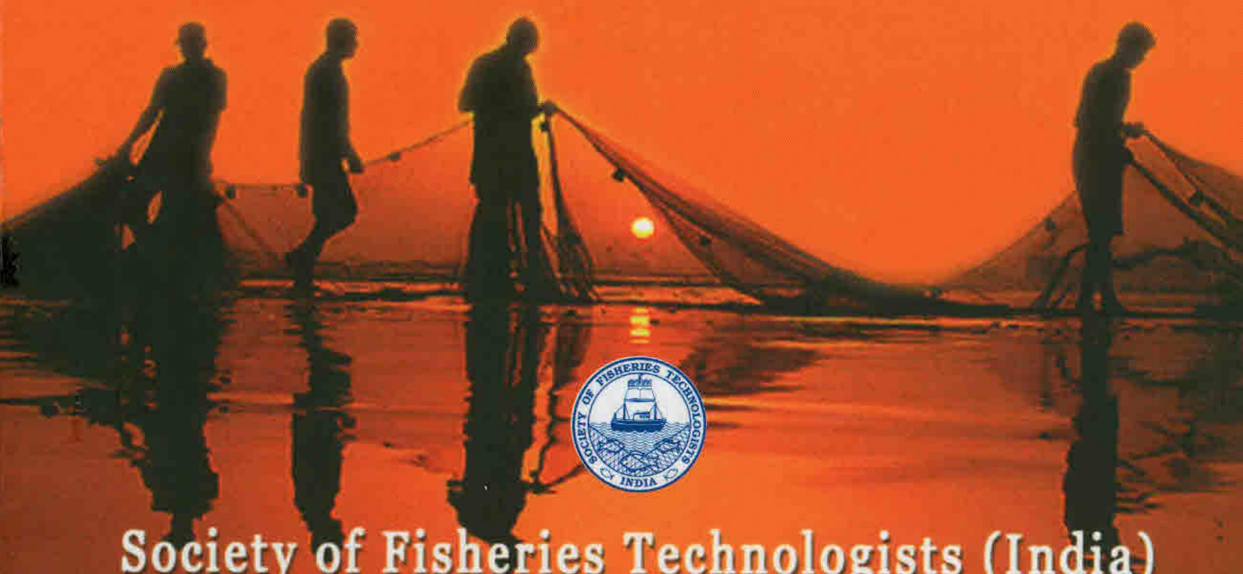
Coastal Fishery Resources of India

Conservation and Sustainable Utilisation



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Biomedical Applications of Collagen and Chitosan

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Biopolymer is a term used to refer to the polymers which are biologically synthesized by nature. Polysaccharides are one such class of polymers comprising of simple monosaccharide molecules connected by ether type linkage to give a high molecular weight polymer. Among the different polysaccharides, cellulose and chitin are the most abundant biopolymers. Professor Henri Braconnot, Director of the Botanical Garden in Nancy, France isolated a fraction called fungine in 1811 from the cell walls of mushrooms (Madhavan, 1992). Odier (1823) found similar material in the cuticle of beetles and re-named fungine as chitin after the Greek word chiton. The discovery of chitosan is ascribed to Rouget in 1859 (Sambasivan, 1992) and he, by boiling chitin in KOH, produced chitosan soluble in organic acids. Now it is understood that chitin is soluble in dimethyl acetamide containing 5% lithium chloride (Rutherford and Austin, 1978) and insoluble in aqueous acetic acid and chitosan's solubility is reverse to that of chitin in these two solvents. The nitrogen content of chitin is usually less than 7%, while that of chitosan is more than 7%. It was only in 1950, the structure of chitosan was resolved. Collagen is produced from air bladder of fish and chitosan from prawn shell.

Structure of chitin and chitosan

The structure of chitin is a linear polysaccharide of â (1-4) 2 amino 2 deoxy D glucopyranose, where all residues are comprised of N acetyl glucosamine residues (Muzarelli,1977). Chitosan is a linear polymer of â (1-4) 2 amino 2 deoxy D glucopyranose where all residues are deacetylated. Traditional sources of polymer, however, do not result in 100% acetylated chitin or 100% deacetylated chitosan and in reality the biopolymer exists as a copolymer. When the acetamido group is more than 50% (more commonly 70-90%), the biopolymer is termed chitin. The number of acetamido group is termed the degree of acetylation. On the other hand, when the degree of deacetylation or the aminogroups are predominant, the biopolymer is termed chitosan. Today producers of chitin and chitosan span the globe and are found in North America, Europe, and Japan, China, India and Southeast Asia.

Uses of chitin and chitosan

The uses of chitin and chitosan are numerous and a few important uses are summarized in Table 1.

