Chitosan-Based Systems for Theranostic Applications

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Abstract

The ideal theranostic approach is capable of several functions ranging from diagnosis to treatment with accurate targeting of cancer-specific cells. Therefore, the newest generation of theranostic systems offers opportunities to combine passive and active targeting, environmentally responsive drug release, molecular imaging, and other therapeutic functions into a single biomedical platform. To achieve this purpose, biomedical researchers have developed various systems composed of organic or inorganic materials. Due to its remarkable physicochemical and biological properties, chitosan and its derivatives have been employed in the development of composite theranostic systems able of prediction, real-time monitoring, and assessment of the therapeutic responses. This review outlines recent developments of chitosan-based systems for theranostic applications and analyzes them in terms of performances and limits. Conclusions and future perspectives will both synthesize the state of the art of the chitosan applications in theragnosis and the authors' point of view about insights toward biopolymer unexploited implications in this field.

Keywords

Theranostic · Chitosan · Bioactivity · Nanoparticles · Scaffolds

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12.1 Theranostics: A Novel Approach to Combine Diagnosis, Treatment, and Subsequent Imaging

12.1.1 Concerns for Theranostic Systems

Cancer is the leading cause of worldwide death for centuries, accounting millions of deaths annually (Jemal et al. 2011). Based on data statistics reported by the International Agency for Research on Cancer (GLOBOCAN) in 2012, there were 14.1 million new cancer cases, 8.2 million cancer deaths, and 32.6 million existing cancer patients worldwide. An increase is estimated to about 16 million new cancer cases per year by 2020. Current promising therapies for cancer, including surgery, chemotherapy, and radiotherapy, are still limited due to the following: (i) lack of selectivity, (ii) multidrug resistance, and (iii) severe toxic side effects (Chowdhury et al. 2016).

In this context, extensive research has been attracted in the development of complex site-specific therapies based on nanotechnology (Liu et al. 2016; Lei et al. 2017; Zhang et al. 2016). Several advantages of nanosystems that can be exploited in cancer management are mentioned below (Zhen et al. 2014):

1. Due to their small size, nanosystems are able to escape renal clearance, easily permeate through the leaky blood vessels of tumor tissues, and accumulate inside the tumoral cells.
2. Their high surface area increases their loading capacity for both therapeutic and imaging agents.
3. They are able to be functionalized with targeting agents in order to selectively accumulate in the cancer cells.
4. They are composed of biocompatible materials, and their biodegradation products are nontoxic.
5. They can be designed for personalized medicine, as the drug therapeutic efficacy can be easily monitored by combining both therapeutic and imaging elements in a single structure.

Recently, a promising site-directed application in the field of nanotechnology relies on a new concept, known as *theranostic*, which involves simultaneous procedure of therapeutic and diagnostic approaches for personalized medicine (Schleich et al. 2013). There are several definitions of *theranostics*, also spelled as *theragnostics*, in some reports. They have been defined as a smart integrated system that assures therapy, diagnosis, and monitoring of the treatment response through imaging techniques (Warner 2004). Therefore, they can provide diagnosis followed by therapy and treatment followed by diagnosis or codeliver imaging and therapy components (Chen and Wong 2014). The ideal theranostic approach is capable of several functions ranging from diagnosis to treatment with accurate targeting of cancer-specific cells. The newest generation of theranostic systems offers opportunities to combine passive and active targeting, environmentally responsive drug release, molecular imaging, and other therapeutic functions into a single biomedical platform.
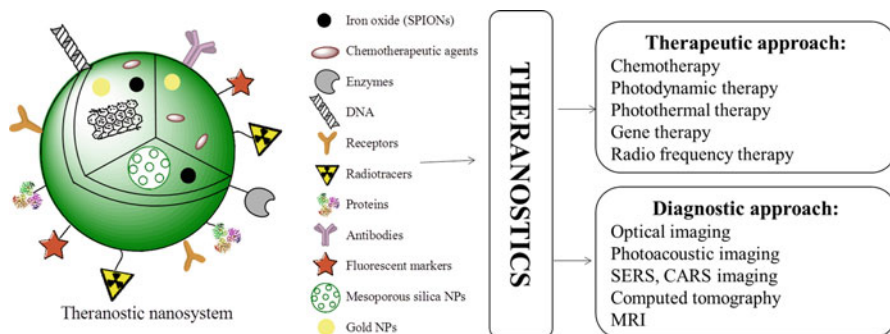
Fig. 12.1 Schematic diagram of the main components for theranostic nanosystems

In particular, a theranostic nanosystem is able to deliver an anticancer agent with triggered or controlled release, coupled to tumor-specific targeting of the microenvironment, while simultaneously allows sensitive tumor imaging with high diagnostic accuracy (Kumar and Srivastava 2015; Jeelani et al. 2014; Zhao et al. 2016). The main components of theranostic nanosystems include the therapeutic agent (payload), payload carrier, targeting ligands, and signal emitter (imaging agent). A schematic representation of the main components for theranostic nanosystems is depicted in Fig. 12.1. The therapeutic agent may be represented by anticancer drugs, nucleic acids, therapeutic proteins, or peptides; meanwhile the signal emitter consists of inorganic nanoparticles, such as gold nanoparticles (AuNPs), carbon nanotubes (CNTs), silica nanotubes, quantum dots (QDs), and superparamagnetic iron oxide nanoparticles (SPIONs). These inorganic nanoparticles exhibit unique optical, electrochemical, and magnetic characteristics. In most cases, the payload carriers are expressed as organic nanosystems, including here polymeric micelles and nanoparticles, liposomes, or dendrimers comprised of biocompatible materials with multifunctional groups such as –OH, –COOH, –NH$_2$, and –SH. Both therapeutic and imaging agent can be loaded in the theranostic nanosystem structure or attached to its surface by covalent or electrostatic bonds. The targeting moieties could be represented by proteins (mainly antibodies and their fragments), nucleic acids (aptamers), or other receptor ligands (peptides, vitamins).

Some of the tumor imaging modalities are represented by magnetic resonance imaging (MRI), positron emission tomography (PET), single-photon emission computed tomography (SPECT), fluorescent imaging through fluorescent agents, ultrasound imaging by nano-/microbubbles, etc.; meanwhile therapeutic approach can be accomplished by chemotherapy, photodynamic and photothermal therapy, and gene or radio-frequency therapy.

12.1.2 Materials Used in Theranostic Applications

12.1.2.1 Iron Oxide Nanoparticles

As a family of high-performance biomedical materials, theranostic nanosystems based on functionalized superparamagnetic iron oxide nanoparticles (SPIONs) could represent the best compromise between excellent magnetic properties and reduced toxicity, evidenced by extensive in vitro and in vivo tests (Mahmoudi et al. 2012) and by quantitative evaluation of biodistribution and local therapeutic effects (Dürr et al. 2013; Tietze et al. 2013). Current studies have been dedicated to the development of functionalized SPIONs for theranostic approaches, and encouraging results have been reported (Dadras et al. 2017; Shen et al. 2013). An important motivation for the choice of theranostic nanosystems in cancer therapy is represented by the patient safety and compliance (Ling et al. 2011).

Indeed, magnetic functionality can be exploited to render the functionalized SPIONs as theranostic nanosystems, particularly as a diagnostic tool and a targeting drug carrier in cancer therapy. If the diagnostic approach is accomplished by magnetic resonance imaging (MRI), the therapeutic path can be achieved by using one of the following techniques described below or by mixing the advantages of all three. First, by using magnetic-field-mediated guidance, it is possible to increase the site specificity and selectivity of the therapy, reduce side effects, and ensure chemotherapy cost-effectiveness (Tietze et al. 2015). Second, by conjugating a targeting molecule, the therapeutic efficacy can be enhanced by site-specific accumulation (Holohan et al. 2013). Third, functionalized SPIONs can be designed to incorporate both hydrophilic and hydrophobic chemotherapeutic agents (doxorubicin, docetaxel, paclitaxel, curcumin, etc.) and deliver them to the tumor cells (Jhaveri et al. 2014).

A strategy to develop a theranostic nanosystem capable of tumor cell-targeting, imaging, and drug delivery was proposed by Sahu et al. (2012). This nanosystem is composed of a magnetic material (magnetite) coated by a polymeric layer represented by N-carboxymethyl chitosan (N-CMC), functionalized with a fluorescent dye and a targeting ligand (folic acid) and loaded with doxorubicin. This targeted delivery system may also be used for tumor cell detection and labeling applications in vivo (Sahu et al. 2012). Likewise, Fan et al. (2011) functionalized magnetic composites based on iron oxide and O-carboxymethyl chitosan (O-CMC) with folic acid in order to improve their biocompatibility and ability to target specific tumor cells. MR imaging and tumor histological analysis demonstrated that this system could target folate receptor-positive tumor cells in vivo (Fan et al. 2011). Si et al. (2010) improved the antitumor effect of genistein using a biocompatible superparamagnetic drug delivery system, based on covalently attached genistein onto cross-linked carboxymethylated chitosan–iron oxide nanoparticles. Their results indicated that this system could be promising for future multifunctional chemotherapeutic applications (Si et al. 2010).

12.1.2.2 Gold Nanoparticles (AuNPs)

Gold nanoparticles (AuNPs) represent an attractive opportunity for developing drug delivery systems with tunable features, due to their size, unique physical properties

combined with chemical inertness, stability, biocompatibility, optical properties in the near-infrared (NIR) region (Xia et al. 2011a, b), and ability to be functionalized with desired targeting ligands, specific antibodies, or drugs (Huang et al. 2013). The most common applications of AuNPs in cancer therapy were photothermal therapy (PTT) and radiation therapy. AuNPs coated with chitosan and polyacrylic acid and loaded with cisplatin were effective as theranostic tool able to combine near-IR photothermal therapy and chemotherapy (Chen et al. 2013a). Moreover, these nanosystems proved an enhanced antitumor effect of cisplatin if photodynamic therapy was included as an additional option (Chen et al. 2013b).

12.1.2.3 Quantum Dots (QDs)

Quantum dots (QDs) are semiconductor nanomaterials with fluorescent, optical, and electronic properties. They exhibit several advantages over conventional organic dyes for both in vitro and in vivo imaging (Sabaeian and Nasab 2012), such as broad excitation spectra, excellent photochemical stability, and narrow, symmetric, and tunable emission spectra as a result of quantum confinement effect. The most commonly used QDs include cadmium-based QDs, carbon QDs (CQDs), and graphene QDs (GQDs). Conventional QDs have a multilayer structure comprised of a metallic core cadmium and its derivatives, cadmium selenide (CdSe) and cadmium sulfide (CdS), and a coating material that prevents the core leaking or photobleaching (Kulkarni et al. 2019). Novel types of QDs particles based on cadmium telluride (CdTe) have been studied and proved to be more stable and efficacious than conventional QDs (Kim et al. 2015a, b). Narayanan et al. (2017) proposed a synergistic therapy of manganese-doped zinc sulfide (ZnS) QDs conjugated with chitosan nanoparticles and cisplatin and proved their effectiveness by in vitro cytotoxicity tests on HeLa cells. Hua et al. (2017) developed QDs functionalized with mercaptosuccinic acid (MSA), ethylenediamine, and chitosan in order to be used as a versatile tool in mitochondrial-targeted photodynamic therapy (PDT).

