting and recognition proteins. The exact machinery of such fusion along with the proteins involved remains unresolved. Chitosome-like structures also have been reported in cell-free system derived from insects (61); however it has not been clarified whether they are involved in *in vivo* chitin formation. Moreover chitosome-like vesicles have not yet been described in intact insect epidermal cells(56).
Different forms of chitin are synthesized by the action of the enzyme, chitin synthase (UDP-N-acetyl-D-glucosamine: chitin4-β-N-acetylglucosaminyl-transferase; EC 2.4.1.16). This enzyme which is highly conserved is expressed in every organism possessing chitin synthesizing activity. Chitin synthase uses UDP-N-acetylglucosamine (UDPGlcNAc) as the activated sugar donor to produce the chitin polymer (62). Candy and Kilby (1962) first proposed a chitin synthesis biosynthetic pathway in insects. The proposed mechanism started with glucose and ended with UDP-GlcNAc. Jaworski et al. (1963) using cell-free extracts from the southern armyworm *Spodoptera eridania*, finally established the whole pathway from UDP-GlcNAc to chitin. Results of many subsequent studies conducted with preparations from various insects supported the molecular mechanism of this pathway.

Industrial processing of chitin:-
The isolation of chitin from crustaceans such as crayfish, crab, shrimp, and other organisms such as fungi is a time-consuming process (63-65). It requires 17–72 h including 1–24h of treatment with HCl and 16–48h of NaOH processing. Lengthy procedures for chitin isolation require more energy, and in return increases the cost of production. Like crabs and shrimps, barnacles also belong to the Crustacea family. The shell structures of barnacle species are less crystalline and they are reported to contain more minerals than other members of the crustacean family (63). These minerals are mainly composed of calcite and calcium phosphate (66, 67).
 It has been shown that these two mineral materials in the carapace can be easily removed from the carapace structure by using HCl. In such creatures, the protein only has a few weak bonds with the chitin so it can be removed from easily because of low-crystalline shell structure of the barnacle species. Therefore, it is expected that the isolation of chitin from barnacle species would be a comparatively quick process. *Chelonibia patulais* is a barnacle species in the subphylum Crustacea which lives epizoically on animals such as turtles, crabs, whales and mollusks, or on rock on the seashore where there is shallow water. In a procedure for the isolation of chitin from *C. patula* shells, they should be demineralized in 1M HCl for 10 min, and deproteinized in 2M NaOH for 20 min. The completion of the whole process takes only half an hour. It starts with dripping 1M HCl solution onto 10g of the dust obtained from ground *C. patula* shells followed by stirring for 10 min by means of a magnetic stirrer at room temperature. If the HCl is added too quickly a vigorous effervescence occurs that may led to overflowing. The samples then should be rinsed with distilled water until a neutral pH value is obtained. In a study, after drying the samples in an oven, 376 mg of material remained at the end of the process. Considering that the majority of the original mass consisted of minerals, these have been removed by means of HCl. Proteins are also found in shells and are removed by a deproteinization process (refluxing with a base for 20 min) which left 311 mg of dry chitin. (Fig.4) The chitin content in the shell was equivalent to 3.11% of the barnacle shell dry weight and the protein content of *C.*

