

# Marine Bior

## 53. Marine Biomaterials

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In recent years, significant development has been achieved in marine biomaterials for various biological and biomedical applications. In the present chapter, we discuss isolation techniques and the application of marine-derived polymers and ceramics in detail. The main marine polysaccharides are alginate, chitin, chitosan, and fucoidan. The marine-derived polymers show substantial biological properties such as those of anti-inflammation, antimicrobial, anticancer, and osteoporosis. In addition, marine-derived ceramics play an important role in bone related treatment. Isolation procedures and the application of hydroxyapatite (HA) and biosilica are also discussed. HA that has been isolated or developed from different sources such as fish bone, fish scales, and coral is discussed along with its biomedical applications. Properly imple-

mented marine-derived biomaterials will be promising materials for mankind.

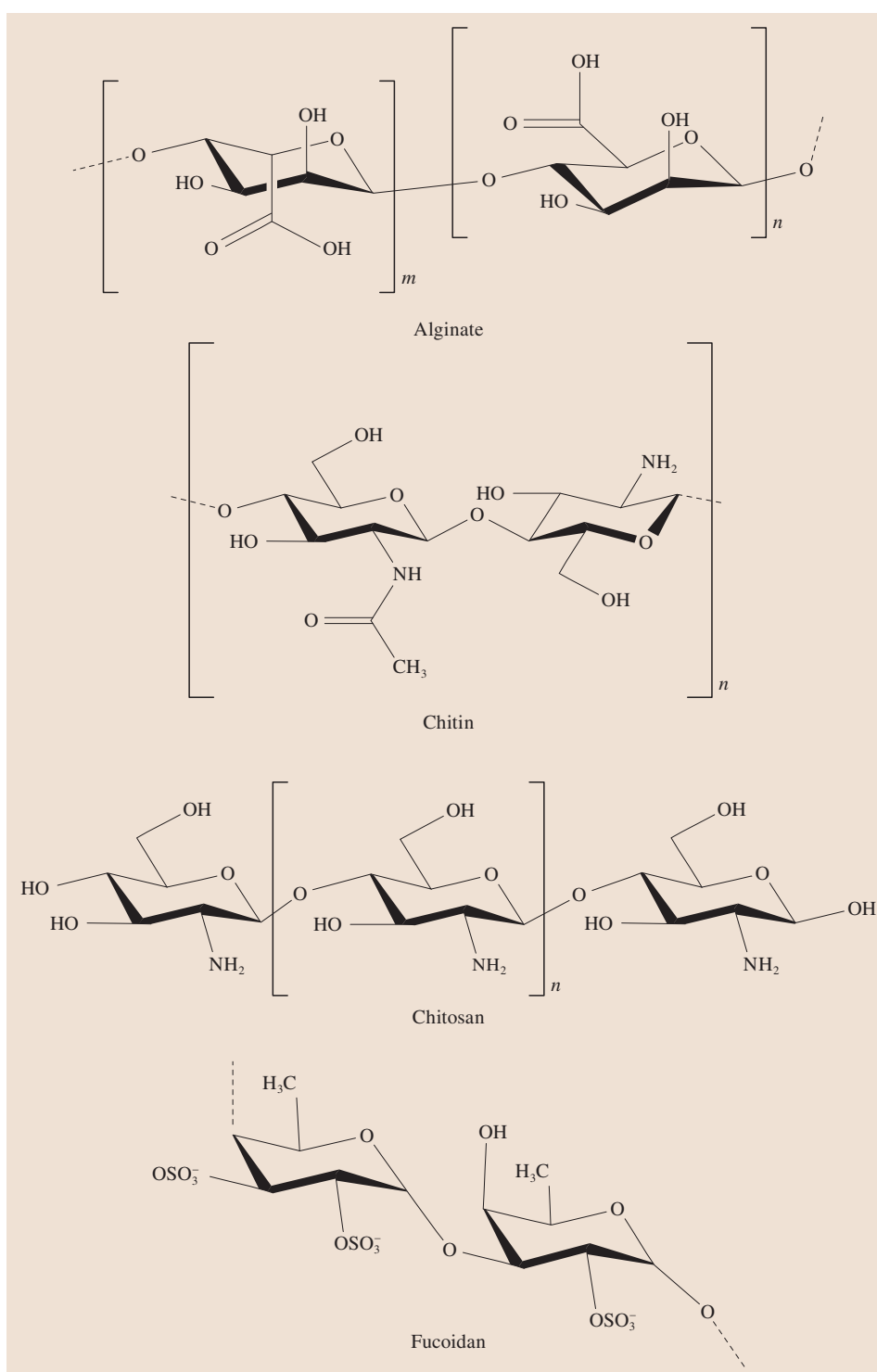
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### 53.1 Examples of Marine Biomaterials

Approximately 72% of the earth is covered by water that is divided by oceans, lakes, rivers, etc. The oceans contain 97% of the earth’s water. They do not only consist of salty water but are an abundant resource for food, medicine, and various raw materials. Marine species are economically important to humans in various ways, including food fish. In recent years, marine-derived biomolecules (proteins, natural compounds, etc.) have been given much importance in medicine and engineering. Marine environments are the household to many exotic biological materials that may inspire biomimetic materials.

Biomaterial science is concerned with the interaction of substances with biological metabolism. Biomaterials can be derived from synthetic sources and

natural sources. Synthetic materials are usually metallic, polymeric, and ceramic, or in the form of composite materials. These materials are often used for biomedical applications, including surgery, tissue engineering, and drug delivery. If the substance comes from a natural source (marine), it can be called marine biomaterial. The important sources of marine biomaterials are fish, invertebrates, mammals, reptiles, fungi, and corals. Fish skin is a rich source of collagen and bone for hydroxyapatite. Algae are a rich source for several polysaccharides. Marine-derived biomaterials have been checked to solve the bone related defects; they include materials from polymers, ceramics, and biomimetic materials<sup>[CE1]</sup>. The main polymers derived include alginate, chitin, chitosan, collagen, fucoidan, etc. (Fig. 53.1).



**Fig. 53.1**  
Structure of im-  
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ical marine  
polysaccharides  
**TS2**

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**TS2** At the bottom “row” of this figure (at the outer left) please check “ $^- \text{O}_3\text{SO}$ ” for correctness. Should this be “ $\text{OSO}_3^-$ ” instead?

polysaccharides!marine	guluronic_acid
alginate	immunoglobulin_G
sodium	wound_dressing
_____alginate	tissue_engineering

## 53.2 Marine Polysaccharides

### 53.2.1 Alginate

Alginate is a biopolymer and found in seaweed and typically extracted from brown algae (*Phaeophyceae*) including *Laminaria hyperborea*, *Laminaria digitata*, *Laminaria japonica*, *Ascophyllum nodosum*, and *Macrocystis prrifere* by treatment with aqueous alkali solutions, typically with NaOH. The extract is filtered with calcium chloride and added to the filtrate in order to precipitate alginate. This alginate salt can be transformed into alginic acid by treatment with diluted HCl. Further purification produces water-soluble sodium alginate [53.1–3] (Fig. 53.2).

Bacterial alginate can be produced from *Azotobacter* and *Pseudomonas* [53.4]. The pathway of alginate biosynthesis is generally divided into four steps:

- Synthesis of precursor substrate
- Polymerization and cytoplasmic membrane transfer
- Periplasmic transfer and modification
- Export through the outer membrane.

Alginate is composed of guluronic acid (G) and manurononic acid, which is considered to be biocompatible, nontoxic, nonimmunogenic, and biodegradable. Alginate extracted from different sources differs in manuronate and gluluronate content, as well as in the length block. The alginate is known to form a hydrogel by hydrogen bonding at low pH. The alginate and alginate chemical modification can bring about new biomaterials, which are useful in cell immobilization, tissue engineering, and drug delivery. Alginate hydrogels have been proven to improve the neo-cartilage and neo-bone formation [53.5–8]. Alginate hydrogels are particulary used for several biomedical applications such as tissue engineering, drug delivery, and wound healing, due to their structural similarity with extracellular tissues [53.1]. Hydrogels are three-dimensionally cross-linked networks composed of hydrophilic polymers with high water contents. Chemical or physical cross-linking of hydrophilic polymers are typical approaches to form hydrogels and their physicochemical properties are highly dependent on the cross-linking type and amount of cross-linking agents [53.9]. The most widely used method to make the alginate gel is with calcium chloride, calcium carbonate, calcium sulfate, and sodium hexametaphosphate (ion cross-linking agents). Covalent cross-linking of alginate with poly (ethylene glycol)-diamines of various molecular weights was first investigated in order to prepare gels

with a wide range of medicinal properties. Thermal gelation and cell cross-linking are some other processing methods to make the alginate hydrogels [53.1].