12.1.2.4 Carbon Nanotubes (CNTs)

CNTs are tube-shaped materials that can be divided into single-walled CNTs (SWNTs, 0.4–2.0 nm in diameters, 20–1000 nm in lengths) and multi-walled CNTs (MWNTs, 1.4–100 nm in diameters, ≥1 μm in lengths). SWNTs exhibit attractive optical properties that can be exploited for biological imaging (Welsher et al. 2008) and can be functionalized by covalent binding, adsorption, and electrostatic interactions in order to develop drug delivery carriers with high biocompatibility and enhanced water solubility. A theranostic nanosystem based on SWCNTs functionalized with fluorescent chitosan and folic acid has been tested by Wu et al. The nanosystem showed water dispersibility, excellent cytotoxicity (60–74%) on HeLa cell lines when exposed to 635 nm laser irradiation, and selective folate receptor-mediated endocytosis (Wu and Zhao 2016). Qi et al. (2015) developed a theranostic nanoformulation using oxidized CNTs wrapped with glycosylated

chitosan and loaded with DOX for hepatic tumors and demonstrated by in vivo studies that the nanosystem showed higher drug accumulation in the tumor as compared to DOX.

12.1.2.5 Mesoporous Silica Nanoparticles (MSNs)

MSNs exhibit unique properties such as stability and resistance to degradations induced by pH, heat, and mechanical stress, uniform and tunable pore size, high surface area and large pore volume allowing for high drug loading, and internal and external functional surfaces available for selective modification (Kumar et al. 2018). Highly charged polymers like chitosan, polyacrylate, and polyaspartate have been frequently employed as capping agents in mesoporous silica nanoparticles in order to achieve pH responsiveness (Shah and Rajput 2018). For instance, chitosan-capped MSNs were used to deliver curcumin in a pH-dependent manner to glioblastoma cells (Ahmadi Nasab et al. 2018); meanwhile pH-responsive alginate/chitosan multilayer-modified magnetic mesoporous silica nanocomposites were designed for gene therapy and cancer treatment (Yang et al. 2017). A photosensitizer chlorin e6 (Ce6) and antitumor drug doxorubicin (Dox) were adsorbed onto the MSNs and successfully achieved reversing multidrug resistance in cancer (Yang et al. 2017).

Using another strategy, Samykutty et al. (2018) developed an acidic pH targeted wormhole mesoporous silica nanoparticle (V7-RUBY) to serve as a novel tumor-specific theranostic nanosystem detectable using multispectral optoacoustic tomographic (MSOT) imaging. These nanoparticles presented both large loading capacity for chemotherapeutic agents (paclitaxel or carboplatin) and favorable release kinetics combined with tumor-specific targeting and gatekeeping. The IR780 imaging dye is carried as cargo, and chitosan controls the release of the dye to orthotopic ovarian tumors. The size and shape of the biocompatible V7-RUBY have essential features including the ability to translocate into the cytoplasmic compartment.

12.1.2.6 Lipid-Based Nanoparticle Platform: Liposomes, Solid Lipid Nanoparticles (SLNs), and Nanostructured Lipid Carriers (NLC)

Liposomes are small spherical-shaped vesicles with bilayer membrane structures composed of phospholipids able to encapsulate either hydrophilic or hydrophobic agents. The most commonly used phospholipids in the preparation of liposomes are polyethylene glycol and phosphatidylcholines (Elgqvist 2017). Noteworthy to mention that a PEGylated liposome formulation of doxorubicin (DOX), namely, Doxil®/ Caelyx, was the first formulation approved for clinical use (Barenholz 2012). Liposomes can be designed to respond to changes in light (Leung and Romanowski 2012), temperature (Park et al. 2013), or acid (Mamasheva et al. 2011). Recently multifunctional and theranostic liposomes have been highlighted in the literature (Charron et al. 2015; Haeri et al. 2016; Perche and Torchilin 2013). However, there are some drawbacks such as rapid clearance from the bloodstream, instability of the carrier, high production cost, and fast oxidation of some phospholipids.

As an alternative, SLN that contains a matrix composed of solid lipid nanoparticle and modified SLN – nanostructured lipid carriers (NLC) – that contains a matrix composed of liquid lipid and solid lipid have been proposed. SLNs exhibit several

advantages including stable formulations, excellent reproducibility, and drug protection from degradation; meanwhile NLC assures enhanced drug loading and less drug expulsion during storage (Iqbal et al. 2012). Both SLN and NLC have been successfully functionalized with targeting agents and achieve efficient drug release in a controlled manner.

Salva et al. (2015) developed chitosan-coated liposomes for the co-delivery of siHIF1-a and siVEGF. The expression level of VEGF mRNA was markedly suppressed in breast tumoral cells (MCF-7 and MDA-MB-435 cells) transfected with the chitosan-coated liposomes designed, and the co-delivery greatly enhanced in vitro gene-silencing efficiency. In addition, chitosan-coated liposomes showed 96% cell viability.

12.1.2.7 Dendrimers

Dendrimers are nano-sized materials (1–15 nm), with regularly and highly branched three-dimensional architecture, monodisperse structure, high water solubility, and high payload. One of the most studied dendrimers was polyamidoamine (PAMAM) dendrimer. Recently, PAMAM dendrimers and chitosan-based nanohybrids were developed, and a significant reduction in the hemolytic toxicity of dendrimers after inclusion into chitosan was noted (Zhou et al. 2015). Zhang et al. (2015) prepared a controllable aptamer-based self-assembled DNA with significant stability, biocompatibility, high drug payload, and intracellular drug delivery efficacy for theranostic applications in cancer. It is important to mention that some dendrimer-based nanohybrids have shown promising outcomes in preclinical studies (Kesharwani et al. 2015; Nounou et al. 2015).

12.2 Chitosan: A Programmable Polymer for Theranostic Applications

12.2.1 Advantages of Chitosan

Chitosan has several advantages in biomedical applications, such as biocompatibility and controlled biodegradability. Due to its antimicrobial, bioadhesive, and bioresorbable nature, chitosan has been tested in medical fields, such as wound dressing, tissue engineering and regenerative medicine, controlled drug delivery, and antimicrobial agent. Its functionality and ability to form complexes with iron oxides and other inorganics and the processability in nanoparticles and nanocapsules made these materials very useful in theranostics, hyperthermia, and biosensing or cancer therapy (Anitha et al. 2014; Vunain et al. 2017). Chitosan has remarkable healing activity, and as all polysaccharides, it is a great moisturizing agent due to its water-retention capacity. It is a cholesterol-lowering product and can inhibit the development of a number of parasites and germs (*E. coli*, *Pseudomonas*, *Candida albicans*) (Gallaher 2003).

Compatibility of chitosan with physiological medium depends on the preparation method (because the residual proteins generate allergic reactions) and on the deacetylation degree – biocompatibility increases at higher deacetylation degree.

Chitosan actually proved to be more cytocompatible in vitro than chitin because the number of positive charges increases and the interaction between cells and chitosan increases as well, which improve biocompatibility (Croisier and Jérôme 2013). Chitosan with a high degree of deacetylation exhibits a high level of biocompatibility with the human biological environment. Thus, polymers with acetylation rates of 4 to 15% were tested, and the inflammatory response of the body after the implantation of the material was monitored. For chitosan with a degree of acetylation of 4–8%, only reduced inflammation was observed which did not cause biocompatibility problems. In contrast, for chitosan with a 15% acetyl degree, a collagen capsule around the implant was observed and infiltration of the implant matrix with neutrophils associated with the inflammatory response. All of that confirmed the significance of degree of acetylation prior to chitosan selection for use in tissue engineering (Barbosa et al. 2010).

Chitosan exhibits a high adhesion to the epithelial and mucosal tissues covering the tissues. Clinical trials on chitosan-containing materials have not reported inflammatory or allergic reactions in the case of implantation, injection, topical application, or ingestion by the human body. In vivo and in vitro cytotoxicity tests revealed the compatibility of chitosan films in contact with keratinocytes and fibroblasts, as well as the insignificant influence of acetylation. Chitosan-based materials in contact with skin lesions adhere to fibroblasts and promote the proliferation of keratinocytes and therefore tissue regeneration (Dai et al. 2011).

The mucoadhesion of chitosan is due to its positive charge which interacts with negatively charged residues in the mucin, the glycoprotein that composes the various mucous. Mucoadhesion is directly related to the positive charge density of chitosan and therefore with deacetylation degree. If the deacetylation degree of chitosan increases, the number of positive charges also increases, and mucoadhesion is improved (Sogias et al. 2018).

The hemostatic activity of chitosan is explained also by the presence of positive charges on chitosan backbone and their interactions with negatively charged membranes of the red blood cells. Due to its positive charges, chitosan can also interact with the negative part of cells' membrane and contributes to the reorganization and opening of the tight junction proteins, explaining the permeation enhancing property of this polysaccharide (Ibrahim and El-Zairy 2015).

The antimicrobial activity of chitosan combines two types of interactions of the positive charges:

(a) The interactions with negatively charged groups at the surface of cells, and as a consequence, the membrane cells' permeability are modified.
(b) Interactions that involve the binding of chitosan with the cell DNA (through protonated amino groups), which leads to the inhibition of the microbial RNA synthesis.

The polycationic nature of chitosan also explains the chitosan analgesic effects: The amino groups can protonate in the presence of protons that are released in the inflammatory area, resulting in an analgesic effect (Cheung et al. 2015).

The chitosan biodegradability is based on its polysaccharidic nature, which consequently contains breakable glycosidic bonds. Chitosan is actually degraded in vivo by several proteases and mainly lysozyme (Muxika et al. 2017). By degradation nontoxic oligosaccharides of variable length are formed which are incorporated in the metabolic pathways and finally excreted. The degradation rate of chitosan is mainly related to its degree of deacetylation but also to the distribution of *N*-acetyl-D-glucosamine residues and the molecular mass of chitosan (Kumar et al. 2004).

Considering all properties of chitosan, it was and will be tested in many biomedical and pharmaceutical applications, such as drug delivery and 3D scaffolds for tissue engineering or 2D scaffolds, especially for wound-healing purposes, and micro- and nanoparticles for diagnostics, cancer therapy, and bone implants (Bružauskaitė et al. 2015). Within the framework of the chitosan biocompatibility, it was notably approved by the Food and Drug Administration (FDA) for use in wound dressings (Gianino et al. 2018).