patula shell dust was low (63). Obtaining chitin from shrimp shells is associated with food industries such as shrimp processing, whereas the production of chitosan–glucan complexes from fungal mycelia is associated with fermentation processes such as that producing citric acid from *Aspergillus niger*. Generally, processing of crustacean shells involves the removal of proteins and then dissolution of calcium carbonate, which is present in the shells in high concentrations. Protocols for obtaining chitosan from these sources also involves deacetylation in 40% sodium hydroxide at 120°C for 1–3h. This treatment yields 70% deacetylated chitosan (41). Recently, a "green conversion" of agroindustrial wastes by the biological activity of *Rhizopus arrhizus* and *Cunninghamella elegans* strains has been reported to produce chitin and chitosan. Such industrial sources have significant advantages including avoiding allergic reactions in individuals susceptible to shellfish antigens and reduction in time and cost of production (68, 69).
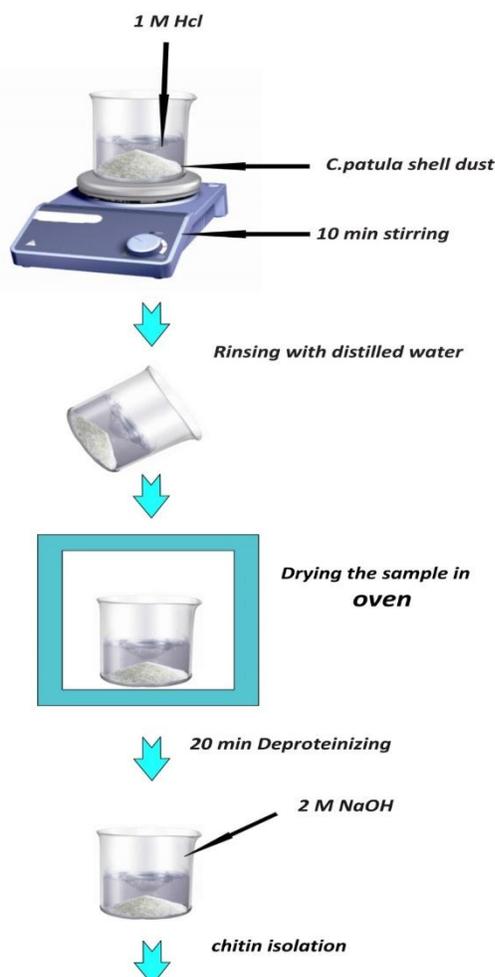
(Fig.4) General steps of chitin processing from *C. patula* shell dust.

Chitin degradation:-
It is believed that bacteria are the major mediators of chitin degradation in nature. Their role can be demonstrated in both soil and water systems. In soil systems, the chitin hydrolysis rate correlates with bacterial population and abundance (70). The degradation in this case depends on factors such as temperature and pH. Not only the bacteria but also fungi may be considered quantitatively important agents in the chitin degradation process (44, 71). The results of plating experiments in aquatic systems demonstrated convincingly that bacteria are the main mediators of chitin degradation (72). Dense fungal colonization of the carapaces of chitinous zooplankton has been observed, and some diatoms were found to be able to hydrolyze chitin oligomers. Furthermore, enzymes released during molting of planktonic crustaceans are believed to be another source of enzymes that can metabolize chitin in aquatic systems (44). The degradation of chitin is a highly regulated process, and (depending on the organism under scrutiny) the hydrolytic enzymes have been found to be induced by the actual products of the chitin hydrolysis (GlcNAc (73) and