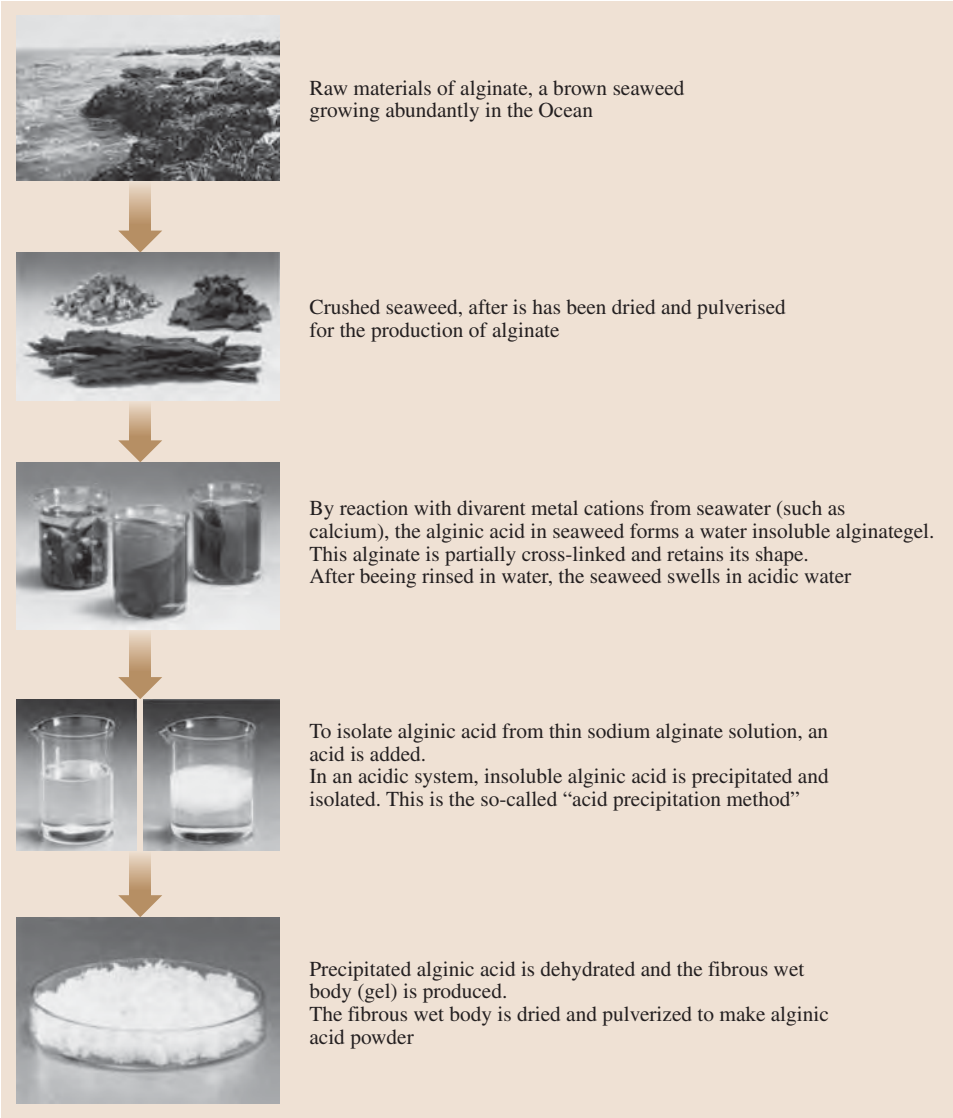
#### Biomedical Applications

**Drug Delivery.** The conventional application of alginate in pharmaceutics is to serve as thickening, gel forming, and stabilizing agents. Multiple drugs have been incorporated with alginate hydrogels with different materials such as poly (caprolactone) [53.10], carbon nanotubes [53.11], and chitosan [53.12]. Alginate is an excellent biomaterial for the delivery of protein drugs. Alginate microspheres are used to encapsulate or load the desired amount of protein for protecting the protein functions, transporting it to the targeted sites, and controlling the kinetics of the protein release. Alginate microspheres were prepared small scale with a water in oil emulsion technique and loaded with fluorescently labeled immunoglobulin G (IgG) [53.13]. Alginate microspheres have been coated with Bombyx mori silk fibroin using layer-by-layer deposition techniques, which provided mechanically stable shells as well as a diffusion barrier to the encapsulated proteins [53.14]. Calcium-cross-linked alginate microspheres and microspheres modified with CpG oligonucleotides are mixed with soluble *matrix* alginate in PBS containing soluble IL-2. Diffusion of calcium ions in the microspheres into the surrounding solution induces cross-linking of the soluble alginate and gel formation. The inset figure outlines the process of calcium reservoir alginate microsphere synthesis via water-in-oil emulsion of alginate in isooctane in the presence of surfactants (Fig. 53.3) [53.15].

**Wound Dressing.** Alginate dressing materials are typically produced by ionic cross-linking of an alginate solution with calcium ions to form a gel, which is subsequently free-dried to obtain a porous sheet<sup>CE3</sup>. Alginate dressing can retain a physiologically moist environment by absorption and desorption of the water from the gels. Several bioactive alginate wound dressing materials have been studied to date [53.16–19].

**Tissue Engineering.** Bone is a complex tissue with a hierarchical structure consisting of hydroxyapatite (HA) and collagen as a major portion. Bone defects can occur in several ways such as through trauma, neoplasm, congenital defects, motor accidents, osteoporosis, arthritis, etc. Various techniques have been used to

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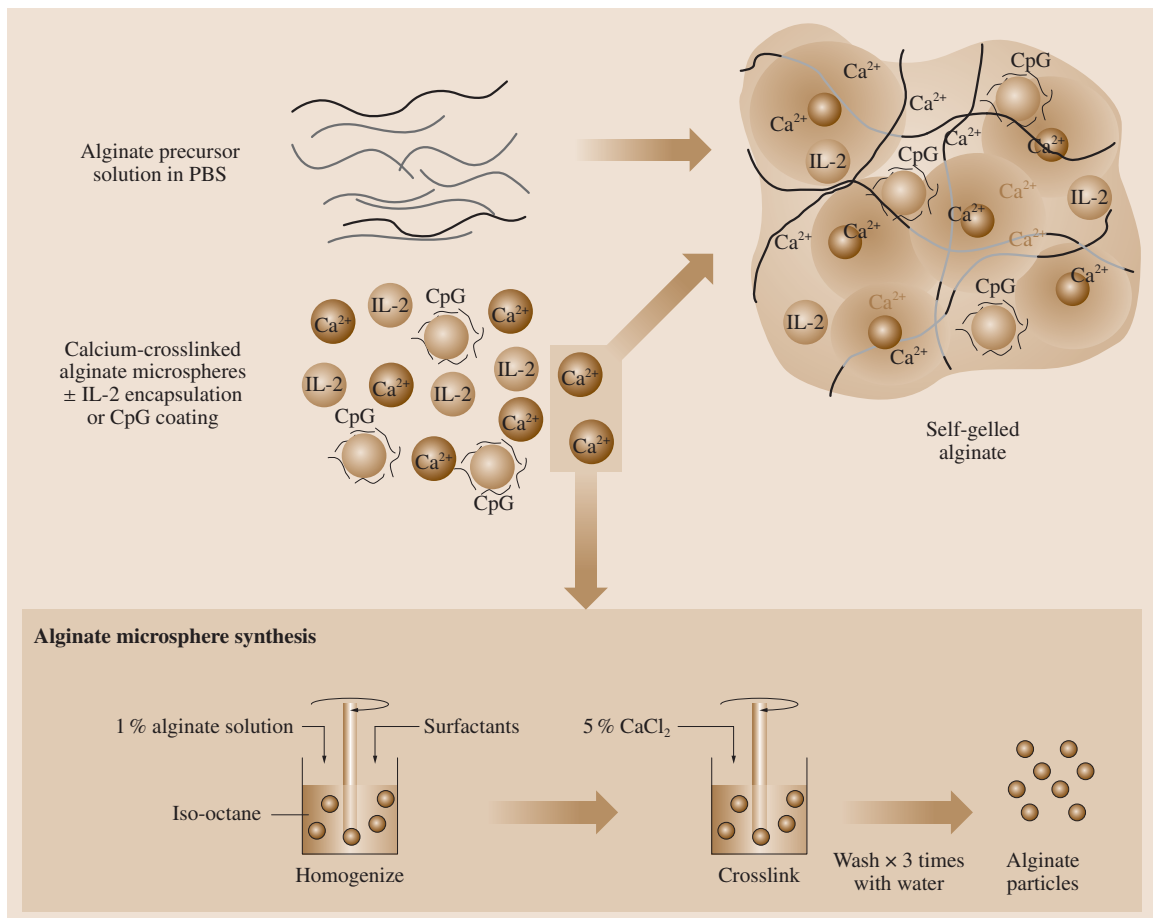


**Fig. 53.2**  
Isolation procedure of alginate from seaweed

solve bone defects, for example, autografting, allografting, and xenografting. However, all these techniques have advantages and disadvantages, for example, insufficient donor sites and transmittable disease [53.20]. Thus, considerable attention has been given by researchers and orthopedists to synthetic and natural materials that can solve the bone defect problem. Experiments are currently taking place at the laboratory and clinical levels. Synthetic materials are often made of hydroxyapatite or other naturally occurring biocompatible materials.