12.2.2 Chitosan Structure and Properties

12.2.2.1 Chitosan Structure

Chitin is one of the most abundant biopolymer and has attractive functional properties such as bioactivity, biocompatibility, biodegradability, and very good mechanical strength. Despite of these important features, it has limited biological applications due to its poor solubility (Saikia et al. 2015; Pillai and Sharma 2009). The solubility is reachable by chitin conversion into CS through chemical or enzymatic processes. Chemical deacetylation involves the chitin reaction with hydroxides at high temperatures, and a wide range CSs can be obtained when the deacetylation degree of CS reaches about 50%. In acidic environment, the amino groups are protonated, and the polymer becomes cationic, allowing it to interact with diverse types of biological substrates (Martinou et al. 1995). Basically, CS is a random copolymer formed by D-glucosamine and *N*-acetyl-D-glucosamine units, linked by 1,4 glycosidic linkages (Fig. 12.2).

Chitosan is a semicrystalline polymer and shows polymorphism depending on its physical condition: A crystallized state the orthorhombic system, like chitin, and two types of chitosan (with a high degree of deacetylation and in the form of free amine
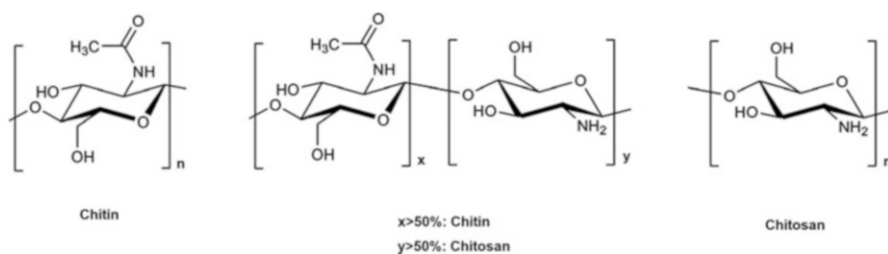

Fig. 12.2 Structure of chitin and chitosan

and chitosan in salt form, with weak degree of deacetylation which is more disordered) can be distinguished. The crystallinity may vary considerably with the origin of the biopolymer and extraction procedure. It is maximum for both 0% deacetylated chitin and fully deacetylated chitosan (100% deacetylated) (Crini et al. 2009).

12.2.2.2 Deacetylation/Acetylation Degree of Chitosan and Molecular Weight

The deacetylation of chitin involves the elimination of acetyl groups from the molecular chain, leaving behind a complex with a high degree of chemical reactive groups (NH_2). Chitosan is a linear polysaccharide, composed of glucosamine and *N*-acetyl glucosamine units linked by 1,4 glycosidic bonds; the content of glucosamine is called the degree of deacetylation (DD).

$$\text{DD is defined as the ratio}: \frac{GlcN}{GlcNAc + GlcN} \times 100,$$

where *GlcNAc* and *GlcN* are the number of *N*-acetyl-D-glucosamine and D-glucosamine units, respectively (Czechowska-Biskup et al. 2012; Correia et al. 2017). DD and the distribution of the acetyl groups along the main chain have a significant effect on the solubility of chitosan and the conformation and flexibility of macromolecular chains, which are important parameters for the use of this biopolymer in biomedical applications. Chitosan has a degree of deacetylation up to 95%, and for a complete deacetylation, repeated alkaline treatments are necessary (Hirai et al. 1991). Some authors reported that the acetylation degree (AD) of chitosan, corresponding to the proportion of *N*-acetyl-D-glucosamine units relative to the total number of glycosidic units, is a structural parameter that influences overall charge, reactivity, and biological properties (Hamdi et al. 2019).

Chitosan can have very high molecular weights (up to 3.000.000 Da), when the polymer was extracted with controlled parameters, but in general they are weaker, between 10.000 Da and 1.500.000 Da. Molecular weight influences the polymer solubility, solution viscosity, and polymer processability. The physical properties of chitosan are dependent on a number of parameters, such as the molecular weight, DD, sequence of the amino and the acetamido groups, and the biopolymer purity (Kaur and Dhillon 2014).

12.2.2.3 Chitosan Solubility

Chitin is insoluble in most organic solvents, but chitosan is soluble in dilute acidic solutions (generally, at pH <6.0). At acidic pH, the amines are protonated and become positively charged; chitosan becomes a water-soluble cationic polyelectrolyte. The primary amino groups of chitosan have a pKa value of 6.3 and make the biopolymer a strong base. At pH above 6.3, the amine group of chitosan is again deprotonated, and the polymer loses the charges and becomes insoluble (Zivanovic et al. 2014).

Chitosan is able to form quaternary nitrogen salts at low pH values. Therefore, organic acids such as acetic, formic, and lactic acids can dissolve CS. The most

frequently used solvent is acetic acid (1–2% in water) at about pH 3.5–4.0. Chitosan is also soluble in 1% hydrochloric acid and dilute nitric acid but insoluble in sulfuric and phosphoric acids. Concentrated acetic acid solutions, especially at high temperature, cause depolymerization of chitosan (Qin et al. 2006).

Due to the presence of hydroxyl and amino groups on its backbone, chitosan can be easily modified through various methods in the aim to improve its physicochemical properties or to extend its applications. Various chemical approaches have been tested for modifying chitosan, including quaternization, N-alkylation, hydroxylalkylation, carboxyalkylation, thiolation, and glycation (Mourya et al. 2010). Physical modifications imply the use of electromagnetic radiation and sonication and enhance properties, such as solubility in aqueous solutions, and modulate surface charges and absorption efficiency.

12.2.2.4 Chitosan Properties

As a biological origin-based polymer, chitosan is a promising biomaterial due to its availability, solubility, modulation of properties, non-toxicity, biocompatibility, and biodegradability. There is a great increasing demand for chitosan for several biological and medical applications. In the last decade, various chitosans and their derivatives have been extensively explored in new applications as antioxidant and free radical scavenging entity (Anraku et al. 2018). Chitosan properties are strongly connected with molecular mass and deacetylation degree (Fig. 12.3).

Degradation of Chitosan

Various enzymes are involved in chitosan degradation, including chitosanases, chitodextrinases, chitotriosidases, chitobiosidases, and chitobiases. Chitosanase converts chitosan in products with lower molecular weight (oligosaccharides) by hydrolysis of glycosidic link β (1-4) of the saccharide units; the oligosaccharides are not harmful and are, often, further degraded by β-glucosaminidases before removing from the body (Guarino et al. 2015). Chitosan has been shown to degrade in vivo, and the degradability by lysozyme is one of the most important properties of chitosan especially for medical and pharmaceutical applications (Szymańska and Winnicka 2015). Enzymatic hydrolysis of chitosan leads to N-acetyl-D-glucosamine and D-glucosamine. Glucosamine plays an important physiological role in biochemical processes that occur in vivo and participates in detoxification functions in the
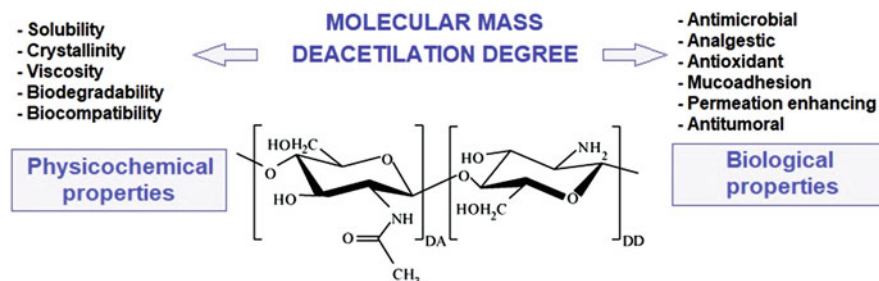

Fig. 12.3 Chitosan properties

liver and kidneys; it present anti-inflammatory properties, hepatoprotective, antireactive and hypoxic effect (Azuma et al. 2015).

Many studies have shown that chitosan is a biodegradable polymer and that it degraded in vivo mainly by lysozyme, which is ubiquitous in the human body (4–13 mg/l in serum and 450–1230 mg/l in tears); however, the biodegradation is dependent on some factors, including pH, type of chitin or chitosan, and chitosan preparation method (Dutta et al. 2011). In general, both rate and extent of chitosan biodegradability in living organisms are dependent on the degree of deacetylation (DD) (Younes and Rinaudo 2015).

It is likely that distribution, degradation, and elimination processes are strongly dependent on molecular weight (MW). Possible sites of degradation, inferred due to the localization of chitosan, are estimated to be located in the liver and kidney. Chitosan has also been administered on tegument. Skin substitutes based on chitosan/collagen were relatively stable over time compared to collagen alone when implanted in rabbits (Ma et al. 2003). After oral administration, chitosan should present some degradation in the gastrointestinal tract. The digestion of chitosan, occurring predominantly in the gut, was found to be species dependent with hens and broilers being more efficient digesters (67–98% degradation after oral ingestion) than rabbits (39–83% degradation). In the same study, the digestion of N-stearoyl chitosan was negligible, indicating that the enzymatic degradation is dependent on chitosan's NH_2 availability. Chitosan degradation rate can be affected by the polymer's MW and degree of acetylation. In these conditions, N-substitution may affect enzymatic degradation, and it should be considered when new derivatives are suggested in formulations for systemic administration (Biswas et al. 2014).

Antimicrobial Properties

Biomass is a naturally abundant source of sustainable biopolymers, and in the last years, increasing environmental awareness has led to growing interest in the development of green compounds with improved performance. Chitosan is a cationic polysaccharide exhibiting both film-forming properties and bioactivity. Due to the quaternization of the amino group, chitosan is known for its natural antifungal and antibacterial activity and also shows abilities to retain included antimicrobial substances. The basic mechanism for its antimicrobial activity is based on the interactions between the positively charged amine groups and negatively charged cell membrane, causing disruption and release of intracellular compounds (No et al. 2002; Vaz et al. 2014). Additionally, two other mechanisms have been also identified as synergists:

1. Chelation of metals by chitosan functional groups, thus inhibiting bacterial enzyme activity.
2. In the case of yeast cells, chitosan segments are able to penetrate the cell membrane and inhibit the RNA synthesis (Kong et al. 2010).

The antimicrobial activity of chitosan against *L. monocytogenes* and yeast *C. albicans* was demonstrated. Chitosan with higher DD (92%) exhibited more remarkable antibacterial property against *S. aureus* (Zhuang et al. 2019). The ability of these compounds to kill or inhibit a wide range of microorganisms makes possible to be used for a number of applications, from hospital surfaces and medical devices to building materials and filtration devices. The biomedical application possibilities for chitin and chitosan into several broad areas of tissue engineering, bone substitutes, and wound dressings have been detailed by Anitha et al. (2014). Laurencin et al. studied biologically active chitosan matrices for tissue engineering application and reported that antimicrobial chitosan matrices could be an alternative to antimicrobial agents because they remove the biofilm problems, like microbe resistance to treatment, the invasion into immune system, and building of bacteria reservoirs which can seed infections elsewhere (Laurencin et al. 2008).