soluble chitin oligomers $(GlcNAc)_{2-6}$. In contrast to $(GlcNAc)_2$, GlcNAc has also been reported to act as a suppressor of the expression of chitinase in a strain of *Streptomyces* (74). This suppressor activity may be connected with GlcNAc occurring in the murein found in fungal cell walls rather than in chitin (44). Despite the insoluble nature of chitin and its relatively resistance to degradation because of its complex crystalline structure, some soil and marine bacteria, such as *Bacillus circulans* WL-12 (75), *Serratia marcescens* (76), *Streptomyces coelicolor* A3 (77), *Aeromonas caviae* (78), *Pseudoalteromonas sp*. strain S91(79) and *Vibrio harveyi* (80) are able to degrade, transport, and utilize chitin as an energy source by the action of chitinases (EC 3.2.1.14). Itoh et al., (2013) studied *Paenibacillus sp*. strain FPU-7 and found cooperative degradation of chitin by extracellular and cell surface-expressed chitinases. This organism grew well in liquid medium and could completely hydrolyze chitin flakes from crab shells. (Fig.5)
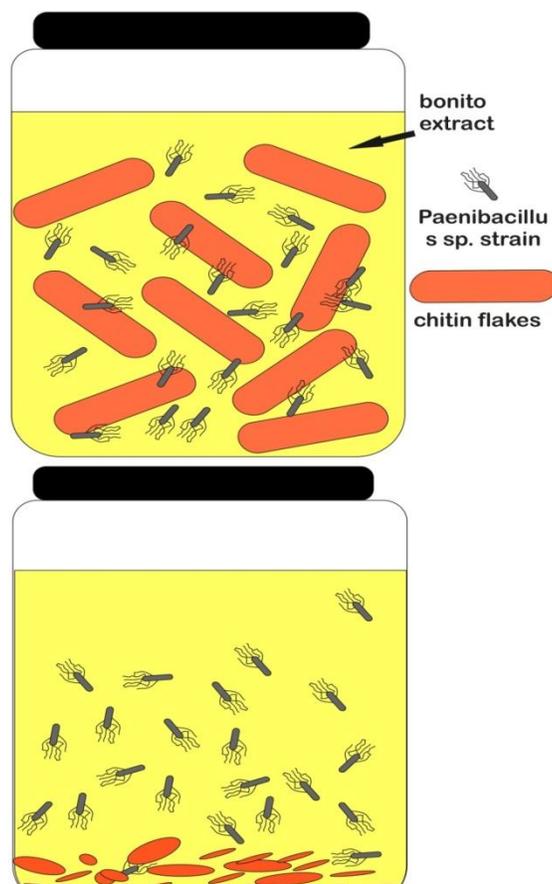
(Fig.5) Chitin degrading ability of Strain FPU-7 was shown after culturing at 30°C for 5 days with shaking, in bonito extract medium containing 5.0% (wt/vol) crab shell chitin flakes. The solid matter (chitin flakes) in the flask disappeared after 5 days of incubation.

Possible chitin degradation pathways have been discussed by Davis and Eveleigh (81). The degradation process is best termed chitinoclastic (chitin breaking) when the degradation pathway is not exactly known, whereas it is best termed chitinolytic when the pathway involves the initial hydrolysis of the $(1{\rightarrow}4)$-β-glycosidic bond. Hydrolysis of this bond is accomplished by chitinase. The *exo*-chitinase enzyme cleaves diacetylchitobiose units from the non-reducing end of the chitin chain, while the endo-chitinase enzyme cleaves glycosidic linkages randomly along the length of the chitin chain.

The common product of this enzyme is diacetylchitobiose, together with some triacetylchitotriose. The latter product may be slowly degraded to disaccharide and monosaccharide units. There may not always be a clear distinction between these two activities and the hydrolysis site strongly depends on the nature of substrate. For instance, *Streptomyces chitinase* only degrades the pure crystalline β-chitin of diatom spines from its end yielding only diacetylchitobiose, while colloidal chitin is degraded to oligomers and disaccharide (37).

Chitin and chitosan: biomedical and nanomedical applications:-
Tissue engineering:-
Tissue engineering involves the use of living cells, which are manipulated and controlled through their extracellular environment to fabricate tissue substitutes for implantation into the body (82, 83). The main purposes of tissue engineering can be categorized as to repair, replace, maintain, or enhance the function of a particular tissue or organ (84).

Chitin-based materials, which can be fabricated into tubular forms, can be successfully applied in tissue engineering of nerves and blood vessels as a template for cells (52, 85-87). Chitin-based scaffolds are versatile products and can be optimized for many regenerative purposes (88).

Chitin and chitosan have been successfully applied to fabricate polymer scaffolds in tissue engineering. Some basic requirements to design polymer scaffolds are: high porosity (with an appropriate pore size distribution); biodegradability (degradation rate should match the rate of neo-tissue formation); structural integrity (to prevent the pores of the scaffold from collapsing during neo-tissue formation); being non-toxic to cells; biocompatibility; interacting with the cells to promote cell adhesion; encouraging cell function (proliferation, migration and differentiation) (24).