Alginate is the best-known material to form a scaffold-forming property, which can be useful to treat loss or failure of organs. Alginate gels have advantages for bone and cartilage regeneration due to their ability to be introduced into the body in a minimally invasive manner, their ability to fill irregularly shaped defects, the ease of chemical modification with adhesion ligands (e.g., RGD), and controlled release of tissue induction factors (e.g., BMP, TGF- $\beta$ ) [53.21–23] (Fig. 53.4). The transplantation of stem cells using alginate hydrogels has been widely explored in bone tissue engi-

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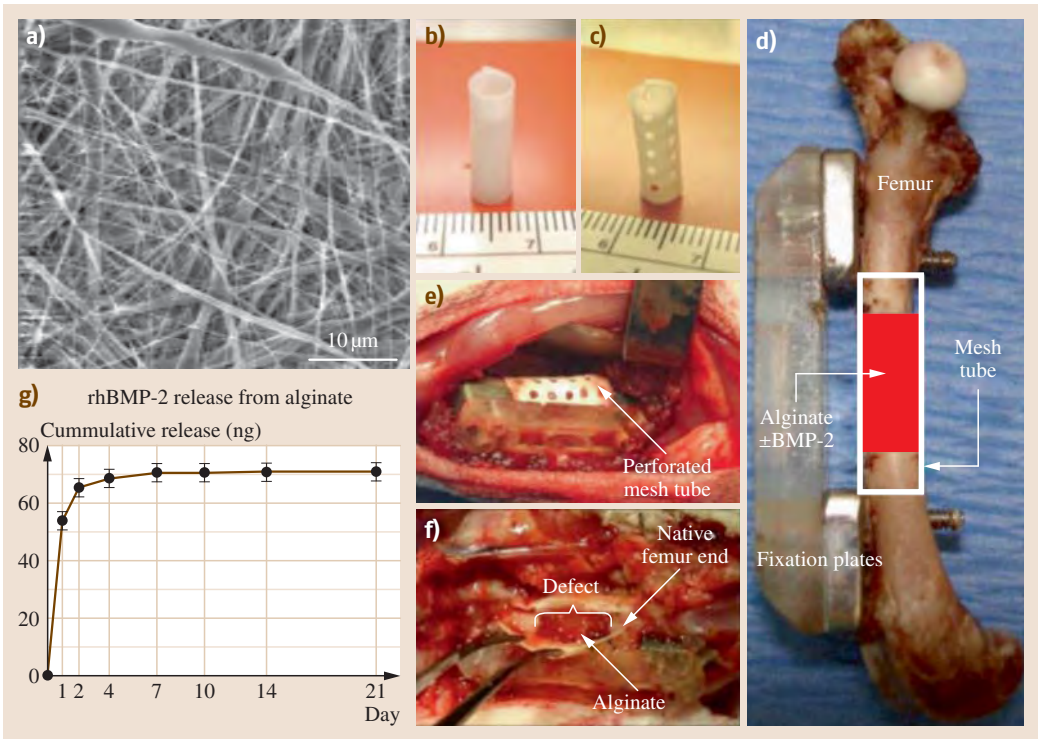
**Fig. 53.3** Schematic of self-gelling alginate formulations based on calcium reservoir alginate microspheres. Reproduced with permission

neering [53.24]. The most widely examined application of alginate gels to promote blood vessel formation has exploited their ability to provide sustained and localized release of heparin binding growth factors such as VEGF [53.25, 26]. Due to a lack of mechanical strength of the alginate scaffold to mimic the natural function of bone, it is combined with inorganic materials to enhance strength as well as bone tissue formation. Alginate with hydroxyapatite is the better combination of the porous scaffold. This has been prepared with the phase separation method, which enhances the cell adhesion of osteosarcoma cells<sup>CE4</sup> [53.27, 28]. Human mesenchymal stem cells (MSCs) encapsulated in the alginate gel bead<sup>CE5</sup> have been cultured in a serum-free medium with the addition of a transforming growth fac-

tor, dexamethasone, and ascorbate, and have been found to form cartilage in large osteochondral defects [53.29–31]. Alginate scaffolds are being actively investigated for their ability to mediate the regeneration of other tissues and organs, including skeletal muscles, nerves, pancreas, and liver. Current strategies for skeletal muscle regeneration include cell transplantation, growth factor delivery, or a combination of both approaches, and alginate was found to be a good candidate in these strategies [53.32–37].

#### Chitin and Chitosan

Chitin are naturally occurring mucopolysaccharides, usually found in fungi, diatoms, nematodes, arthropods, shrimps, crabs, lobsters, krill, and squid [53.38–47].



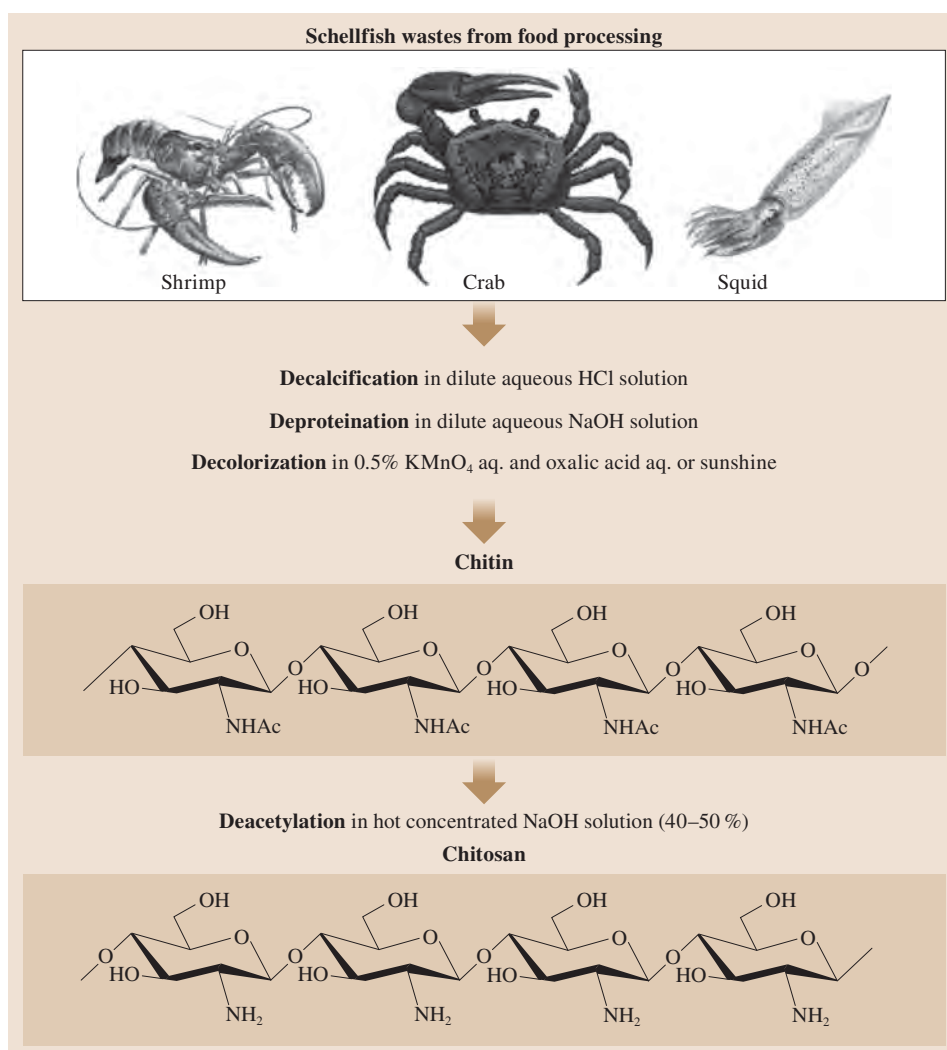
**Fig. 53.4** (a) Nanofiber mesh tubes and alginate hydrogel for surgery. SEM (scanning electron microscope) image of an electrospun nanofiber mesh illustrating the smooth and bead-free nano-scaled fibers. (b) Hollow tubular implant without perforations made from nanofiber meshes. (c) Tubular implant with perforations. (d) Implants in a segmental bone defect. Modular fixation plates are used to stabilize the femur. A nanofiber mesh tube is placed around the 8 mm defect. In some groups, alginate hydrogel, with or without rhBMP-2, is injected inside the hollow tube. (e) Defect after placement of a perforated mesh tube. The alginate inside the tube can be seen through the perforations. (f) A specimen was taken after 1 week and the mesh tube was cut open. The alginate was still present inside the defect, with hematoma present at the bone ends. (g) Alginate release kinetics over 21 d in vitro. Sustained release of the rhBMP-2 was observed during the first week

