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Review

Biomedical applications of chitin and chitosan based nanomaterials—A short review

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ABSTRACT

Chitin and chitosan are biopolymers having immense structural possibilities for chemical and mechanical modifications to generate novel properties, functions and applications especially in biomedical area. Chitin and chitosan are effective materials for biomedical applications because of their biocompatibility, biodegradability and non-toxicity, apart from their antimicrobial activity and low immunogenicity, which clearly points to an immense potential for future development. These candidate biopolymers can be easily processed into gels, sponges, membranes, beads and scaffolds forms. This review emphasizes recent research on different aspects of chitin and chitosan based nanomaterials, including the preparation and applications of chitin and chitosan based nanofibers, nanoparticles and nanocomposite scaffolds for tissue engineering, wound dressing, drug delivery and cancer diagnosis.

Contents

1. Introduction

Chitin, poly (β-(1-4)-N-acetyl-D-glucosamine), is a natural polysaccharide of major importance, first identified in 1884 (Fig. 1). This biopolymer is synthesized by an enormous number of living organisms; and considering the amount of chitin produced annually in the world, it is the most abundant polymer after cellulose. Chitin occurs in nature as ordered crystalline microfibrils forming structural components in the exoskeleton of arthropods or in the cell walls of fungi and yeast. It is also produced by a number of other living organisms in the lower plant and animal kingdoms, serving in many functions where reinforcement and strength are required. The most important derivative of chitin is chitosan (Fig. 1), obtained by (partial) deacetylation of chitin in the solid state under alkaline conditions (concentrated NaOH) or by enzymatic hydrolysis in the presence of chitin deacetylase. Because of the semicrystalline morphology of chitin, chitosan obtained by solid-state reaction has a heterogeneous distribution of acetyl groups along the chains. Chitin and chitosan are biocompatible, biodegradable and non-toxic poly-

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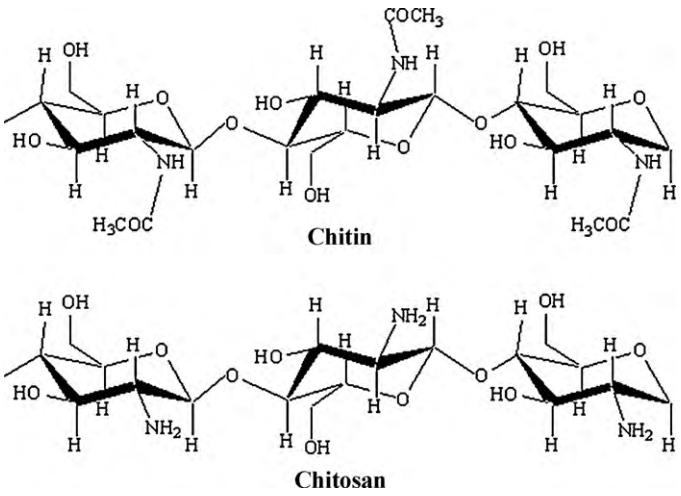

Fig. 1. Structure of chitin and chitosan.

mers. These properties, find several biomedical applications in tissue engineering (Jayakumar, Prabaharan, Reis, & Mano, 2005; Madhumathi, Binulal, et al., 2009), wound healing (Madhumathi et al., 2010), as excipients for drug delivery (Jayakumar, Nwe, Tokura, & Tamura, 2007; Jayakumar et al., 2005) and also in gene delivery (Gerrit, 2001; Jayakumar, Chennazhi, et al., 2010). Chitin and chitosan are easily processed into gels (Nagahama et al., 2008), membranes (Jayakumar, Nwe, Nagahama, & Tamura, 2008; Jayakumar et al., 2007, 2005; Jayakumar, Rajkumar, Fretias, Selvamurugan, et al., 2009; Jayakumar, Rajkumar, Fretias, Sudheesh Kumar, et al., 2009; Madhumathi, Binulal, et al., 2009), nanofibers (Jayakumar, Prabaharan, Nair, & Tamura, 2010; Shalumon et al., 2009), beads (Jayakumar, Reis, & Mano, 2006), microparticles (Prabaharan & Mano, 2005), nanoparticles (Anitha et al., 2009), scaffolds (Madhumathi et al., 2010; Madhumathi, Sudheesh Kumar et al., 2009; Peter, Binulol, Soumya, et al., 2010; Peter et al., 2009; Prabaharan & Jayakumar, 2009; Jayakumar, Prabaharan, Nair, Tokura, et al., 2010) and sponges (Muramatsu, Masuda, Yoshihara, & Fujisawa 2003). The above forms provide a wide variety of biomedical applications in tissue engineering, wound dressing, cancer drug delivery and targeting, in the area of nanobiotechnology. This review proposes to consolidate and discuss the recent applications of novel chitin and chitosan based nanofibers, nanocomposite membranes and scaffolds as well as nanoparticles.