Mucoadhesive Properties of Chitosan

The excellent mucoadhesive properties of chitosan have often been linked to its ability to interact with negatively charged mucins by electrostatic attraction (Hejjaji et al. 2018). However, some authors have highlighted the complexity of the mucoadhesive interactions of chitosan and have suggested that hydrogen bonds and hydrophobic effects may also have certain functions (Nikogeorgos et al. 2015). Mucins are a family of complex high molecular weight glycoproteins secreted by the epithelial tissues (intestinal, respiratory, urogenital tracts). They consist of linear or branched oligosaccharides attached to the protein backbone. These glycoproteins are mostly carbohydrate (five sugars *N*-acetyl glucosamine, *N*-acetylgalactosamine, galactose, and fucose and sialic acid (*N*-acetylneuraminic), and traces of mannose and sulfate esters (Varki et al. 2009). The high concentration of sialic acid and sulfate ester leads to a negative charge on mucin which is the main reason for its mucoadhesive properties. Depending on the physiological conditions and physiochemical properties such as pH, the carboxylate group of sialic acid residues on mucin can interact with the positive charges (protonated amino group $-NH_3^+$) on the chitosan through electrostatic and hydrogen bonds (Leal et al. 2017). The pH of the medium as well as the presence of other chemicals can change the relative contributions of each type of physical interaction. Hydrophobic and hydrophilic interactions are also very important (Szymańska and Winnicka 2015). The presence of active functional groups (such as amines and hydroxyls) in chitosan opens broad opportunities for its chemical derivatization. There are a number of well-known chitosan derivatives; some of these are even commercially available. The most important chitosan derivatives relevant for pharmaceutical applications include trimethyl chitosan, glycol chitosan, carboxymethyl chitosan, and semi-acetylated chitosan. Numerous papers have been published on the use of chitosan-based materials for the preparation of transmucosal medications (Ways et al. 2018; Tzaneva et al. 2017).

Antioxidant Activity of Chitosan

In the last decade, extensive researches have been focused on the antioxidant and free radical scavenging abilities of chitosan and its derivatives and in vitro but also in vivo using animal models, and clinical trial tests strongly suggest that chitosan and

its derivatives will find new applications as natural biological defenses against the consequences of oxidative stress (Anraku et al. 2017; Anraku et al. 2018). Chang et al. studied the influence of chitosan molecular mass on antioxidant and antimutagenic properties. The authors used seven chitosan samples with MWs ranging from 2.2 to 300.0 kDa and demonstrated that the antioxidant and antimutagenic properties of chitosans were inversely related to chitosan MW. Chitosan generally exhibited higher antioxidant property when scavenging H_2O_2 and DPPH (2, 2-diphenyl-1-picrylhydrazyl radical, superoxide anion) free radical than when chelating Fe^{2+} (Chang et al. 2018).

Some derivatives of chitosan were tested as materials with improved antioxidant properties. Tan et al. prepared cationic chitosan derivative with 1,2,3-triazole and *N*, *N*,*N*-trimethyl moieties using cuprous-catalyzed azide–alkyne click chemistry approach and tested the antifungal and antimicrobial properties. The chitosan derivative bearing 1,2,3-triazole exhibited enhanced antifungal activity against four kinds of plant-threatening fungal strains and strong scavenging effect against superoxide radical as well as improved reducing power. The authors consider that the behaviors of the cationic chitosan derivative with 1,2,3-triazole are due to the introduction of quaternary ammonium and 1,2,3-triazole moieties and consider the new combination a very promising material for biomedical applications (Tan et al. 2018).

Chitosan could significantly reduce serum free fatty acid and malondialdehyde (MDA) concentrations and increase antioxidant enzymes' activities such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px), indicating that this biopolymer regulated the antioxidant enzymes' activities and decreased lipid peroxidation. Chitosan and its derivatives have been indicated as dietary food additives and functional factors for their antimicrobial, hypocholesterolemic, and immune-stimulating effects (Xia et al. 2011a, b). Antioxidant activity of chitin, chitosan, and their derivatives can be attributed to in vitro and in vivo free radical scavenging activities, and therefore, these polymers may be used as functional ingredients in food formulations to promote consumer health and to improve the shelf life of food products (Ngo and Kim 2014).

Concerning the disadvantages of using chitosan-based material for biomedical applications, the variability and heterogeneity of the polymer is of great importance. Indeed, the difficulty of controlling the distribution of the acetyl groups along the backbone makes it difficult to get reproducible initial polymers (Bellich et al. 2016). There is a need for a better standardization of production processes in order to prepare reproducible initial polymers with the same characteristics; changes in the specifications of the polymer may significantly change its performances.

12.2.3 Chitosan Modification for Theranostics

Taken into consideration its biocompatibility and vast derivative potential, chitosan has proven to be an excellent choice for the design of theranostic systems. In order to accomplish its purpose as an efficient vehicle for both therapeutic and imaging

agents, chitosan must undergo a series of modifications. Depending on the final purpose, chitosan can be derived using specific molecules to enhance its properties. In Fig. 12.4 a few chemical modifications of chitosan are presented, and the remarkable properties of obtained derivatives are highlighted.

For instance, hydrophilic molecules such as glycol or PEG (polyethylene glycol) are being used as a means to enhance the water solubility of chitosan in basic or neutral conditions. As a result, PEGylated chitosan nanoparticles possess improved hemocompatibility and higher cellular uptake and are excellent vehicles for hydrophobic agents such as ibuprofen or paclitaxel. The enhanced properties of the glycol-modified chitosan nanoparticles have major implications in the passive targeting of tumors via the EPR (enhanced permeability and retention) effect, ensuring a longer circulation time and a higher accumulation of the nanocarriers at the tumor site (Zhang et al. 2002).

Choi et al. (2018) have developed a series of echogenic nanoparticles based on glycol chitosan for the dual imaging of cancer. The team has chemically conjugated iodine-contained diatrizoic acid to the glycol chitosan backbone for CT imaging purposes, followed by the encapsulation of perfluoropentane, a US imaging agent, through the oil-in-water (O/W) emulsion method. In addition to the comprehensive diagnosis provided by the CT/US dual-modal imaging, the study has shown an accurate and rather fast accumulation of the nanocarriers at tumor level, which can be assessed as a result of the glycol-modified chitosan and its properties. Furthermore, as seen in a study conducted by Min et al. (2015), echogenic glycol chitosan nanoparticles can be engineered not only for diagnostic purposes but also for the treatment of cancer. The study focuses on the development of glycol chitosan nanoparticles that can simultaneously be employed as a vector for cancer-targeted ultrasound imaging and ultrasound-triggered drug release. The nanoparticles were synthesized by O/W emulsion method and encapsulate both perfluoropentane and a hydrophobic anticancer drug (docetaxel).

It is essential for theranostic nanosystems that are not characterized by active targeting to exhibit proficient tumor-homing abilities and reduced nonspecific uptake by other tissues. Therefore, to assure a precise treatment and monitoring of the disease, the nanoparticles should have to benefit from an efficient EPR effect. The EPR effect is strictly dependent on their characteristics such as particles' size, morphology, and chemical interactions with different biological media. As proven in many studies, glycol and PEG modifications of chitosan increased the nanoparticles' abilities to delivering therapeutic or imaging agents to specific cancer sites via EPR (Prabaharan 2015).

In order to induce the formation of nanoparticles through self-assembly, hydrophobic molecules such as palmitoyl or caproyl can be added to the chitosan backbone through the N-acylation of the NH_2 group. These modifications resulted in improved polymeric network stability and increased the ability to encapsulate hydrophobic agents inside the nanoparticle core. In addition, the N-acylation of chitosan entails a rather simple process, making it both a time- and resource-efficient method (Fathi et al. 2018).
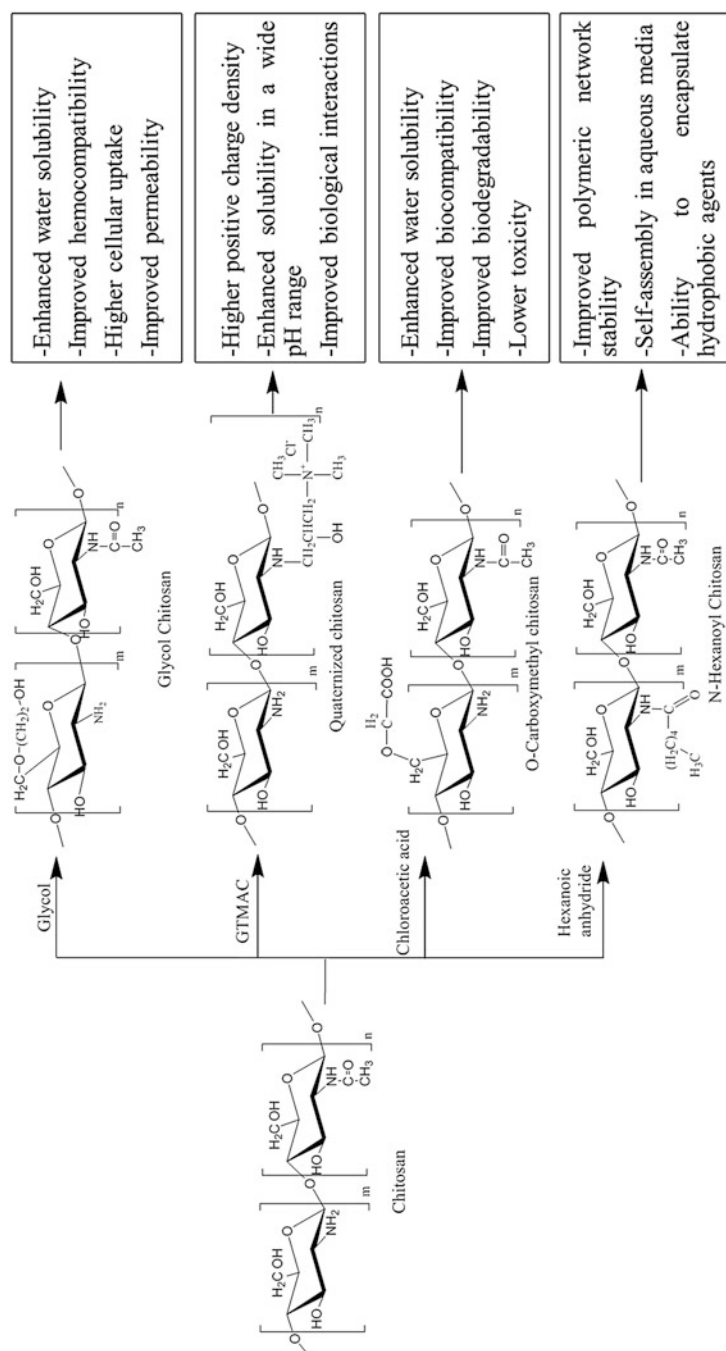
Fig. 12.4 Chemical modifications of chitosan for theranostic applications

Hsiao et al. (2015) proposed a nanosystem that combines oleic acid-stabilized iron oxide nanoparticles (OA-IONP) with a functionalized polysaccharide – an amphiphilic copolymer of hexanoyl-chitosan-PEG (CP6C) – and encapsulates paclitaxel (PTX) in its hydrophobic region. Chlorotoxin was then conjugated onto the nanocarrier surface to act as a targeting peptide which binds to a certain molecule (viz., MMP-2) that is overexpressed in the majority of brain tumors. The inner core of the nanocarrier comprising of OA-IONP is bound to the outer shell through hydrophobic interactions. These particles have excellent superparamagnetic properties and can be tracked via MR imaging, thus enabling real-time observation of the ongoing treatment.