The chemical formula of chitin is 2-acetamido-2-deoxy- $\beta$ -D-glucose through  $\beta$  (1–4) linkages (Fig. 53.1). This linkage can be easily degraded by the chitinase enzyme [53.48, 49]. Chitosan is a linear polysaccharide composed of randomly distributed  $\beta$ -(1–4)-linked glucosamine and *N*-acetyl-D-glucosamine. Chitosan can be obtained from chitin by a chemical method or an enzymatic production method. Chitosan can be isolated directly from the cell wall of certain fungi, but commercially available chitins are usually prepared from chitin. Chitin and chitosan are white, hard, inelastic nitrogenous polysaccharides and the major source of surface pollution in coastal areas. Chitosan

is produced commercially by deacetylation of chitin, which is the structural element in the exoskeleton of the crustaceans crab and shrimp, and the cell walls of fungi. The degree of deacetylation can be determined by NMR spectroscopy; it ranges from 60% to 100%, and the molecular weight is between 3800 and 20 000 Da.

Several methods have been introduced to isolate chitin and chitosan from shellfish waste, the most traditional and well-developed method is a chemical, enzymatic, fermentative method for industrial production, which is simple and convenient for large production [53.50–52].



**Fig. 53.5**

Chemical method for the production of chitosan

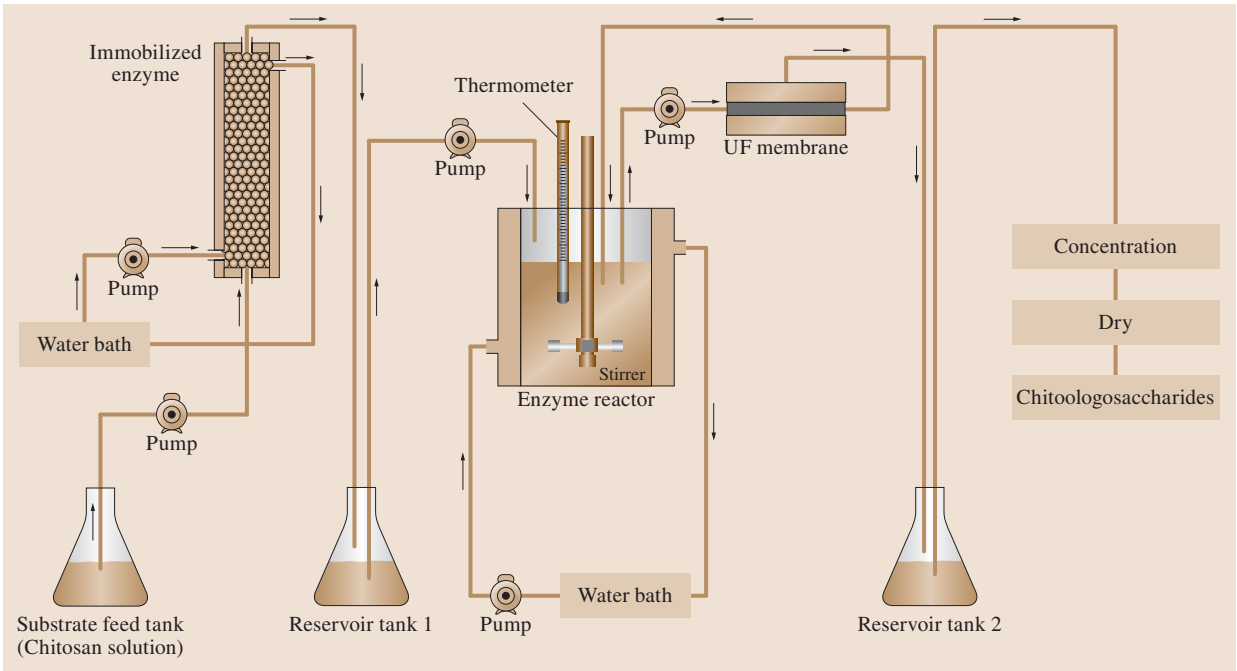
#### Production of Chitosan by Chemical Methods

In the chemical hydrolysis method, four main steps are involved in the production from marine crustacean shells, as depicted in Fig. 53.5 [53.53–55]. They are:

- Decalcification in dilute aqueous HCl solution
- Deproteinization in dilute aqueous NaOH solution
- Discoloration in 0.5% aqueous KMnO<sub>4</sub> and aqueous oxalic acid or sunshine<sup>CE6</sup>
- Deacetylation in hot concentrated NaOH solution (40–50%).

In general, proteins are first removed from ground shells by treatment with mild sodium hydroxide or potassium hydroxide solution at elevated temperature. Alkali concentration is usually between 1 to 10% with temperatures ranging from 30 to 100 °C, independent of the starting materials. These are the most common, and reaction times usually varies from 30 min to 12 h. Higher temperature reduces the molecular weight of the resultant chitosans. The removal of calcium carbonate, calcium phosphate, and other mineral salts found in shell waste is accomplished by extraction with dilute acids. To produce 1 kg of 70% deacetylated chitosan

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**Fig. 53.6** Schematic diagram of the dual reactor system used for continuous production of chitooligosaccharides

from shrimp shells, 6.3 kg of HCl and 1.8 kg of NaOH are required.

**Enzymatic Methods**  
Enzymatic methods are an alternative to the chemical method for chitin and chitosan production. In addition, the protein often remains high and reaction times are significantly increased compared to chemical methods. Enzymatic methods are limited in industrial production of chitosan, due to higher cost of enzymes [53.52]. Several commercially available enzymes such as alcalase, chymotrypsin, and papain are also used for the production of chitosan [53.56].

**Fermentation Methods**  
Fermentation with bacteria producing proteolytic and chitinolytic enzymes has been researched as an alternative method [53.57]. Organic acid and protease produces a soil isolate of *Pseudomonas aeruginosa* F722 with crab shells<sup>CE7</sup>. With an optimal fermentation temperature of 30 °C and a 10% glucose supplementation, the degree of demineralization was 92% and the degree of deproteinization was 63% after 7 days incubation [53.58].

**Production of Chitooligosaccharides**  
Lower molecular and water soluble chitosan obtained by continuous hydrolysis of chitosan by chitinolytic enzymes, such as chitinase, chitosanase, papain, and lysozyme are widely used for the production of chitooligosaccharide (COS). Several research groups developed a method for the production of COS with a higher yield and a higher degree of polymerization [53.59–63]. For the continuous production of COS by an enzymatic method, ultrafiltration reactors have been employed. The advantages of continuous production of COS is higher efficiency and greater enzyme productivity; it was found that high the viscosity of chitosan restricted continuous operation due to membrane fouling [53.62].

The continuous production of COS from chitosan has been attained with a dual reactor system with an ultrafiltration membrane reactor and a column reactor packed with an immobilized enzyme. The production of the COS was performed in two steps (Fig. 53.6):

1. Preparation of the partially hydrolyzed chitosan from viscose chitosan in the column reactor packed with an enzyme

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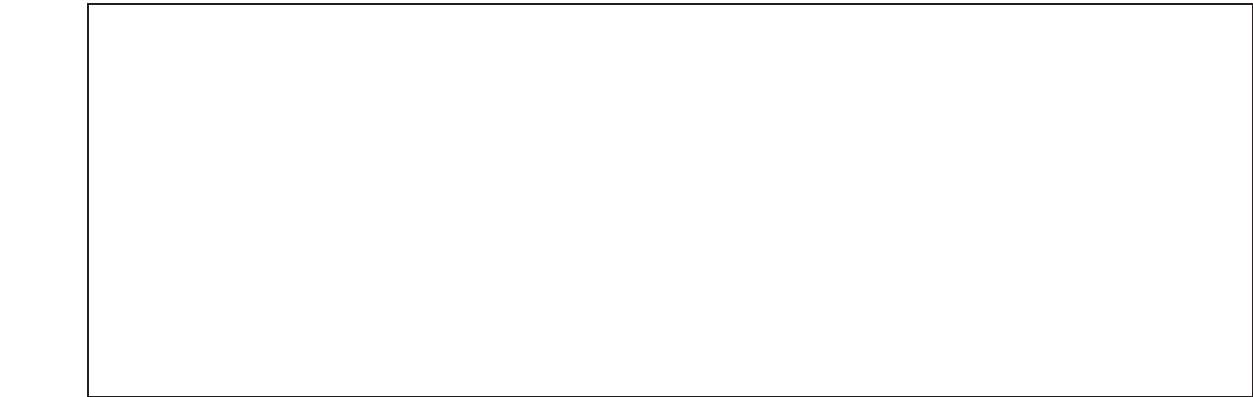