Table 1: Uses of chitin and chitosan

Area of application	Uses
Water treatment	As coagulating/flocculating agents for polluted waste water
	For removal and recovery of metal ions from waste water
Agriculture	As antimicrobial agents
	As plant seed coating
	As fertilizer
Textile and paper	Fibres for textile and woven fabrics
	In paper and film industry
Biotechnology	As enzyme immobilizing material
Food and health supplement	As natural thickeners
	As food additives including in pet foods
	As filtration/clarification agents
	As hypocholesterolemic agent
Cosmetics	Ingredients for hair and skin care (as conditioners)
Biomedical dressings	For treatment of burns and wound
	In the preparation of absorbable surgical sutures
	In coatings of blood contact tubings
	As antithrombogenic material
	As a haemostatic agent and in tissue engineering applications
	As a bone substitute and in drug delivery for the controlled release of drugs
Analytical applications	Thin layer chromatography

Source: Muzzarelli (1977); Knapczyk (1991); Karnicki, *et al.* (1994)

Biomedical applications of chitosan

A biomaterial is a material used in a medical device intended to interact with biological systems. Metals, ceramics, and polymers (synthetic and natural) are used as biomaterials in medical field. Cellulose is used as a gauze material in dressings. The advantages of materials derived from nature are that they exhibit great compatibility with humans. Many studies have been carried out in the Central Institute of Fisheries Technology (CIFT), Cochin on the biomedical applications of chitosan. An attempt was made for the use of chitosan along with another copolymer collagen for the preparation of biological membranes.

Collagen-chitin membrane

Collagen is an extra-cellular matrix protein, playing a major role as connective tissue. It is the most abundant protein in humans and performs multiple roles. The word collagen has been derived from the Greek words kolla and genos meaning glue and formation (Suseela, 2002). In the muscle, the myocommata covering muscle segments, the basement membrane covering muscle fibres and the tendons are made up of connective tissue and collagen forms its major part. An acetic acid extraction process can successfully yield a collagen substance, which can be used to form biodegradable collagen (O'Sullivan *et al.*, 2006.)

Collagen molecule is rod like in shape, 290 nm long. The molecule has a triple helical structure with three long polypeptide chains. Each polypeptide is a left handed helix, but the three helices are wrapped around each other towards right. Each polypeptide is made up of nearly 1000 amino acid residues with a repeated Glycine–X–Y sequence, where X and Y are normally iminoacids, proline and hydroxyproline and are arranged towards the outside of the triple helix. The low molecular weight aminoacid glycine is located at the centre of the triple helix. The three polypeptide chains are stabilized by interchain hydrogen bonds. Collagen also contains rather large amounts of polar aminoacids ie arginine, lysine, and aspartic acid. Tropocollagen molecules associate in a staggered fashion to form collagen fibrils, which are stronger than steel wire of similar size. Collagen fibrils are strengthened and stabilized mainly by covalent cross-links, existing both within and between individual tropocollagen molecules. These crosslinks represent the enzyme-catalyzed formation of covalent bonds involving lysine and hydroxylysine side chains. The extent of cross-linking depends on the specific function of the collagen. Older animals have more highly cross-linked and therefore more rigid collagen. CIFT has developed a membrane made up of collagen and chitosan to treat the wounds due to burns.

The main problem in extensive burns is the relative lack of donor sites for skin drafting. Before the burnt areas can be covered, infection often sets in and the patients may then succumb to death. Biological dressings can be used to buy crucial time till donor sites become available. They prevent water loss and bacterial infection, relieve pain and stimulate epithelialization. Various biological dressings like sterilized human amnion, biosynthetic wound dressing, deep frozen porcine split skin, human fresh and cadaver skin grafts are currently in use. Among these sterilized human amnion is in the process of rejection due to the possibility of spreading aids. Biosynthetic materials have the disadvantage of being synthetic in nature and there is the necessity for a second surgery. Skin cultures and grafts are difficult to be manufactured and are expensive. The biological dressing material prepared in CIFT has several advantages and was to be of tremendous use in the treatment of burns. Collagen in the soluble form was prepared from airbladder of fish. Chitosan was incorporated to the collagen and both crosslink each other to make a film of desired properties.

A good biological dressing material should be absorbable, prevent body fluid loss, control bleeding, prevent infections, provide oxygen permeability and should not cause allergic reactions. Collagen-chitosan membrane prepared in CIFT was proved to have all these properties. Animal studies were conducted in the animal house of CIFT to study the tissue reactions. No adverse effects were noticed in these studies. The studies in Medical College, Calicut, proved that the membrane has easy adhesion and stickiness, proper wound closure, prevention of fluid and blood loss, protection from microorganisms, oxygen permeability and better healing.

Dental surgeons came up with the proposal to try this material in dental surgery for guided tissue regeneration (GTR) in case of furcated gums. A detailed study on the physical properties was carried out and to improve the strength by adding gluteraldehyde. It was decided to use this membrane as a barrier device in GTR for periodontal infrabony and furcation defects. The advantages of this membrane over the traditional open flap dedridement were many. The parameters studied included probing pocket depth, gingival marginal level, probing attachment level, horizontal probing depth, vertical probing depth, condition of soft tissue, plaque index, any untoward reactions, and radiographic studies. It was found that the probing pocket depth got reduced, when collagen chitosan membrane was used as the GTR membrane. More horizontal gain in furcation site, more bonefill, less pathology, better healing, and no adverse reactions were also noted when collagen chitosan membrane was implanted. The membrane had excellent handling characteristics, ease of placement and more biological acceptance.