There are several methods that have been used to produce chitosan scaffolds including: phase separation and lyophilization technique (88-94); particulate leaching techniques; freeze gelation technique; rapid prototyping technology; formation of microparticles and microspheres. (Fig.6)
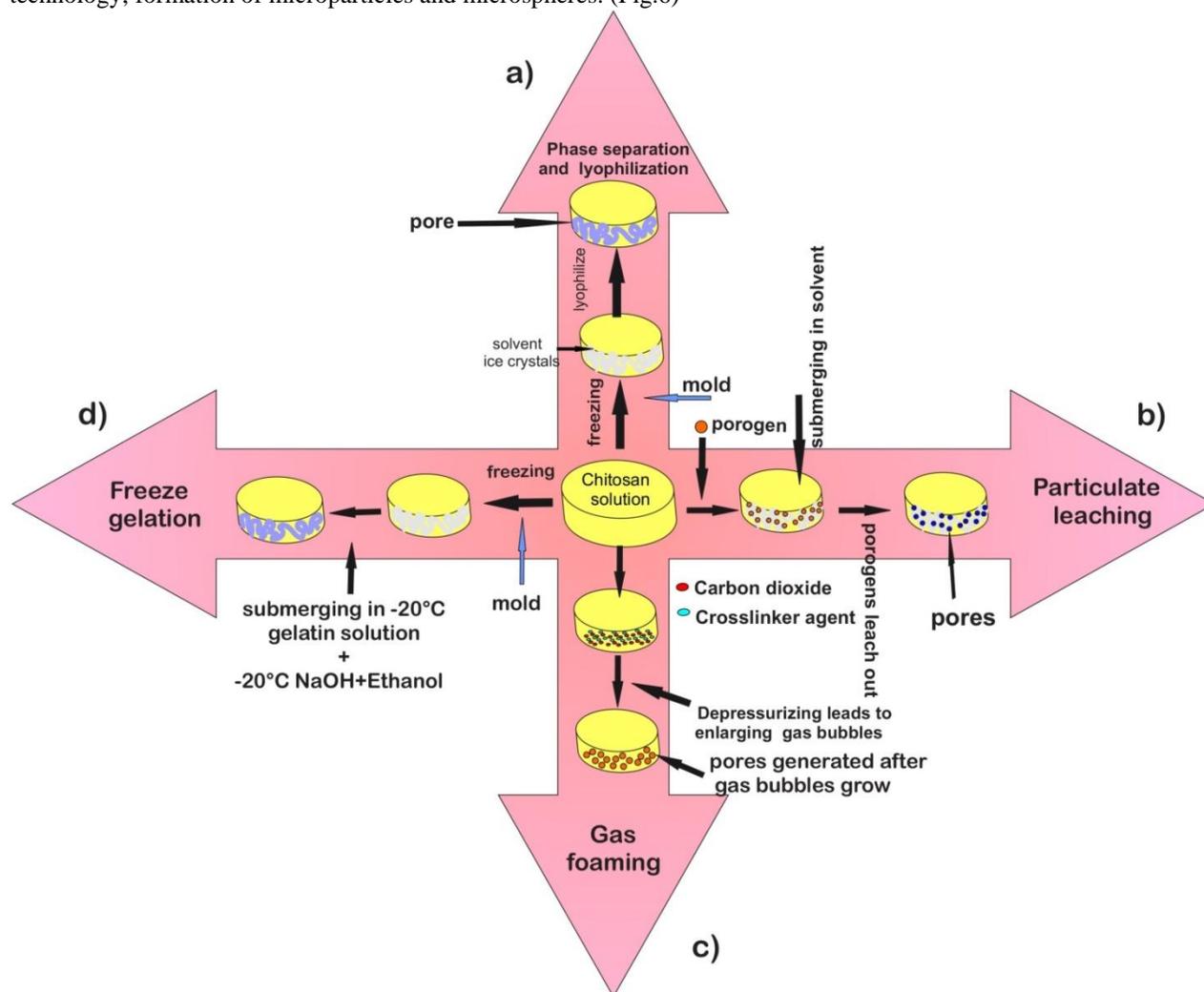
(Fig.6) Four commonly used methods of chitosan scaffold fabrication. (a) Phase separation and lyophilization technique, a chitosan solution is introduced into a mold, then a freezing step makes it ready for phase separation with acetic acid solvent and chitosan acetate salt. Lyophilization is the final step. (b) Particulate leaching technique, is frequently used combined with phase

separation. In this method, a porogen (i.e. gelatin) is mixed with chitosan solution prior to phase separation and lyophilization steps. Through submerging in a solvent, the resultant scaffold is prepared for porogen leaching. The obtained scaffolds would then possess additional porosity.