Table 53.1 Properties of chitosan and their applications

	Properties	Applications
Cationic	Linear polyelectrolyte with high charge density Chelates toxic metal ions	Water purification Excellent flocculent
Chemical	High molecular weight Reactive amino and hydroxyl groups	High viscosity, film forming Chemical modification for species end uses
Biological	Biocompatible and biodegradable Bioactivity	Non-toxic, packaging films, coating Antimicrobial, antitumor, anticancer, immuno potentiator
Pharmaceutical	Biocompatible, biodegradable	Wound, healing, drug delivery, tissue engineering, contact lens
General cosmetics	Moisture retention, excellent feed, protective film-coating, excellent tactile properties	Skin care products, hair lotions, natural ingredient
Food and Agriculture	Binds anions (bile acids or free fatty acids)	Hypocholesterolemic agents, anticarcinogenic agents, dietary fibre, antiulcer
	Fungistatic	Increased crop production
Biotechnological	Extrapment and adsorption	Immobilization matrix, chitosan beads/gels

2. Production of the oligosaccharides from partially hydrolyzed chitosan in the ultrafiltration membrane reactor.

Three kinds of partially hydrolyzed chitosan were obtained from three different outflow rates (3, 5, and 9 ml/min) in the column reactor and were supplied to a substrate feed tank of the following UF reactor in order to identify the influence of the feed on membrane fouling. The partially hydrolyzed chitosan obtained with a 5 ml/min overflow rate was the most suitable substrate for alleviation of membrane fouling and efficient hydrolysis under the operating conditions of the dual reactor system [53.62].

Some of other methods are also used for the production of chitin, chitosan and COS such as, gamma irradiation [53.64]. The method of preparation as follows, heads and shells from prawns found on the Algerian coast were collected, dried at 60 °C, and cut into small pieces that were then irradiated at a dose of 75 Gy/min to a dose of 25 kGy. Irradiation reduced the time needed for deproteinization from 3 to 1 h using 1 N sodium hydroxide and a reaction temperature of 85 °C.

Applications

Because it is a natural resource and is thus biologically reproducible, biodegradable and environmentally nonpolluting, biocompatible, nontoxic, and biologically functional, chitosan is a versatile material for various biological and biomedical applications [53.54] (Table. 53.1).

Antimicrobial Activity

The antimicrobial activity of any substance is always directed toward its applicability. The film forming ability of any polymers with antimicrobial property can be used for food packaging. Antimicrobial packaging is one of the most promising active packaging systems that have been found to be highly effective in killing or inhibiting spoilage of pathogenic microorganisms that contaminate food [53.65]. Chitosan is best known for its antimicrobial property from the literature [53.66–69].

Variations in chitosan’s bactericidal efficacy arise from various factors. According to the roles played, these can be classified into four categories as follows:

- 1. Microbial factors, related to species and cell age
- 2. Intrinsic factors of chitosan including positive charge density, molecular weight, concentration, hydrophilic/hydrophobic characteristic, and chelating capacity
- 3. Physical state, namely water solubility and solidity<sup>CE8</sup> of chitosan
- 4. Environmental factors, involving the ionic strength in the medium, pH, temperature, and reaction time.

Chitosan is an ideal biopolymer for developing such antimicrobial films due to its nontoxicity. The inherent antibacterial/antifungal properties and the film-forming ability of chitosan make it ideal for use as a biodegradable, antimicrobial packaging material. The antimicrobial properties of chitosan can be enhanced

<sup>CE8</sup> Please check that this is the intended meaning.

by irradiation, ultraviolet radiation treatment, partial hydrolyzation, chemical modifications, synergistic enhancement with preservatives, synergistic enhancement with antimicrobial agents, or in combination with other hurdle technologies.

#### Anti-Inflammatory Activity

Anti-inflammatory refers to the property of a substance or treatment that reduces inflammation. Chitin is rarely checked for anti-inflammatory property due to its lack of solubility.

Yang et al. reported that COS have shown considerable anti-inflammatory activity with different molecular weights [53.70]. The effects of COS on nitric oxide (NO) production and the cytokine expression tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), and interleukin-6 (IL-6) have been checked as **CE9** LPS stimuli from RAW 264.7. Stimulation with increasing concentrations of COS, the LPS-stimulated TNF- $\alpha$ , and IL-6 secretion recovered significantly within the incubation media of RAW 264.7 cells [53.71].

#### Anticancer Activity

Chitosan shows several biological activities, as discussed above. Moreover, chitosan significantly inhibits the tumor growth [53.72]. The intra tumoral administration of chitosan compounds alone has been shown to promote antitumoral effects in a metastatic breast cancer model [53.73]. Chitosan was also found to activate macrophages into cytotoxic macrophages and suppressed Meth-A tumor growth in Balb/c mice [53.74]. COS is also shown to inhibit the growth of Meth-A solid tumors transplanted in mice [53.75].

#### Biomedical Application of Chitin, Chitosan, and COS

The wide array of tissue engineering applications exacerbates the need for biodegradable materials with broad potential. Chitosan is an excellent biodegradable and biocompatibility biomaterial. Natural polymer composite materials are promising scaffolds for bone tissue engineering [53.76]. Next generation biomaterials should combine bioactive and bioresorbable materials, which mimic the natural function of bone and activate in vivo mechanisms of tissue regeneration. Composite materials based on combinations of biodegradable polymers and bioactive ceramics are highly suitable for bone regeneration [53.77]. The important biomedical applications of chitin and chitosan are tissue engineering and drug delivery [53.53, 78–83].

#### Tissue Engineering

Tissue engineering has been a fascinating area of research in recent years to develop the artificial organs [53.84]. Several materials have been widely used to develop artificial organs; these are synthetic and natural derived materials. Chitosan is a promising biomaterial used for various biomedical applications. Chitosan can be modified to any form such as film, fibers, beads, and scaffolds (Fig. 53.7).

Dimensional structures of scaffolds are used to simulate the extracellular matrices naturally found in the body. The design consists of a large surface area so that cells can be seeded and are able to penetrate the pores. The pores are interconnected so that wastes and nutrients can be exchanged between the scaffold and the surrounding environment, thereby promoting cellular development. The properties of the scaffold are influenced by the method used in the creation of the scaffold. There are several methods used to create highly porous scaffolds, such as supercritical fluid technology [53.85] and the freeze drying method [53.86]. While chitosan has many desirable properties, its mechanical strength is poor, and to enhance the mechanical strength, it is often blended with other polymers and ceramics. Ceramics such as hydroxyapatite are biomaterials that are widely used with chitosan to make the scaffolds [53.76, 87–90].

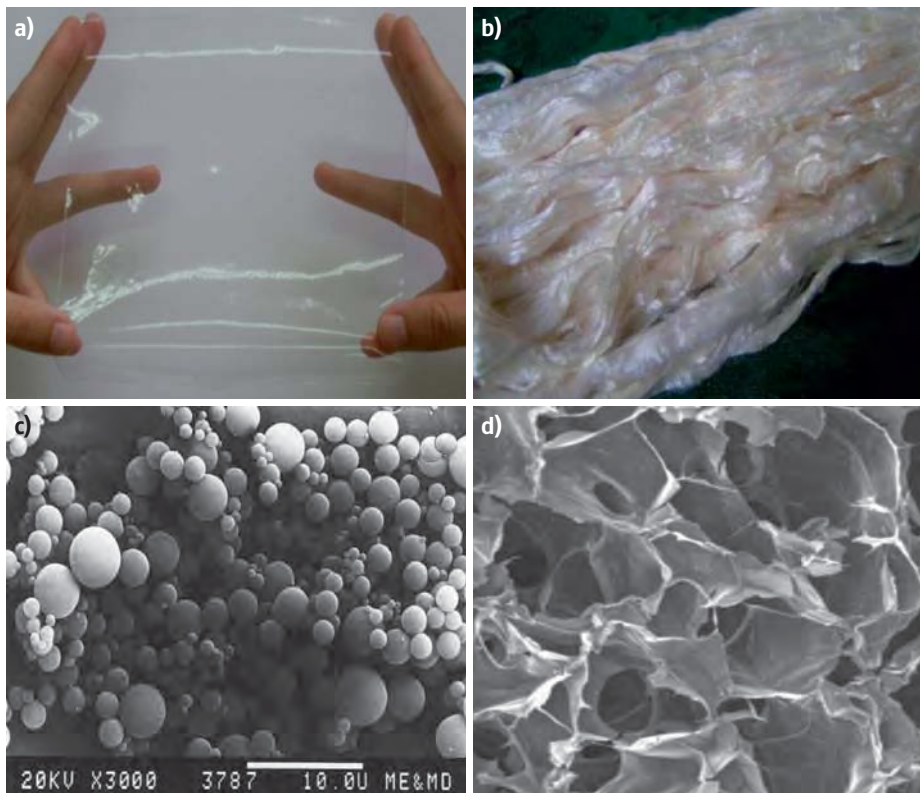
#### Drug Delivery

Considerable research efforts have been directed towards developing safe and efficient chitosan-based particulate drug delivery systems [53.91–97]. Chitosan has been used as excipient in oral formulations and vehicles for parenteral drug delivery devices. Chitosan has further been used to manufacture sustained release systems deliverable by other routes (nasal, ophthalmic, transdermal, and implantable devices) [53.98]. Chitosan forms colloidal particles and entraps bioactive molecules through chemical cross-linking, ionic cross-linking, and ionic complex formation for the association of bioactive molecules to polymers and to control drug release [53.99, 100].