The selection of the material used in the development of a drug carrier is of critical importance. The selected polymer must possess specific characteristics such as the ability to protect its cargo from enzymatic or hydrolytic degradation and to enhance its interactions and permeability across biological barriers. Native chitosan is a polycationic saccharide, which makes it soluble in various acidic media, but its positive charges are limited to the number of conjugations and ionic complexations it undergoes, and so are its interactions and stability. Quaternized chitosan, instead, exhibits a higher density of positive charges and enhanced solubility in a wider pH range, making possible better interactions at a biological level and also to effectively permeate gastrointestinal junctions (Desai 2016).

Nanocarriers based on quaternized chitosan (*N*-trimethyl chitosan, *N*-diethylmethyl chitosan, *O*-(2-hydroxyl) propyl-3-trimethyl ammonium chitosan chloride/O-HTCC, *N*-(2-hydroxyl) propyl-3-trimethyl ammonium chitosan chloride/HTCC, etc.) have been previously designed. For instance, Soares et al. (2016) developed O-HTCC iron oxide nanoparticles. The team of researchers synthesized O-HTCC-coated Fe_3O_4 nanoparticles loaded with anticancer drug DOX through ionotropic gelation method. Taking into consideration the behavior of chitosan compared to its quaternized counterpart at different pH values, the current study has concluded that drug release profile is influenced, with O-HTCC-Fe_3O_4 nanoparticles exhibiting an increase in the amount of DOX released compared to CS-Fe_3O_4 at a pH value of 4.5. Also, it has been observed that the presence of Fe_3O_4 fastens the release.

Another study conducted by Huang et al. (2019) explored the synthesis of CuS nanoparticles as a photothermal agent stabilized using quaternized chitosan. CuS is responsive to near-infrared (NIR) light and is able to convert photon energy to thermal energy, which results in the destruction of the cellular medium. Also, Cu ions, when activated by an appropriate light wavelength, can generate reactive oxygen species (ROS) through redox reactions and undergo oxidative processes at protein and DNA level. These processes can generate inflammation and initiate apoptosis, leading to extensive tumor damage. Moreover, fluorescence emission occurs as a result of relaxation from the excited state of the compound, which can be used as a source for imaging applications. On the other hand, CuS nanoparticles present high levels of biological toxicity and are insoluble in water; therefore, their surface has to be modified in order to achieve more desirable characteristics. Thanks to its unique properties, quaternized chitosan has been chosen as a stabilizer and

proved to be an excellent choice for enhancing CuS nanoparticles' therapeutic activity. The study concluded that engineered nanoparticles induced low toxicity in all vital organs compared to the control group.

To further enhance the properties of chitosan in terms of water solubility, biocompatibility, biodegradability, and low toxicity, the biopolymer can undergo certain modifications which enable better interactions at macromolecular level and increased cellular uptake. Carboxymethyl substituents can be added at the primary hydroxyl group of chitosan (*O*-carboxymethyl chitosan) or both amino and hydroxyl sites (*N*,*O*-carboxymethyl chitosan). Depending on the purpose meant for the nanocarriers, *O*-carboxymethyl chitosan can be an option if further conjugations are needed, as it presents amine and carboxyl sites available for modifications (Ji et al. 2011).

In a study conducted by Li et al. (2014a, b), the team engineered a theranostic system based on a core–shell structure, composed of $Fe_3O_4@SiO_2$ coated with *O*-carboxymethyl chitosan-folic acid (OCMCS-FA). The main challenge when dealing with $Fe_3O_4@SiO_2$ nanoparticles is their low biocompatibility and low specific cellular uptake. Hence, OCMCS has been chosen as coating for its excellent water solubility, low toxicity, and reduced immunogenic response; meanwhile FA has proved to enhance targeting potential. The novel nanosystem not only combines two targeting mechanisms (magnetic- and receptor-mediated targeting) but is also characterized by improved biologic interactions and increased safety, thanks to its OCMCS outer layer.

Thiolated derivates of chitosan are the main choice for engineering nanosystems with enhanced mucoadhesion. A higher mucoadhesivity is a desirable feature for a theranostic system, as it enables a prolonged contact which results in increased concentration of nanosystems at their specific delivery site. The enhanced mucoadhesion of thiolated derivates of chitosan is a result of the covalent interactions that take place between the thiolated polymer and the cysteine-rich subdomains of mucus glycoproteins via disulfide bonds (R–S–S–R′) (Kast and Bernkop-Schnurch 2001).

Yang et al. (2016) have designed hybrid self-assembled nanoparticles based on the modification of chitosan with folic acid. The team studied the possibility of engineering a complex nanostructure that includes both an encapsulated chemotherapeutic drug (docetaxel) and VEGF shRNA (vascular endothelial growth factor short hairpin RNA) gene-silencing therapy as a means to combat multidrug resistance in cervical cancer. Furthermore, in order to assess the evolution of the synergistically applied therapy, researchers included in the structure two fluorescence imaging agents, fluorescein isothiocyanate (FITC) and carbon quantum dots (C-dots).

12.2.4 Types of Chitosan-Based Systems for Theranostic Applications

12.2.4.1 Self-Assembled Nanoparticles

Self-aggregated nanoparticles, also known as polymeric micelles, have unique core–shell structure, including a hydrophobic core that provides a loading space for the lipophilic and poorly water-soluble drugs and hydrophilic shell that assures stability in the aqueous media. Polymeric micelles based on biocompatible and biodegradable polymers represent a promising research area, as remarkable properties of these biopolymers can significantly improve their biological behavior as theranostic nanosystems able to defeat cancer drug resistance (Yang et al. 2014; Zhu et al. 2015).

Several representative works have explored the self-assembly ability of amphiphilic modified chitosan (AMC) derivates as a feasible strategy to design various drug delivery platforms, with promising results as chemotherapeutic systems (Chiu et al. 2010; Yhee et al. 2014), the AMC self-assembled micelles being more stable than the most of low molecular weight surfactant-based micelles (Larsson et al. 2013).

The nanoscale size of polymeric micelles allows their efficient accumulation in tumor tissue through the EPR effect by passive targeting approach, but at the same time, their surface may be functionalized with a series of small compounds with targeting functions such as folic acid (Wang et al. 2011), arginine–glycine–aspartic acid (Zhao and Zhai 2013), or biotin (Yang et al. 2014). Among all, biotin (vitamin B7 or H) is known to be involved in various cellular functions such as cell growth or signal transduction (Vadlapudi et al. 2012). Furthermore, it has been revealed that biotin receptors are overexpressed on the tumoral cell surface, especially breast cancer cells.

In this context, Balan et al. (2016) have synthesized biotinylated N-palmitoyl chitosan through the reaction of *N*-palmitoyl chitosan with biotin, via carbodiimide chemistry, and developed self-assembled nanoparticles loaded with docetaxel. Nanoparticles exhibited a good drug loading ability and a pH-dependent release profile of drug, susceptibility to biodegradation, and hemocompatibility, properties which can be further exploited in drug delivery applications.

Recently, Yu et al. (2018) proposed an effective strategy to deliver gambogic acid (GA) to the liver using *N*-octyl-*N*-arginine-chitosan micelles. Gambogic acid (GA) is a potent anticancer agent that suppresses the signaling pathway of nuclear factor-kappa B and connects to the transferrin receptor. After exposing the human hepatocellular carcinoma, HepG2 cells, to GA-loaded micelles for 2 h, a high cellular uptake was noted. Furthermore, in vivo studies indicated that the micelles could increase the half-life (1.5–2.0-folds) and showed a high biodistribution to the liver.

Because cancer exhibits abnormally high local acidities compared to normal tissues (pH 7.4), smart drug delivery systems that are triggered by environmental conditions have been developed to enhance cancer therapeutic efficacy while limiting side effects. For instance, Lim et al. (2013) designed smart chitosan-based magnetic nanocomposites capable of pH-sensitive doxorubicin release and

MR-guided imaging. In vivo therapeutic efficacy trial of these theranostic nanocomposites showed effectiveness in delaying tumor regression.

12.2.4.2 Cross-Linked Chitosan-Based Nanoparticles

Self-assembled polymeric micelles are formed and held together by weak physical interactions, which entails poor stability for in vivo administration of hydrophobic drugs. Hence, there are a number of risks associated with the use of these micelles as carriers, such as the possibility of dissociation if the dilution is made below the critical micelle concentration (CMC) and, as a result, the release of the therapeutic agent off-site. Micelle dissociation can occur also due to nanoparticle–blood interactions, when certain blood components may cause variations in the kinetic stability of the suspension. It is essential for a drug delivery system to exhibit a high stability profile for as long as needed before reaching its target (Lu and Park 2013). Therefore, stability-enhancing strategies have been extensively used as a means to enhance nanoparticle stability in vivo and maintain their integrity. Some of these strategies include crosslinking and mineralization through deposition of inorganic materials.

Chitosan is a polycationic polymer with several highly reactive functional groups that enable the formation of three-dimensional networks through both ionic and covalent crosslinking, which result in structurally stable nanoparticles. Ionic crosslinking of chitosan is a rather facile process, which can be conducted in mild conditions, at room temperature, in the absence of organic solvents (Swierczewska et al. 2016). The ionic complexation may occur between the positively charged chitosan and other negatively charged structures, which can be either polymers or small molecules. Sodium tripolyphosphate (TPP) is commonly used as a crosslinking agent for chitosan-based drug delivery systems and has been broadly used for this purpose. Its polyanionic structure enables the formation of physical bonds between ionized hydroxyl and amine moieties. Similarly, ionic complexation can take place between chitosan and other negatively charged polysaccharides: hyaluronic acid, (Deng et al. 2014) alginate (Thu et al. 1996), or chitosan and certain inorganic salts, such as calcium chloride (Kalliola et al. 2017).

Zahraei et al. (2016) developed a theranostic nanosystem composed of nanoparticles that combine a manganese zinc ferrite core as a negative contrast agent and an outer shell made of ionically cross-linked chitosan. The crosslinker was TPP, a safe, nontoxic polyanion. For comparison, PEG and dextran have also been used as coating materials, as their behavior is different at certain pH values. The study concluded that the hydrophilicity of the coating material is responsible for the proton relaxation rates. Thus, the closer water molecules are to the magnetic moment, the better the nanoparticles act as an imaging agent. Chitosan and dextran have been found to exhibit higher relaxivity values compared to PEG, but in turn, the PEG coating reduces particle aggregation. Furthermore, dextran and chitosan nanoparticles have shown interesting potential for hyperthermia.