(c) In the gas foaming technique, a chitosan solution containing a cross-linker (glutaraldehyde) is supersaturated with carbon dioxide at high pressure to induce cross-linking. Gas bubbles are generated; for example by mixing a foaming or blowing agent such as sodium bicarbonate with a pre-polymer where gas is generated upon chemical decomposition (89, 95, 96). When the system is depressurized, thermodynamic instability leads to nucleation and gas bubble growth. Pores are generated while the bubbles escape the polymer solution. (d) Freeze gelation technique, which initially involves phase separation due to freezing. The obtained scaffold is placed in a gelation solution of sodium hydroxide and ethanol below the chitosan freezing temperature. The last step following the gelation, is scaffold air-drying to remove residual liquid (89).

Chitosan scaffolds also can be fabricated through the fusion or the sintering of chitosan microparticles or microspheres (97, 98). An electrospun water-soluble blend of carboxymethyl chitin (CMC) with PVA for tissue engineering applications was reported by Shalumon et al. (2009). In order to fabricate appropriate nanofibers, the concentration of CMC (7%) and PVA (8%) was optimized, blended in different ratios (0–100%) and electrospun. Fibers were made water-insoluble by cross-linking with glutaraldehyde vapors followed by thermal treatment. The nanofibers were found to be bioactive and biocompatible. Scaffold properties such as cytotoxicity and cell attachment were evaluated using human mesenchymal stem cells (hMSCs) by the MTT assay. These studies revealed ability of cells to attach and spread on the nanofibrous scaffolds. Peter et al. reported chitosan/gelatin/nanophase hydroxyapatite (nHAp) composite scaffolds produced by blending chitosan and gelatin with nHAp (24).

Wound healing:-
Madhumathi et al. (2010) developed α-chitin/nanosilver composite scaffolds for wound healing applications. These scaffolds were found to possess antibacterial activity against
S. aureus and *E. coli*, as well as blood-clotting ability. Such properties have made them useful nanostructures for wound healing applications. Similarly, Sudheesh Kumar et al. (2010) developed and characterized β-chitin/nanosilver composite scaffolds for this application using β-chitin hydrogel containing silver nanoparticles. In addition, these scaffolds were evaluated for their cell adhesion properties using Vero cells, and the results revealed that nanosilver incorporated chitin scaffolds were ideal for wound healing applications (24).

Drug delivery
Water-soluble carboxymethyl chitin (CMC) was used for drug delivery applications. CMC nanoparticles were prepared through a cross-linking approach using $CaCl_2$ and $FeCl_3$. A spherical morphology was observed in SEM images of CMC nanoparticles, with diameters ranging from 200 to 250 nm. 5-fluorouracil (5-FU) drug-loaded nanoparticles also showed similar morphology. MTT assay results showed that they were non-toxic to normal fibroblast L929 mouse cells. The hydrophobic anticancer drug 5-FU was loaded into CMC nanoparticles via an emulsion cross-linking method, and these were found to have a controlled and sustained drug-release profile at pH-6.8. An anti-HIV drug delivery application was also reported by Dev, Binulal, et al. (2010) using poly(lactic acid) (PLA)/CS nanoparticles. In addition, lamivudine (a hydrophilic antiretroviral drug) was loaded into the PLA/CS nanoparticles. In this case absorption spectrophotometry was used to evaluate the encapsulation efficiency and the in vitro drug release behavior of drug loaded PLA/CS nanoparticles (24).