#### 53.2.2 Fucoidan

Fucoidan is sulfated polysaccharides, mainly in found brown algae such as mozuku, kombu, limu moui, bladderwrack, wakame, hijiki, and sea cucumber. Other common fucoidans are sourced from edible species such as *Fucus vesiculosus*, *Cladosiphon okamuranus*,

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**Fig. 53.7 a–d**  
Chitosan in different forms. **(a)** Film, **(b)** fiber, **(c)** beads, and **(d)** scaffolds

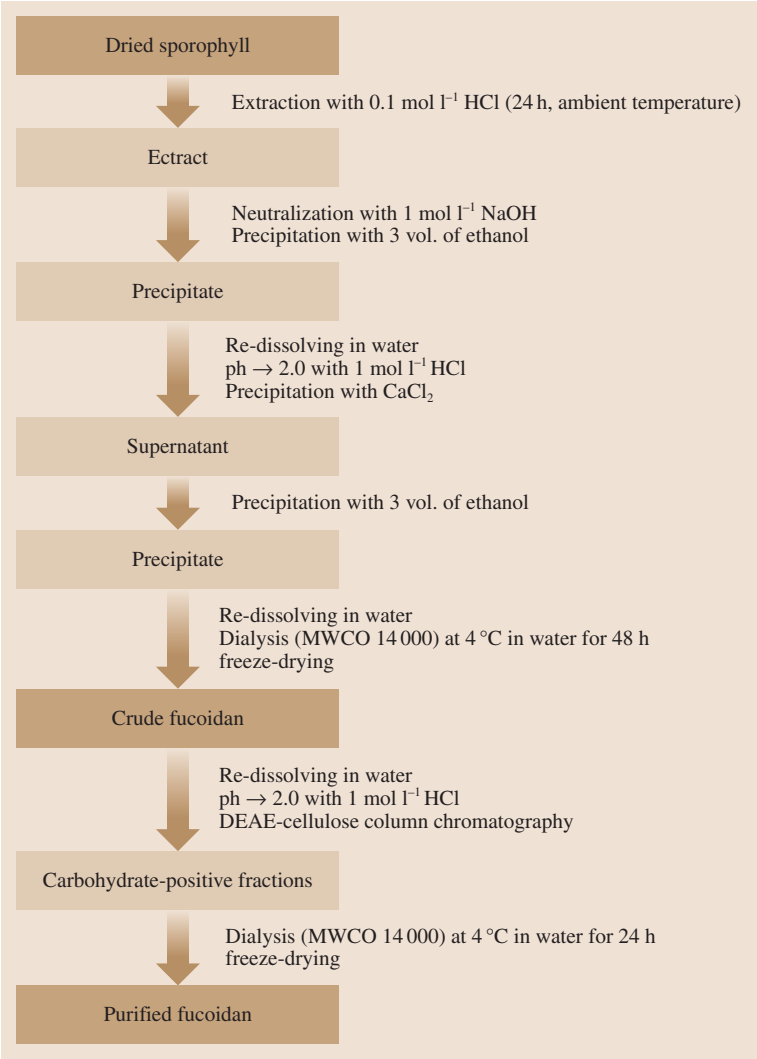
*Laminaria japonica*, and *Undaria pinnatifida*. The main skeleton of fucoidans consists of  $\alpha$ -1,3-linked sulphated l-fucose; a repeating sequence of alternating  $\alpha(1 \rightarrow 3)$  and  $\alpha(1 \rightarrow 4)$  glycosidic bonds is also possible. Published research articles on fucoidans increased threefold between 2000 and 2010. Fucoidan plays a significant role in the control of acute and chronic inflammation via enzyme inhibition and inhibition of the complement cascade [53.101]. Fucoidans contain a high proportion of fucose in the sugar backbone of the polymer. They are sulfated, may be acetylated, and may also contain uronic acids. The yield of a crude first fraction of fucoidan is generally 2–10% by weight. Fucoidans derived from seaweed are all highly branched. A second source of linear rather than branched fucoidans is echinoderms, in particular sea cucumbers [53.102–104].

The chemical composition and structure of fucoidans are very diverse and vary significantly depending on the algae source, place of cultivation and habitat, harvesting time, etc. The biological activity and medicinal impact of fucoidans depends strongly on their

structural properties. The extraction and purification procedures of fucoidan are shown in the Fig. 53.8. Fucoidan was isolated from the raw material by dilute acid extraction, ballast alginates were removed by  $\text{CaCl}_2$  precipitation, and crude extract was purified by chromatography on DEAE-cellulose [53.105].

#### Biological Applications of Fucoidan

**Anticancer Activity.** Cancer is known to be one of the worst diseases that threaten human's lives. Unfortunately, drugs used for cancer therapy are toxic and affect not only cancer cells but also normal cells and tissues. It has been reported that fucoidans effectively inhibit proliferation and colony formation of cancer cells in vitro [53.106, 107] and also inhibitory activity in tumors growing in vivo [53.108]. Studies suggest that sulfate content, molecular weight, monosaccharide composition, and the structure of the main polymer chain of fucoidan have a great influence on their biological activities. A higher amount of sulfated content in fucoidan shows a higher antitumor activity with low degree substitution **CE10** [53.109–113].



**Fig. 53.8** Isolation and purification of the Miyeokgui fucoidan

**Anti-inflammatory Activity.** Park et al. checked the inhibitory effects of fucoidan on production of lipopolysaccharide (LPS)-induced pro-inflammatory mediators in BV2 microglia. Results indicated that fucoidan treatment significantly inhibited excessive production of nitric oxide (NO) and prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) in LPS-stimulated BV<sub>2</sub> microglia [53.114, 115].

**Osteoporosis.** Fucoidan has several biological activities and recent studies indicate that fucoidan can be useful in treating osteoarthritis. Park et al. used an animal model of collagen-induced arthritis and showed that

orally administered *Undaria pinnatifida* fucoidan successfully inhibited pain [53.116]. Osteoarthritis symptoms were significantly inhibited by oral administration of fucoidan-rich seaweed extract; the symptoms were reduced by 52% [53.117].

**Biomedical Applications of Fucoidan.** Changotade et al. [53.118] reported that low molecular weight fucoidan explored<sup>CE11</sup> to bone extracellular matrix to support human osteoblastic behaviors in 3-D culture. Fucoidan promotes cell proliferation, collagen type I expression, alkaline phosphatase activity, and mineralization [53.118, 119].

<sup>CE11</sup> Please check and reformulate. This sentence is unclear.



53.3 Marine Ceramics

Ceramics of marine origin<sup>CE12</sup> such as corals, naces, fish bone, and sponges provide significant amounts of ceramic materials for biomedical applications.

53.3.1 Hydroxyapatite

Hydroxyapatite (HA), is a naturally occurring mineral form of calcium apatite with the formula  $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ . Several sources have been identified and used for isolation of HA, such as fish bone [53.120–123]. Considerable interest has been given to fish bone for the production of HA, which has several advantages such reduction of environmental pollution and results in value-added products. Several synthetic methods have been reported in the literature such as hydrother-

mal [53.124], liquid membrane [53.125], precipitation [53.126], radio frequency thermal plasma [53.127], ultrasonic precipitation [53.128], reverse microemulsion [53.129], sol-gel [53.130] and polymer-assisted methods [53.131], for example.