Polyelectrolyte complexation can also take place between chitosan and an anionic polysaccharide such as alginate. The formation of this complex is based on the interaction between the protonated NH_2 groups of chitosan and deprotonated COOH

groups of alginate. Also, it has been found that the network of the newly formed complex can be strengthened by combining polyelectrolyte complexation with ionotropic gelation. TPP can be used as a crosslinker, but studies show that Ca^{2+} ions help alginate to bind to the chitosan 100 times better than in their absence (Sankalia et al. 2007; Tiwari et al. 2015). These findings were also confirmed by a study conducted by Türkoğlu and Taşcıoğlu (2014). The team has reached the conclusion that Ca^{2+} considerably influences the formation of a stable network between chitosan and alginate. Moreover, they have discovered that combining Ca^{2+} ions with anionic salts in the gelation process can result in stronger and more stable complexes rather than using Ca^{2+} or anions alone.

Theranostic systems based on this specific type of crosslinking are very promising and have proven to be efficient in both imaging and therapeutic approaches. For example, Yang et al. (2017) have engineered a novel multifunctional colloidal nanosystem which encompasses the photosensitizer chlorin e6 (Ce6) and the chemotherapeutic agent doxorubicin (DOX). The nanocarriers have a complex structure, with a magnetic Fe_3O_4-Au core, a functionalized silica mesoporous shell, alternatively covered by biocompatible chitosan–alginate multilayers and functionalized with P-glycoprotein small hairpin RNA (P-gp shRNA) in order to fight multidrug resistance. The layers of chitosan–alginate not only ensure the nanosystem's biocompatibility but are also pH-sensitive gatekeepers, having the important role of releasing the therapeutic agent in specific acidic environments.

Covalent or chemical crosslinking means that two or more molecules engage in the formation of covalent bonds with the purpose to obtain a stronger three-dimensional structure. Chemical bonds are far stronger than physical bonds and ensure more stability to prepared nanomaterials (Li et al. 2018). However, one of the main concerns is that covalent crosslinking might be too stable, and therefore the obtained nanoparticles might release insufficient amounts of therapeutic agent or might even negatively influence normal physiology due to prolonged circulation (Qiu et al. 2007). Chitosan can be chemically cross-linked using agents such as glutaraldehyde (Laroui et al. 2010) or genipin (Ding et al. 2017).

A theranostic strategy based on the synthesis of microspheres capable of delivering radiotherapy for unresectable liver tumor has been developed by Cho and Choi (2018). The so-called in vivo generator is comprised of chemically cross-linked chitosan microspheres loaded with $^{166}Dy/^{166}Ho$, a nontoxic parent–daughter isotope pair. This pair is able to participate in enzymatic reactions, and more than that, ^{166}Ho's paramagnetic properties can be exploited for medical imaging purposes using single-photon emission computed tomography and magnetic resonance imaging. The microspheres were prepared by W/O emulsion, and glutaraldehyde-saturated toluene was added as crosslinker. The study concluded that the biocompatible particles have real prospects of treating intractable liver tumors through high-energy radioactive decay as well as monitoring malignant cells via MRI or SPECT.

Song et al. (2015) developed nanocarriers for photothermal therapy (PTT) based on PEGylated chitosan. The ultrasmall nanoparticles (5 nm) were obtained by reverse microemulsion, and genipin was used as a covalent crosslinker. Indocyanine green (ICG) has been used as an imaging and PTT agent as it possesses remarkable

photochemical and photobiological properties (Li et al. 2013). In spite of these characteristics, ICG has several drawbacks regarding thermal degradation, aqueous degradation, and photodegradation which can be successfully overcome by its encapsulation in a chitosan-based nanocarrier. The particles exhibited good dispersibility, good stability at various pH values, and low cytotoxicity and, most of all, succeeded in suppressing tumor growth in mice.

12.2.4.3 Chitosan-Based Nanocapsules

The most common protocols of drug loading on the theranostic nanosystems imply either covalent binding or entrapment of the drug into a polymer layer (Mahmoudi et al. 2011). In some cases, covalent linkage can be too strong to be cleaved by cellular enzymes, in particular if access to the drug is hindered by the polymeric coating (Shkilnyy et al. 2010); meanwhile the entrapment of the drug within a polymer often leads to a burst effect (fast initial release) and/or to a low release in the absence of stimuli (Yang et al. 2010).

The use of nanocapsules can overcome these drawbacks. Nanocapsules can be defined as nano-vesicular systems that exhibit a typical core–shell structure in which the drug (in liquid/solid form or as a molecular dispersion) is confined to a reservoir or within a cavity surrounded by a polymer membrane or coating (Anton et al. 2008; Letchford and Burt 2007). These structures are usually prepared by nanoprecipitation or double emulsification methods. Nanocapsules can also carry the active substance on their surfaces or be embedded in the polymeric membrane (Khoee and Yaghoobian, 2008). The advantages of nanocapsule systems as nanotherapeutic tools include high encapsulation efficiency, maintenance of drug levels within a desired range by reducing their systemic distribution, and the possibility to administrate lower but more accurately targeted doses of the cytotoxic compounds (Pinto et al. 2006). The preparation and biomedical applications of nanocapsules have been reviewed in several excellent articles (Mora-Huertas et al. 2010; Levine et al. 2008). For diagnostic purposes, nanocapsules have also been applied in a variety of imaging modalities including fluorescence microscopy, PET, and MRI for noninvasive monitoring (Sun et al. 2007; Kelly et al. 2005). The theranostic applications of nanocapsules using NIR agents in combination with different chemotherapeutics (e.g., paclitaxel and irinotecan) have been tested in different cancer models.

In the last years, magnetic nanocapsules based on biocompatible polymers are emerging as excellent nanocarriers, especially for the delivery of chemotherapeutic drugs (Ashjari et al. 2012; Chiang et al. 2014; Ling et al. 2011). In this regard, Balan et al. (2015) prepared, through a double emulsion method, doxorubicin-loaded magnetic nanocapsules based on N-palmitoyl chitosan and magnetite. Magnetic nanocapsules exhibited suitable magnetic saturation, superparamagnetic behavior, and good ability to incorporate a chemotherapeutic agent. These characteristics, correlated with the biocompatibility and biodegradability of magnetic nanocapsules, are good premises for the suitability of proposed magnetic nanocapsules as drug delivery system for therapeutic agents.

12.3 Chitosan in Clinical Applications: Key Challenges

12.3.1 Drug Delivery, Bioimaging, and Hyperthermia

The progress in nanotechnology has contributed to the development of multifunctional nanosystems that enable specific delivery of imaging agents and therapeutic drugs to target diseased tissues for cancer imaging and therapy. A series of noninvasive imaging modalities is employed for the detection of cancer at early stages and monitoring the drug fate in living systems, in real time (Fang and Zhang 2010). These noninvasive imaging modalities include positron emission tomography (PET), magnetic resonance imaging (MRI), X-ray computed tomography (CT), single-photon emission computed tomography (SPECT), ultrasound (US), optical fluorescence, and near-infrared fluorescence (NIRF) imaging (Lee et al. 2012; Rhee et al. 2014). Among them, primarily molecular imaging techniques including PET/SPECT and optical imaging are characterized by high sensitivity; meanwhile primarily morphological/anatomical imaging techniques like CT, US, and MRI are highlighted by high spatial resolution (Huang et al. 2012). Recently, integrated molecular/anatomical systems (e.g., SPECT/CT) have been adopted to combine their strengths in order to obtain more accurate data (de Smet et al. 2013; John et al. 2013).

In another study, Agyare et al. (2014) designed targeted theranostic nanocarriers for MRI-based early detection and treatment of cerebrovascular inflammation. They developed polymeric nanocarriers loaded with Magnevist®-conjugated chitosan and cyclophosphamide and further functionalized with putrescine-modified F(ab′)2 fragment of anti-amyloid antibody, IgG4.1 (pF(ab′)24.1). The developed theranostic nanosystem-enabled imaging of cerebrovascular amyloid by both MRI and SPECT and caused decrease in pro-inflammatory cytokine production by amyloid beta-challenged BBB endothelium than free drug.

Glycol chitosan-based nanoparticles (GC NPs) have been intensively studied as theranostic nanosystems. For instance, Kim et al. (2010) developed a promising nanoconstruct based on GC-5β-cholanic acid conjugate labeled with a near-infrared fluorophore (cyanine 5.5, Cy5.5), with high tumor-targeting characteristics, good colloidal stability, and strong NIRF signals in tumor tissues after passing through the in vivo filtration system of the liver and spleen (Na et al. 2011; Kim et al. 2010). Likewise, Kim et al. (2015a, b) prepared a fibrin-targeted CT based on GC NPs for visualization of cerebrovascular thrombus and guiding thrombolytic therapy. Micro-CT imaging allowed quick detection of cerebrovascular thrombi and tracking of tissue plasminogen activator-induced thrombolysis.

Yoon et al. (2014) utilized two distinctive formulation strategies of modified glycol chitosan-based NPs (GCNPs) with the aim to encapsulate doxorubicin (DOX) or a complex Bcl-2 siRNA for gene therapy (siRNA-GCNP) while carrying imaging agents on their surfaces. The team reported that theranostic nanosystems were able to overcome drug resistance in cancer by downregulating antiapoptotic defense mechanisms of cancer cells while triggering apoptosis. A similar siRNA delivery strategy was also proposed to suppress P-glycoprotein, the protein responsible for

the efflux of chemotherapeutics in multidrug resistance, and in vivo results indicated that a combination therapy with subtherapeutic doses of DOX can effectively inhibit tumor growth in MCF-7/ADR tumor-bearing mice (Yhee et al. 2015).

Hyperthermia, used alone or in combination with other cancer therapies, treatment is generally well tolerated and, if the temperature does not exceed 45 °C, rarely affects normal tissues, this being one of the main advantages over other treatment techniques (Habash 2018). The approach pursued by Srinivasan et al. (2013) to obtain a multifunctional vector capable of in vivo imaging and also hyperthermic effects in different cancer cell lines consisted in conjugating IR820, a NIR fluorescent dye, with chitosan.

Lin et al. (2015) designed a protease-mediated drug release nanosystems based on PEG-/chitosan-coated magnetic nanoparticles with the cytotoxic agent – methotrexate (MTX) – attached to the surface via a protease-cleavable amide linker. The structural similarity between MTX and folate-enabled cellular uptake into folate-receptor-overexpressing HeLa cells and intracellular protease-mediated drug release. At the same time, the nanosystems allowed in vivo-targeted MR and fluorescence imaging in HeLa tumor growth in Sprague-Dawley rats.