Cancer diagnosis :-
Semiconductor nanocrystals (or quantum dots) can be bioconjugated to a variety of biological recognition ligands, and can replace conventional organic fluorescent dyes in immunostaining applications and bioimaging of cancerous cells and tissues. However, quantum dots include the heavy metals, cadmium sulphide, cadmium selenide, zinc selenide, etc. and can be cytotoxic and even hazardous. A heavy-metal-free luminescent quantum dot (QD) based on doped-zinc sulphide (ZnS), conjugated with a cancer-targeting ligand, folic acid (FA) was developed for targeted cancer-imaging. Folate receptors are over-expressed on many cancer cells, and can mediate receptor-based endocytosis when they interact with folate-conjugated nanoparticles, providing intracellular uptake. Similarly, mannose receptors, have been used for cancer diagnosis (24). A novel FA-conjugated carboxymethyl chitosan (CMCS) coordinated to manganese doped zinc sulphide (ZnS:Mn) quantum dots (FA-CMCS-ZnS:Mn) forming composite nanoparticles was developed by Manjusha et al. (99). This multifunctional system could be used for targeting, controlled drug-delivery and cancer cell imaging. The selected anticancer drug was 5-FU, which is used for breast cancer treatment. L929 cells were used to confirm the non-toxicity of FA-CMCS-ZnS:Mn nanoparticles. Furthermore, the MCF-7 breast cancer cell line was used to study imaging, specific targeting and cytotoxicity of the

drug loaded nanoparticles. The in vitro imaging of cancer cells with the nanoparticles was studied using fluorescence microscopy(100).

Chitin- and chitosan-based dressings:-

Sparkes and Murray (101) developed a chitosan–gelatin complex   desu eb dluoc taht as a surgical dressing. The procedure involved dissolving the chitosan in water in the presence of a suitable acid in order to maintain the solution pH at about 2–3, followed by addition of gelatin dissolved in water. The ratio of chitosan and gelatin was between 3:1 and 1:3. A certain amount of a plasticizer such as glycerol or sorbitol could be added to the mixture to reduce its stiffness. A film was cast from this solution onto a flat plate and dried at room temperature so it could be used as a dressing. This experimental dressing displayed excellent adhesion to subcutaneous fat. The British Textile Technology Group (BTTG) patented a procedure for making a chitin-based fibrous dressing (102). In this method the chitin/chitosan fibers were obtained from micro-fungi (instead of shrimp shells) and were not fabricated by the traditional fiber-spinning technique. The procedure can be summarized as follows.
(1) Preparation of micro-fungal mycelia from a culture of *Mucor mucedo* growing in a nutrient medium.
(2) Washing and deproteinizing of the mycelial matt with NaOH to precipitate chitin/chitosan.
(3) Bleaching and further washing.
(4) Preparation and dispersion of the fibers using paper-making equipment.
(5) Filtration and wet-laid preparation of a fiber matt; additional mechanical strength provided by mixing with other fibers.

Ophthalmology:-

Some of the particular characteristics of chitosan such as its optical clarity, mechanical stability, sufficient optical correction, gas permeability (particularly towards oxygen), wettability, and immunological compatibility make it ideal to be used for fabricating contact lenses for use in the eye. These lenses are made from partially depolymerized and purified chitosan isolated from squid pen and are clear, tough and possess other required physical properties such as Young's modulus, tensile strength, tear strength, elongation, water content and oxygen permeability. The antimicrobial activity, film-forming capability, and wound-healing properties of chitosan also make it suitable for development of ocular bandage-lenses for traumatic injuries(59).

Antibacterial properties:-

The growth of *E. coli* was inhibited in the presence of chitosan with a concentration greater than 0.025%. Chitosan is also able to inhibit the growth of other microbial species such as *Fusarium*, *Alternaria* and *Helminthosporium*. The cationic amino groups of chitosan probably bind to anionic groups of microorganisms and prevent their growth (41). Low-molecular-weight chitosan after penetrating into bacterial cell walls, can bind with DNA and inhibit DNA transcription and mRNA synthesis (103). The natural antimicrobial characteristics of chitosan derivatives have resulted in their use in commercial disinfectants and topical antimicrobials (104, 105).