Hydroxyapatite from Fish Bone and Scales

The simplest method for the production of HA from fish is thermal treatment. HA is the main component of fish and the other is collagenous and noncollagenous protein. The amount HAp at different temperatures 600 °C, 900 °C, and 1200 °C were 62.12%, 59.33%, and 57.64% (Table. 53.2 and Fig. 53.9). HA was isolated from *Thunnus obesus* bone using alkaline hydrolysis and thermal calcination methods. The

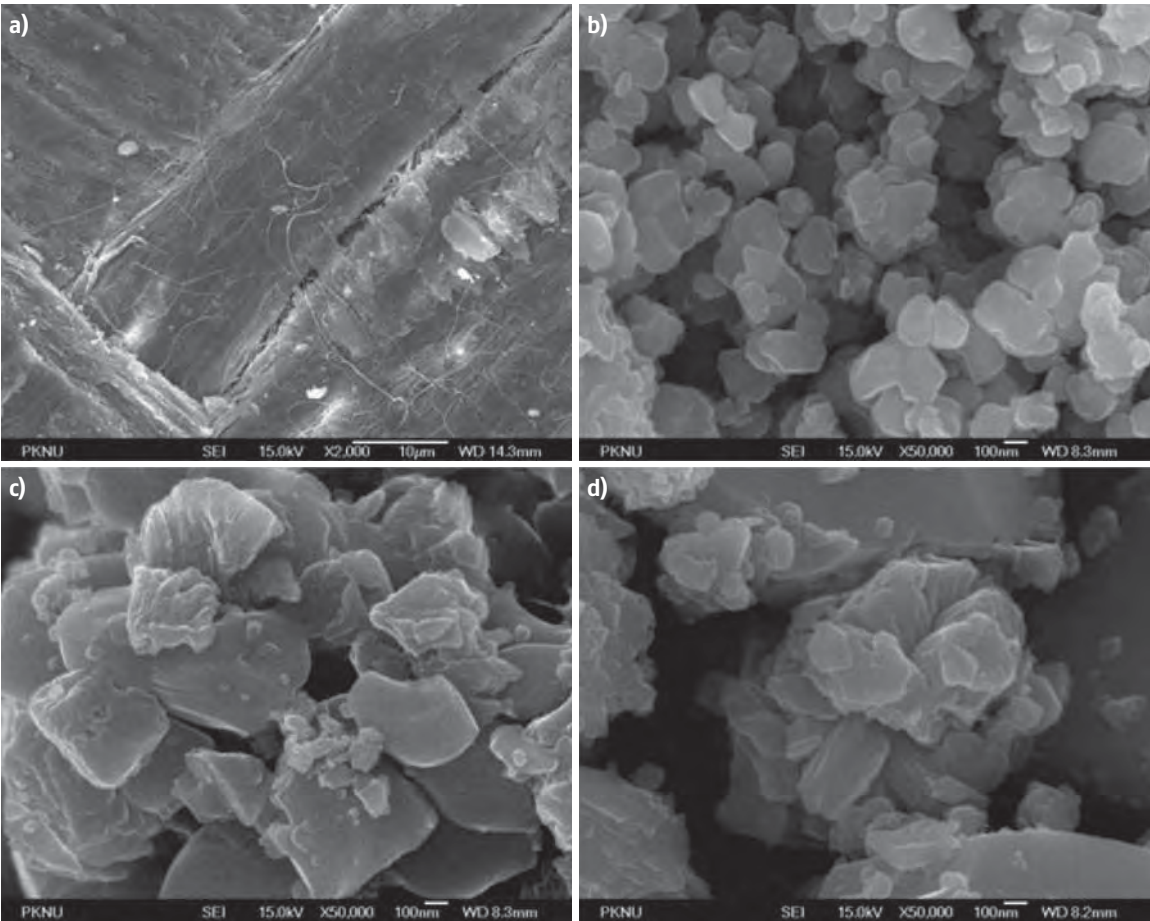


Fig. 53.9 a–d SEM results of (a) raw fish bone and treated at (b) 600 °C, (c) 900 °C, (d) 1200 °C

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Table 53.2 Residues and color of calcined *Thunnus Obesus* bone

Sample No.	Calcination temperature (°C)	Calcination period in (h)	Initial weight (g)	Weight after calcination (g)	Residue (%)	Description
1	1200	5	2.0000	1.1527	57.6350	White
2	1100	5	2.0020	1.1529	57.5874	White
3	1000	5	2.0024	1.1771	58.7845	White
4	900	5	2.0011	1.1872	59.3274	White
5	800	5	2.0030	1.1936	59.5906	White
6	700	5	2.0032	1.2129	60.5481	Off-white
7	600	5	2.0017	1.2434	62.1172	Off-white
8	500	5	2.0052	1.2688	63.2755	Tan
9	400	5	2.0031	1.3402	66.9063	Tan
10	300	5	2.0061	1.5162	75.5795	Black
11	200	5	2.0000	1.7360	86.8000	Black
12	Raw fish bone	—	—	—	—	Yellow

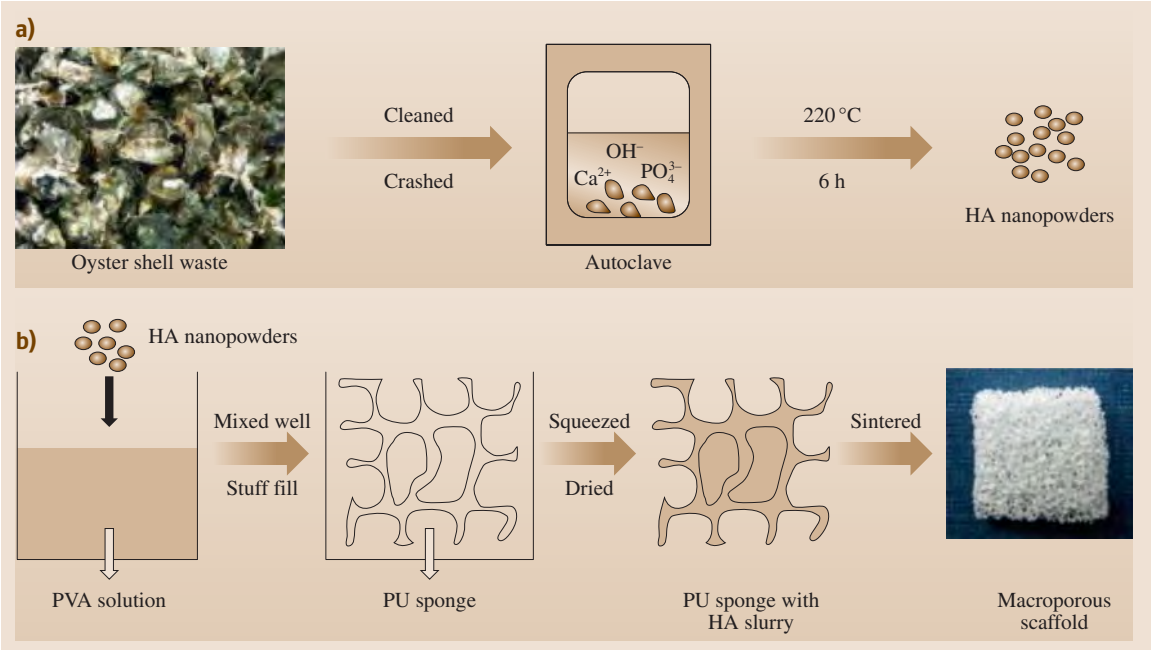
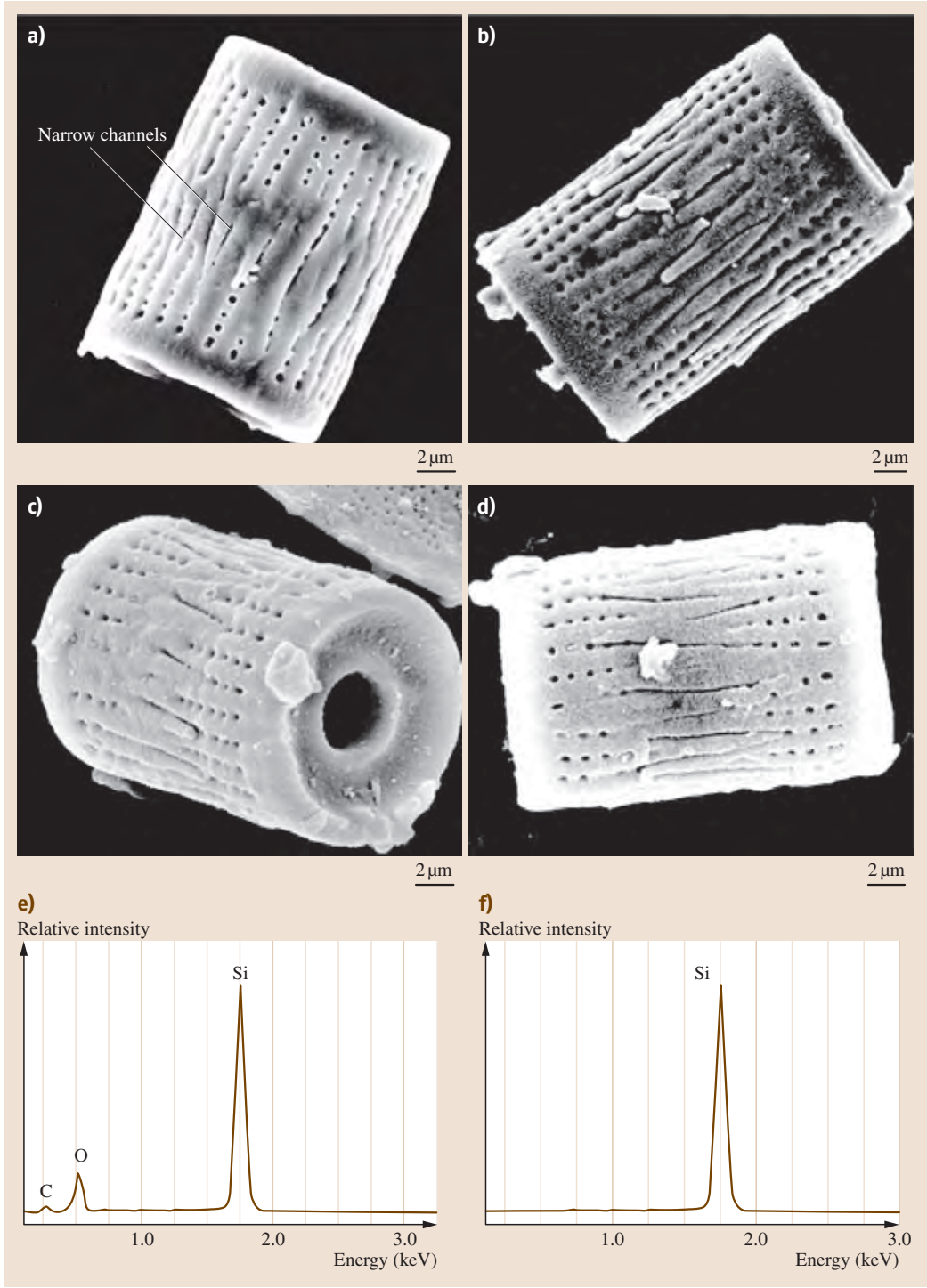
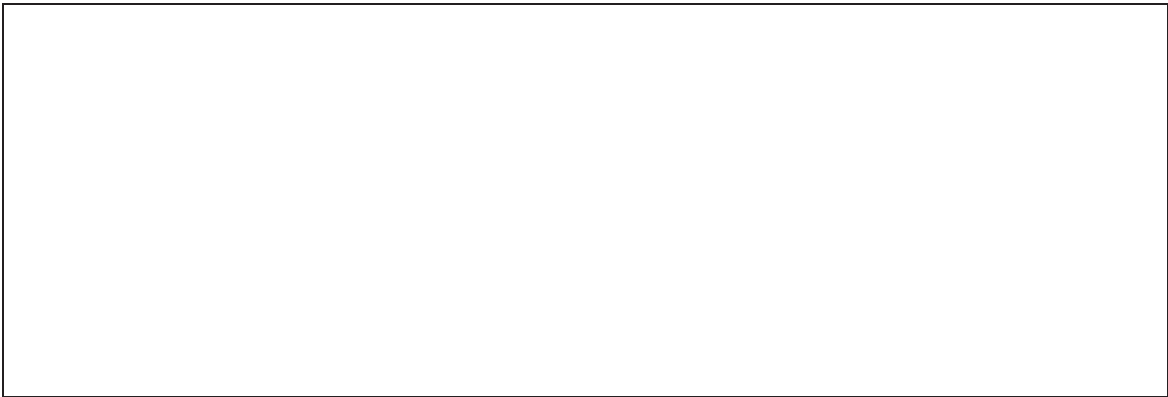