Another approach was proposed by Wang et al. (2016a, b) who used SPIO nanoparticles coated with chitosan-PEG-grafted polyethyleneimine as nanovectors for the delivery of targeted siRNA to orthotopic hepatocellular carcinoma (HCC) xenografts in a mouse model. In this case, the negatively charged Luc gene-targeting siRNA was loaded to polymer-coated magnetic nanoparticles through electrostatic interaction with PEI segments. PEG linkers were further conjugated on the surface to protect siRNA and facilitate conjugation with antibody against human glypican 3 (GPC3) receptor. Upon intravenous injection, the functionalized nanovectors enabled specific targeting ability to HCC and significantly suppressed Luc expression of the tumor.

A facile approach to fabricate a tumor-specific theranostics for targeted DOX delivery and magnetic resonance (MR) imaging was established by Xie et al. (2019). The team developed a hybrid cluster bomb by co-precipitation of polyethylene glycol (PEG)-modified chitosan (CS), oleylamine-modified Fe_3O_4 (OA-Fe_3O_4) nanoparticles, and DOX. The in vitro studies indicated that clusters could be uptaken into HepG2 cells to deliver DOX into the cell nuclei with enhanced anticancer efficacy in comparison with free DOX. In the tumor intracellular microenvironment, the stimuli-responsive hybrid cluster bombs disassembled and re-self-assembled into the OA-Fe_3O_4 nanoparticle clusters with higher Ms for MR imaging-guided diagnosis or hyperthermia.

12.3.2 Chitosan in Bioresponsive Tissue-Engineered Scaffolds

The main objective of tissue engineering is to obtain functional tissues that allow implantation, regeneration, or replacement of functionally inactive biological tissue. In order to achieve this objective, some conditions (mechanical, structural, and functional) must be respected. At the tissue level, cells are organized in a three-
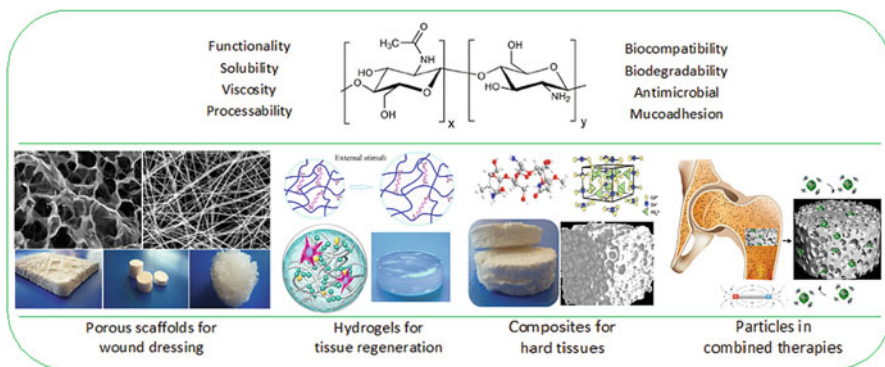
Fig. 12.5 Chitosan in tissue engineering

dimensional form and are surrounded by macromolecules of the extracellular matrix (collagen, proteoglycans, glycosaminoglycans, glycoproteins). On the other hand, the extracellular matrix is the supportive environment for the growth and differentiation of cells. Therefore, in tissue engineering, specific cells, support matrix, and signal biomolecules work together for a successful treatment or implantation. A scaffold should stimulate the adhesion and growth of cells that migrate from neighboring tissues or allow the cell differentiation into porous structure, ensuring cellular nutrition and excretion of metabolites. Some important properties should be met by any biomaterials for tissue engineering: (i) biocompatibility; (ii) porous structure with interconnected pores; (iii) promoting cell attachment, differentiation, and proliferation; and (iv) suitable mechanical properties for handling and stimulating the cells' adhesion and differentiation of mechanical properties (O'Brien 2011). The scaffold should not induce acute or chronic response, should be biodegradable, and, finally, should be manufactured into a variety of shapes (Zhu et al. 2005).

Due to its remarkable properties, chitosan is a good candidate for the preparation of scaffolds, which could substitute the missing or damaged tissue and organ and allow cell attachment and proliferation (Cai et al. 2017). Several methods have been developed to finely shape chitosan hydrogels and foams (or sponges) as 3D scaffolds applicable for soft or hard tissue engineering (Ikeda et al. 2014; Chaudhari et al. 2016). Various types of scaffolds with potential applications in soft and hard tissue engineering are presented in Fig. 12.5.

Hydrogels are interesting biomaterials considering their compatibility with a majority of living tissues. Generally, they are soft and bendable, which minimizes the damage to the surrounding tissue during and after implantation in the human body (Feksa et al. 2018). The mechanical properties of hydrogels can mimic those of soft body tissues, which allow the gels to insure both functional and morphological characteristics of the repairing tissue (Geckil et al. 2010), and therefore hydrogels are often used as biomedical scaffolds for tissue replacements and drug and growth factor delivery systems (Tang et al. 2012). Various chitosan hydrogels have been

developed, presenting either reversible or irreversible gelation. Chitosan can be physically associated, coordinated with metal ions, or irreversibly/chemically cross-linked into hydrogels (Ahmadi et al. 2015).

The amino and hydroxyl functional groups of chitosan provide chitosan derivatives with improved properties and applications for tissue engineering. Acrylic acid-grafted chitosan exhibits improved swelling properties, higher mesh mechanical resistance, and reduced cytotoxicity (Rusu et al. 2016). Chitosan crosslinking is a necessary process to improve the stability and durability of chitosan in applications as tissue engineering-controlled drug delivery. The chemical crosslinking of this polymer is irreversible and is achieved either by covalent or ionic crosslinking or both. Each type of crosslinking has advantages and disadvantages, chemical crosslinking being more resistant, but even more toxic, while the mechanical strength of physical hydrogels is lower but does not present biocompatibility problems. Physical hydrogels are preferred for achieving soft tissues and controlled drug delivery systems due to higher susceptibility to changes in pH, temperature, or ionic strength (Sood et al. 2016).

Chitosan is biocompatible and biodegradable, the products resulting from the degradation process being nontoxic. This biopolymer is used as a dressing material and hemostatic agent in treating skin lesions. A commercial product using this feature is the HemCon bandage that has the ability to stop severe bleeding by adhering to negatively charged cells as well as by attracting negatively charged red blood cells that seal the wound. The dressing is applied by pressing on the wound, bleeding, letting it act for up to 48 h. Removal occurs when saline is applied over dressing (Khoshmohabat et al. 2016). Chitosan can be used as a tissue engineering matrix with applications in the regeneration of bone, cartilaginous, cutaneous, and nervous tissue. Network porosity is of great importance for the swelling capacity, cell adhesion, and cell proliferation. The interconnected pore network allows cell migration and proliferation to the damaged tissue, replacing the tissue in the injured area. Various types of chitosan-based hydrogels with biomedical and pharmaceutical applications are reported in literature. They show some common features such as biocompatibility, biodegradability, and lack of cytotoxicity, which recommend chitosan in further tests to improve the products obtained from this biomaterial (Hamedi et al. 2018; Pellá et al. 2018).

Hydrogels containing different ratios of chitosan and collagen were made by initiating gelation using β-GP and temperature by Cho et al. (2005). This process was performed at physiological pH and temperature, so living cells could be incorporated directly into the hydrogel matrix. The presence of collagen in chitosan-collagen-like materials has been associated with increased cell proliferation as well as increased gel density and resulting rigid matrix. Chitosan content has been associated with improved osteogenic differentiation, as assessed by gene expression and the presence of osteogenic markers. Beta-glycerophosphate has been shown to be an osteogenic supplement when added to stem cell cultures derived from human bone marrow stromal cells (hBMSC). It was also used as a catalyst to cause a sol–gel transition in chitosan solutions at physiological pH and temperature. This form of chitosan hydrogel has been investigated for use in tissue engineering of cartilage

because the polysaccharide nature of chitosan resembles cartilage glycosaminoglycans and has shown good compatibility with hBMSC and chondrocytes. The use of β-GP as an agent to cause both the gel-to-gel transition in chitosan and the collagen fibrinogenesis presents the possibility of pure chitosan composites, pure collagen, and chitosan–collagen composites without the need for freeze-drying or chemical crosslinking. Therefore, it is of great interest to manufacture a chitosan–collagen composite that supports cell survival but can also be injected and gelled in situ to effectively fill tissue defects (Wang and Stegemann 2010; Wang et al. 2013).

Neovascularization is an essential part of regenerating tissues that provide food and nutrients to cells. There is a real need for proangiogenic biomaterials to help heal wounds. Degradation studies were tested in three different environments, such as phosphate-buffered saline (PBS), lysozyme, and hydrogen peroxide, and relatively greater degradation was observed in hydrogen peroxide. The hydrogel loaded with thyroxine (TLH-1)-stimulated angiogenesis. The collagen/chitosan hydrogel has a good biocompatibility with effective epithelial and nerve restoration. Both collagen (Coll) and chitosan (CS) have been used in a variety of bioactive materials for different biomedical applications (Aleem et al. 2017).

Angiogenesis is a complex process involving the formation of new blood vessels from the preexisting ones. Davis et al. (2006) reported the proangiogenic effect of thyroxine (T4) at physiological concentrations. They reported evidence that the hormonal effect was initiated at the endothelial cell plasma membrane. In addition, thyroid hormones regulated cellular metabolic activity as well as cell proliferation. The preparation, biocompatibility, and angiogenic potential of the chitosan/collagen loaded with thyroxine were reported. Synthesis of essentially three CS/Coll hydrogels with variable concentrations of thyroxine and control (without T4) was compared. Porous hydrogels were synthesized by freeze gelation, and thyroxine was loaded by the physical loading method. The synthesized materials showed a good level of degradation: 16% weight loss in PBS solvents and over 60% weight loss in lysozyme, after 6 days. In addition, these materials were placed on the chorioallantoic membrane in fertilized chicken eggs from day 8 to day 14 to investigate their angiogenic growth and tissue in this membrane potential. It has been observed that low-concentration thyroxine material (TLH-1) significantly stimulates angiogenesis compared to the high-concentration thyroxine material (TLH-10). Overall, TLH-1 has been shown to be suitable materials for future tissue engineering applications. The hydrogels have shown good levels of in vitro degradation (Aleem et al. 2017).

Chitosan can be used in regenerating surgery as a substitute for cartilage or bone (osteocompatible and osteoconductive properties). The bone consists of an organic collagen matrix and a mineral component. The bone is composed of 2030 wt% collagen and 60–70% wt% inorganic hydroxyapatite-like crystals and water. The formation and the maintenance of the bone is controlled by a variety of factors (genetic, dietary, hormonal, and physical activity) (Palmer et al. 2008).

The bone thus undergoes constant remodeling. Normally there is a balance between bone formation and bone breakdown. Globally, rates of bone disease are dramatically increasing due to as follows:

1. The elderly populations living longer, outliving their joint replacements.
2. Failure due to loosening of implant (5–10 years lifetime).
3. A non-negligible percentage of hip replacement operations are repeat procedures.