In a study conducted at Dental College, Trivandrum, controlled release of the drug chlorhexidine was attempted, for treating periodontal defects. Chlorhexidine was incorporated to collagen chitosan membrane at a level of 2.5 mg for a 4x5 mm chip of 0.23-0.32mm thickness. Drug release was studied spectrophotometrically. At 255 nm, initial burst was 35%, 24%, 12% and 1.2% on 1st, 2nd, 3rd and 4th days. SEM photographs showed a spherical and uniform distribution of chlorhexidine particles on the collagen-chitosan membrane. It was concluded that teflon membrane can be replaced with collagen chitosan membrane in dendistry. Investigations in the same lines at Meenakshi Ammal Dental College, Chennai; Yenspoya Dental College and Hospital, Mangalore; Nair Hospital Dental College, Mumbai; Ahmed Dental College, Calcutta; Tamil Nadu Government Dental College and Hospital, Chennai, have also been encouraging. The technology for collagen-chitosan membrane was recently transferred to Eucare Pharmaceuticals Ltd., Chennai, for commercial production.

Anti-ulcerognic effect of chitin and chitosan

Assessment of anti-ulcerogenic effect of chitosan on mucosal antioxidant defense system in HCl–ethanol induced ulcer in rats has given promising results (Anandan *et al.*, 2004). Peptic ulcer is caused by *Helicobacter pylori*, which weakens the protective mucous coating of the stomach and duodenum allowing the acid to get through the sensitive lining beneath. In the experiment conducted in CIFT, ulcer was induced in male wistar rats using HCl and ethanol. Levels of acid output (both volume and acidity) and lipid peroxide content in the mucosal tissue and number of lesions were increased when ulcer was induced. Peptic activity was decreased, along with the activities of antioxidant enzymes like glutathione peroxidase (GPx), glutathione S-transferase (GST), catalase (CAT) and superoxide dismutase (SOD) and reduced glutathione. Even the levels of mucosal proteins and glycoproteins were reduced when ulcer was induced. The pretreatment with chitin and chitosan at 2% level has reversed the changes to near normal levels and thus proved to have anti-ulcerogenic effect. The animals were divided into four groups. Group-I received standard diet for 30 days. In group-II, ulcer was induced after feeding with normal diet. In group-III, ulcer was induced after feeding with chitin at 2%

level for 30 days. In group-IV, ulcer was induced after feeding with chitosan at 2% level for 30 days. Acid neutralizing capacity of chitin and chitosan might be due to the gradual release of glucosamine residues into the gastric mucosa. Peptic activity was found to be elevated by chitin and chitosan treatment, indicating the cytoprotective activity of chitin and chitosan. Antioxidant nature and free radical scavenging properties of chitin and chitosan help in the reduction of lipid peroxidation and the elevation of reduced glutathione. In the experiment, it was found that chitin was found to be more effective compared to chitosan.

Effect of chitin-chitosan on fatty liver disease in rats

Asha and Nair (2005) studied the effect of chitin-chitosan treatment on fatty liver in rats with a high fat diet. Diet induced non-alcoholic fatty liver disease (NAFLD) is now widespread in affluent societies, in which up to 24% of the general population has been estimated to have NAFLD. This can lead to end stage liver disease. 20% of the affected subjects may progress to cirrhosis and some of them require liver transplantation. Aim of the study was to study the hepatoprotective effect of 2% chitin-chitosan mixture on high fat diet induced fatty liver, an experimental model for non- alcoholic fatty liver disease in wistar strain male rats. The hepatoprotective property was determined based on the criteria: total lipid in liver, concentrations of total cholesterol, triglycerides, free fattyacids of serum and liver tissue, liver lipid-protein ratio, serum and tissue activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), faecal fat determination, and activity of pancreatic lipase.

Chitin-chitosan fed animals had less body weight and a significant reduction in the liver lipid-protein ratio. Concentrations of blood and liver fat, cholesterol, triglycerides, free fattyacids decreased significantly by chitin–chitosan supplementation. Enhanced fat excretion was also noted in chitin–chitosan experimental group. No change in the pancreatic lipase activity was observed. This indicates that there is interference in the gastrointestinal absorption of dietary fat and there is no direct role on fat metabolism. Chitin and chitosan are not hydrolysed by human digestive enzymes and behaves as digestive fibre. Chitosan can increase the amount of fat eliminated in the stools. Chitosan can bind bile acids reducing the amount of cholesterol containing bile acids available for re-absorption by enterohepatic circulation in the lower intestine. This would result in more endogenous cholesterol being used to make bile, thus lowering the blood and tissue cholesterol.

Conclusion

There are several existing and emerging applications for biopolymers such as collagen and chitosan. Investigations at CIFT, Cochin have demonstrated the application of collagen-chitosan membrane in periodontal interventions and as artificial skin in treating burn injuries. Animal studies have indicated the potential of chitin-chitosan in the control of ulcer and diet induced non-alcoholic fatty liver disease. Collagen and chitosan are the molecules for the future and they can be tailored to suit the requirements in pharmaceutical and dental industries. More research has to be planned to utilize these biomolecules, which will tackle the problems of utilization of wastes from fishery resources.

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