Fig. 53.10 a,b Schematic of the basic process: (a) Hydrothermal conversion of oyster shells to hydroxyapatite (HA) nanopowders and (b) the polymer replication technique used to fabricate macroporous scaffolds

Fig. 53.11 (a) Secondary electron image of an *Aulacoseira* diatom frustule. (b) Secondary electron image of a MgO/Si composite replica after reaction of an *Aulacoseira* frustule with Mg(g) at  $650^\circ\text{C}$  for 2.5 h. (c) Secondary electron image of a silicon-bearing replica produced by selective dissolution of magnesia from a MgO/Si replica in an HCl solution. (d) Secondary electron image of a silicon replica after HCl treatment and additional treatment in a HF solution. (e,f) Energy dispersive x-ray analyses obtained from silicon frustule replicas of the type shown in (c) and (d) , respectively ►CE13





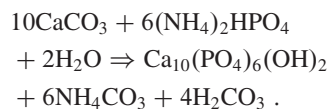
results indicate that there are significant differences between the ceramics and *Thunnus obesus* bone, the thermal calcination method produces good crystallinity with dimensions 0.3–1.0 μm, whereas the alkaline hydrolysis method produces nanostructured HAp crystals with 17–71 nm length and 5–10 nm width [53.120–123].

HA ceramics isolated from natural sources like cuttlefish bone [53.132], bovine bone [53.133–138], and fish bone [53.122, 139–142] have the advantage of providing inexpensive raw materials from bone and teeth. Ooi et al. developed HAp from a bovine source with the thermal calcination method at a temperature range 600–1000 °C, which exhibited HA in a pure form [53.135]. The most important parameters that can affect the properties of HA are the temperature and the duration of the heat treatment [53.134]. While synthetic materials have been widely used in the biomedical field with great success, natural structural materials are now providing an abundant source for novel biomedical applications. Carbonate groups present in carbonated HA are eliminated by heating, which affects the biological properties of the apatite extracted by thermal calcination method [53.143]. Ozawa et al. reported that HA was isolated from Japanese sea bream by the thermal calcination method up to 1300 °C. Weight loss was observed at the three temperature ranges 30–250 °C, 250–380 °C, and 380–520 °C. The first one corresponds to water content and other two are removal of organic substances [53.144]. Some researchers use fish scale for the production of HA [53.145]. Some researchers have used cuttlefish for the production of HA [53.146–148].

#### Hydroxyapatite from Corals

Coral is widely used for the production of HA [53.149, 150]. The main component of coral is calcium carbonate, normally in the form calcite. Some chemical reaction is required for conversion of coral to HA. Usually phosphate-containing substances are

used.



Several commercially available HA based products are available based on the above reaction; they are ProOsten and Interpore200. These materials are widely used in several biomedical applications [53.151]. The above reaction condition can be varied according to the coral substance [53.152, 153] (Fig. 53.10).

#### Biomedical Applications

The natural trabecular structure of coralline HA is similar to that of bone by the hydrothermal conversion of the calcium carbonate skeleton of coral to HA. The benefits of CHA for bone grafts are predominantly its safety, biocompatibility, and osteoconductivity; it is used as a bone graft substitute and bone void filler [53.154–158].

#### 53.3.2 Biosilica

Biogenic silica, commonly known as biosilica, consist of glassy amorphous silica and are formed in many aquatic organisms (and in terrestrials as well), such as sponges, diatoms, radiolarians, and choanoflagellates [53.159]. In addition to being inspiring and a valuable source of marine collagen, as mentioned above, some sponge species are also an important source of biosilica.

There are two classes of sponge that have a silica skeleton: demospongiae and hexactinellida. The third class, calcarea, has a calcium carbonate skeleton. The process of biosilica formation in sponges is enzyme-mediated. The axial filament consists predominantly of the silicatein, which mediates the silicification process around it through the formation of the said concentric layers [53.160] (Fig. 53.11).

### 53.4 Current Understanding and Future Needs

Recent screening techniques have revealed the vast chemical diversity of oceans, which is much higher than what can be achieved by synthesis and standard chemical approaches; this opens new and exciting research scenarios. In fact, the real value of marine-derived materials and compounds can only be roughly checked; it is still to be discovered. Thus, the sus-

tainable exploitation of ocean diversity for industrial and medical purposes is of enormous interest and promises a huge impact not only on research, but particularly on the progress of society, which is reflected on the emergence of marine biotechnology, also known as blue biotechnology, as a fast-growing sector [53.161].

### 53.5 Conclusions

Oceans not only consist of water, but it has been proven that they are an abundant source of various materials. There is a need to develop several bioactive biomaterials from the marine source. Isolation procedures need to be developed in order to obtain medical grade materials for human purposes. In recent years, several biotech-

nological processes have improved to help us obtain medical grade biomaterials from the marine source. Chemical derivatization is one promising approach to modify marine-derived polymers for use in tissue engineering, tissue delivery, or biosensors.

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