Bone disease, such as osteoarthritis (degradation of joints, including articular cartilage) or osteoporosis (reduced bone mineral density leading to increased risk of fracture), is dramatically increasing. As a result, chitosan-based matrices, associated with other polymers, might be used to elaborate implants with required properties. Recently, chitosan and its derivatives were described as good candidates to regenerate cartilage because they degrade while new tissues are formed, with no inflammatory reaction or toxic degradation products (Bressan et al. 2011). As the chitosan is transformed into glucosamine in the body, many studies concern its use in the form of injections into the knee to stimulate cartilage repair. Indeed, the surgical techniques of damaged cartilage repair require the formation of a blood clot on the lesions. Incorporation of chitosan in liquid blood allows an adherent and more stable blood clot, which leads to better repair of the cartilage, thanks to this chitosan-blood implant. Glucosamine is well-known for its anti-pain and anti-inflammatory effects and for preventing deterioration of the articulations. Chitosan derivatives ensure a progressive release of the monomer (Marchand et al. 2009).

Chitosan has a critical role in cell attachment and growth. Indeed, epithelial cells can grow on a film of chitosan. In addition, it is also a good candidate for synthesizing scaffolds because of its biocompatibility, biodegradability, non-toxicity, high affinity toward proteins, and antimicrobial affinity. The main disadvantage of chitosan-based matrices is its poor mechanical properties. To improve them, crosslinking can be carried out. To overcome this problem, researchers are studying new hybrid scaffolds made up of chitosan and synthetic polymers, prepared by chemically cross-linked chitosan and synthetic polymer for potential use in skin engineering applications (Costa-Júnior et al. 2009).

12.3.3 Bio-sensing Applications

Biosensors are finding diverse applications in clinical diagnosis, environmental monitoring, and specific biomolecule release (Prasad et al. 2016). Extensive researches have been done toward biosensor designing due to its specificity, fast detection time, and high selectivity to detect analytes (proteins, DNA/RNA, cells), within the miniaturized settings (Yager et al. 2008). A biosensor consists of a transducer combined with a biologically active molecule that converts the biochemical response into a quantifiable signal. It is comprised of three basic components: a detector, a transducer, and a signal processor. The transducer can be optical, acoustic, electrochemical, and calorimetric, depending on diagnosis and the analyte characteristics. Biosensors have been broadly studied based on various detection principles: conductometric, amperometric, potentiometric, and voltammetric (Malhotra et al. 2017). Different types of sensing systems have been tested, like enzymes (Sakamoto et al. 2015), DNA/RNA (Linardy et al. 2016), antibodies,

receptors, organelles, and animal cells/tissues (Darsanaki et al. 2013). Some considerations should be followed in biosensor designing: (i) it should work in a wide range of pH and temperature conditions, (ii) it should have a wide dynamic range and high sensitivity, and (iii) it should involve facile fabrication steps (Jolly et al. 2016). Various biomaterials have been tested to develop various types of personalized biosensing prototypes. These include natural biomaterials such as chitosan and collagen, metal oxides and carbon nanotubes (CNT), quantum dots, and grapheme (Maxwell et al. 2015; Li et al. 2014a, b).

Chitosan has proven to be a suitable modifier of transducer surface by enabling mild yet stable immobilization of biological recognition elements (BREs). Other biological polymer that can act as surface modifier (such as polylysine, polyglutamate, and poly-arginine), alginic acid, and hyaluronic acid have been tested. Chitosan seems to have the greatest potential to be used in sensor development by offering attractive benefits like nontoxicity, low cost, and solubility in aqueous solution at mild acidic pH (6.3) which ensures immobilization of BREs without actually causing their denaturation (Baranwal et al. 2018).

Substrate selection is a key step in device fabrication as it provides the foundation on which to construct the biosensor. A substrate that conforms more the contours of the surface indicates a large contact area for sample collection such as body fluids. Such devices can often also be used in combination with added functionalities (e.g., drug delivery) (Wang et al. 2016a, b).

Material selection considers the location of intended use (internal or external to the biological system). Characteristics of bendability, stretchability, conformability, and fracture resistance are obtained by two approaches: by selection/synthesis of flexible and stretchable materials with good inherent or supplemented electronic characteristics or by using rigid conductive or semi-conductive materials, imparting flexible attributes via specific geometry or structural designs (Zhu and Moran-Mirabal 2016).

Bio-derived materials have received recent consideration for green and sustainable bioelectronics because of their biocompatible and biodegradable characteristics (Muskovich and Bettinger 2012). Various, naturally derived biomaterials have been proposed as substrates for flexible devices such as silk proteins, polysaccharides, and gelatin. Bio-derived materials such as chitin and chitosan have been reported for electrochemical biosensing as components, substrates, or surface modifiers for thin film electrodes (Xu et al. 2019).

The availability of aqueous chitosan solutions near-physiological conditions contrasts with its parental polymer, chitin, and makes exploitation of chitosan more biocompatible and thus widens the range of its applications. Well-adherent thin films of both materials can be simply prepared using their dilute solutions in casting, spinning, or dip-coating procedures, resulting in structures of density and porosity that are adjustable by the number of casting/dipping/spinning repetitions and by the specific composition of the casting/dipping/spinning solutions (Muzzarelli et al. 2003).

Chitin and chitosan possess good biocompatibility, have potential to form uniform films and hydrogels, and contain multiple oxygen- and nitrogen-based

functional groups that can be chemically modified. A stable and reproducible long-term response is achieved only when the matrix-induced degradation of the immobilized BRE occurs at a negligible rate. The compatibility of the immobilizing layer with the BRE is still crucial for durable sensor performance, but in addition, the sensor's composition and tip design should not trigger a local inflammatory host response, as there is a risk of sensor fouling by cellular release of absorbable proteins and lipids or immunoprotective fibrous tip encapsulation. For in vivo applications, the aim must therefore be to construct the biosensor from materials that are as nontoxic as possible for both the BRE and the tissue under study. As expected for biological polymers, the tissue compatibility and low immunogenicity of chitin and chitosan have been confirmed in many clinical trials (Cheung et al. 2015).

Chitin and chitosan contain abundant functionalities ($-OH$, $-NHCOCH_3$, and $-NH_2$). These reactive function entities allow the derivatization of the biopolymers if chemical tailoring would benefit sensor performance. Chitin and chitosan derivatives have been synthesized for various applications by covalent modification of the backbone that include sugar-modified, phosphorylated, quaternized, cyclodextrin-linked, thiolated, sulfated, azidated, and ferrocene-branched (Jayakumar et al. 2006; Mourya and Inamdar 2009).

Wu et al. prepared thin film of chitosan by electrodeposition on an electrode by cathodic hydrogen evolution from water electrolysis, which consumes protons and generates hydroxide ions at the interface between the negatively polarized electrode and the electrolyte. The local increase of the interfacial pH neutralizes the positive charges on the protonated groups in chitosan chains reaching the cathode through electrostatic attraction. Continuous electrophoretic delivery and concomitant removal of positive charges produce chitosan thin film formation on the sensor surface, as the polymer solution is heading to the point of precipitation ("sol–gel transition") (Wu et al. 2010).

Due to their behavior, chitosan-based Schiff bases (CSBs) are functioned as excellent pH stimuli sensors (Zhao et al. 2017; Zhang et al. 2017). Zhang et al. (2017) have studied the different voltage response of CSB (from chitosan and catechol) at different pH conditions. Based on the destruction-formation of imine bonds under different pH conditions, a CSB gel (derived from phenyl boronate-modified chitosan and dialdehyde polyethylene oxide) was tested as glucose-responsive material (Zhao et al. 2017). Different to this, the morphology of gold nanoparticle–CSB composite is tuned between spongy and wire morphologies at different pH values due to the destruction-formation behavior of imine bonds (Zhang et al. 2017). Fluorescent CSBs (prepared via the reaction between chitosan and highly conjugated aldehydes) and their reduced forms can detect the different chemical molecules (Jatunov et al. 2015).

CSB-modified glassy carbon electrode can act as the electrochemical sensor for the detection of hydrogen peroxide within 4 s. The aldehyde groups are initially introduced in chitosan through oxidation, and the oxidized chitosan is allowed to react with the amino groups of thionine for the synthesis of CSB (Feng et al. 2010). Chitosan modified with 2,5-dihydroxybenzaldehyde was tested as coatings on carbon electrode were tested as electrochemical sensor for the detection of lead

(II) ions (Kocak et al. 2012). The electrochemical sensor based on chitosan and salicylaldehyde can effectively detect the presence of tryptophan (Deng et al. 2011).

12.4 Conclusions and Future Perspective

Theranostics involves smart integrated system and materials that assures therapy, diagnosis, and monitoring of the treatment as a platform for personalized medicine. The performing theranostic systems offer opportunities to combine passive and active targeting, environmentally responsive drug release, or other therapeutic functions into a single biomedical platform. High-performance biomedical materials have been tested as theranostic nanosystems (superparamagnetic iron oxide nanoparticles (SPIONs), gold nanoparticles (AuNPs), quantum dots (QDs), carbon nanotubes (CNTs), mesoporous silica nanoparticles (MSNs), lipid-based nanoparticles platform, dendrimers), biological-sensitive hydrogels, 3D scaffolds, and micro- and nanowires with promising performances for medical applications.

Chitosan is a natural polymer extensively tested for theranostic platforms because of several advantages in biomedical applications, such as biocompatibility and controlled biodegradability, antimicrobial properties, and bioadhesive and bioresorbable nature. Its functionality and ability to form complexes with iron oxides and other inorganics and the processability in nanoparticles and nanocapsules made these materials very useful in theranostics, hyperthermia, and biosensing or cancer therapy. The polycationic nature based on protonated amino groups explains the chitosan biological properties: adhesion of cells and tissue, analgesic effect, and antioxidant effect. Compatibility of chitosan with physiological medium depends on the preparation method, deacetylation degree, molecular weight, crystallinity, solubility, and, eventually, the chemical modification with small or large molecules with specific properties. Various kinds of modification of chitosan have been investigated in recent years, and examples using acylation, alkylation, quaternarization, carboxymethylation, or functional derivative synthesis by reaction chemical modifications have been reported in the aim to increase the solubility in water, encapsulation of bioactives, cell penetrability, and blood interactions.

Pure and modified chitosans have been used in compositions for biomedical and pharmaceutical applications, such as drug delivery systems and 3D scaffolds for tissue engineering or 2D scaffolds, especially for wound healing purposes, stimuli-responsive hydrogels, micro- and nanoparticles, and bone implants. Huge progress in the application of chitosan in theranostic is based on surfaces' activation of chitosan-based nanoparticles or immobilization of cellular receptors, bioactives, or membrane penetrators. The future challenges include the strategies for balancing the scale of the carrier with the quantities of bioactive able to be included and that are clinically necessary. Other directions of investigations consider formulation as scaffolds, imprinted polymers, membranes, emulsions, and liposomes, which are biocompatible and nontoxic in human body and are able to respond to external stimuli.

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