Anti-thrombogenic and haemostatic materials:-

Blood clot formation is an area where chitosan may have completely opposite effects depending on its functional type and formulation. Chitosan has been shown to be a highly effective hemostatic agent when formulated as microspheres (106), as bandages or dressings (107), or as absorbable sponges (108). In fact a chitosan dressing was able to control an experimental arterial hemorrhage in dogs (109). The efficiency of chitosan as a hemostatic agent was shown to be dependent on its degree of hydration (110). However there are also reports that different chitosan preparations can be anti-thrombogenic and anti-coagulant. N-hexanoyl and N-octanoylchitosan fibers can be used as anti-thrombogenic materials (41). A phthalized chitosan derivative was used to prepare an anti-thrombogenic material for the fabrication of vascular grafts (111). Sulfate derivatives of chitin and chitosan have blood anticoagulant and lipoprotein lipase (LPL)-releasing activities. Chitin 3,6-sulfate showed about two-fold anticoagulant activity and also 0.1-fold LPL-releasing activity over those of heparin; the sulfate derivatives might be usable as heparinoids for artificial blood dialysis (112).

Antiaging cosmetics:-

Morganti et al. (113) developed block-copolymer nanoparticles (BPN) composed of phosphatidylcholine and linoleic acid nanocomplexed with hyaluronan and chitin nanofibrils (PHHYCN) Thee nanoconstructs were used to encapsulate cargos comprising cholesterol, creatine, caffeine, melatonin, vitamins E and C, and the amino acids glycine and arginine. The idea was to use these nanocarriers for skin rejuvenation, as all the individual ingredients had shown some activity in this regard. Subjects whose skin was treated with the active chitin nanofibrils containing

loaded BPN were shown to be softer and more hydrated after one month of treatment. Both fine wrinkles and crease lines were reduced soon after the first 15 days of treatment with injectable active chitin nanofibril containing BPN, as well less presence of telangiectasia, so that the general appearance of the face was notably ameliorated during the regression period (113).

Antitumor activity:-
Chitosan and its derivatives have exhibited antitumor activity in both in vitro tests and in vivo animal models. Tokoro et al (114) suggested that this activity was due to an increase in secretion of interleukins-1 and 2 which caused maturation and infiltration into the tumor of cytolytic T-lymphocytes. It was further suggested by Dass and Choong (115) that chitosan could increase lymphokine production and enhance proliferation of cytolytic T-lymphocytes (103).

Vaccine Adjuvant:-
Studies in mice and guinea pigs have demonstrated that intranasal chitosan glutamate can significantly enhance antibody responses to a diphtheria antigen (a non-toxic cross-reacting derivative of diphtheria toxin, CRM_{197}) (116). Mills et al. studied the impact of this chitosan adjuvant to a diphtheria vaccine in healthy human volunteers (117). Mann et al. conducted a live-virus challenge study in ferrets, which is a recognized preclinical model for human influenza. Both chitosan glutamate (CSN) and N,N,N-trimethylated chitosan (TM-CSN) were investigated as adjuvants for an inactivated NIBRG-14 (H5N1) subunit antigen (derived from influenza antigen A/Vietnam/1194) (25, 118).

Conclusion:-
The unique biochemical properties of chitin and chitosan suggest they could be seen as almost ideal biopolymers with numerous applications in biomedical research. These materials can be processed into various products and on the other hand it is possible to fabricate scaffolds and nanoparticles with increasing applications in the burgeoning field of nanomedicine. In this review we have tried to present an overview of the biosynthesis, isolation and technical applications of chitin and chitosan. However in the limited space available we have been able to cover only a fraction of the biomedical applications, and there is an even wider variety of applications in food packaging and processing, agriculture, biotechnology and cosmetics.

Acknowledgments:-

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