2. Applications of chitin and chitosan based nanomaterials

2.1. Tissue engineering

Tissue engineering is one of the most multidisciplinary research areas today. It involves the use of living cells, manipulated through their extra cellular environment or genetically, to develop biological substitutes for implantation into the body and/or to foster remodeling of tissues in some active manner. The purpose of tissue engineering is to repair, replace, maintain, or enhance the function of a particular tissue or organ. There are a few basic requirements that have been widely accepted for designing polymer scaffolds. Firstly, a scaffold should possess a high porosity, with an appropriate pore size distribution. Secondly, a high surface area is needed. Biodegradability is yet another requirement, with the degradation rate matching the rate of neo-tissue formation. Fourth, the scaffold must possess the required structural integrity to prevent the pores of the scaffold from collapsing during neo-tissue formation, with the appropriate mechanical properties. Finally, the scaffold should be non-toxic to cells and be biocompatible, positively interacting with the cells to promote cell adhesion, proliferation, migration, and differentiated cell function. It is now well known that many biologically functional molecules, extracellular matrix (ECM) components, and cells interact at the nanoscale level. For example, collagen is a major natural ECM component, and possesses a fibrous structure with fiber bundles varying in diameter from 50 to 500 nm (Hay, 1991). In morphology, electrospun nanofiber mat is very similar to human native ECM (Jayakumar, Prabaharan, Nair, & Tamura, 2010), and hence can be a promising scaffolding material for cell culture and tissue engineering applications. Electrospinning process makes it possible to produce complex, seamless and three-dimensional (3D) nanofiber scaffolds that support diverse types of cells to grow into the artificial tissues.

Shalumon et al. (2009) reported an electrospun water-soluble carboxymethyl chitin (CMC)/PVA blend for tissue engineering applications. The concentration of CMC (7%) with PVA (8%) was optimized, blended in different ratios (0–100%) and electrospun to get nanofibers. Fibers were made water insoluble by cross-linking with glutaraldehyde vapors followed by thermal treatment. The prepared nanofibers were found to be bioactive and biocompatible. Cytotoxicity and cell attachement studies of the nanofibrous scaffold were evaluated using human mesenchymal stem cells (hMSCs) by the MTT assay. Cell attachment studies revealed that cells were able to attach and spread in the nanofibrous scaffolds (Fig. 2). These results indicated that the nanofibrous CMC/PVA scaffold supports cell adhesion/attachment and proliferation and hence this scaffold can be a useful candidate for tissue engineering applications (Shalumon et al., 2009).

Chitin and chitosan inherently have poor mechanical property. Therefore chitin can be used as bone substitute for bone repair and reconstruction only if its mechanical property can be improved with addition of biomaterials like hydroxyapatite (HAp), bioactive glass ceramic (BGC), etc. BGC are a group of osteoconductive silicate based materials used for bone repair. Bioglass was developed by Hench as a biomaterial to repair bone defects (Hench, 1991) and is widely used in orthopaedics and dentistry. Bioactive glass ceramic coatings on the surface of titanium are superior to HAp in their ability for osteointegration (Wheeler, Montfort, & McLoughlin, 2000). Moreover, BGC can also bond to soft and hard tissues (Verrier, Blaker, Maquet, Hench, & Boccaccinia, 2004). The bonding ability of these materials is attributed to the formation of carbonated apatite layer on the surface of the coated materials (Kokubo, 1991). BGC have been reported to influence osteoblast and bone marrow stromal cell proliferation and differentiation (Bosetti & Cannas, 2005; Foppiano, Marshall, Marshall, Saiz, & Tomsia, 2007). It has been reported that bioactive glass could directly influence cells at the genetic level (Hench, 2009). Many groups have reported that BGC influences osteoblastic cell differentiation with an increase in the level of differentiation markers like ALP, osteocalcin and osteopontin (Valerio, Pereira, Goes, & Leite, 2004).

Sol–gel method is a versatile technique to produce nano sized ceramic particles by tuning the precipitation reaction. Nanobioactive glass ceramic (nBGC) has been prepared using sol–gel method (Xia & Chang, 2007). Nanosurfaces are known to influence the cell behavior considerably. Cell–material interactions are known to be better on nanophase ceramics compared to microphase ceramics (Webster et al., 2000). The research on chitin/nBGC (Fig. 3A) and chitosan/nBGC composite scaffolds for tissue engineering provides an important reference to this fact (Peter, Binulol, Soumya, et al., 2010; Peter et al., 2009). Chitin or chitosan/nBGC composite scaffolds were developed by lyophilization technique. The composite scaffolds showed adequate porosity when the nBGC were homogenously distributed on the pore walls. The developed nanocomposite scaffolds showed adequate swelling and degradation properties aside of its ability to become bioactive. Cytocompatability of the chitin/nBGC and chitosan/nBGC scaffolds was assessed using MTT

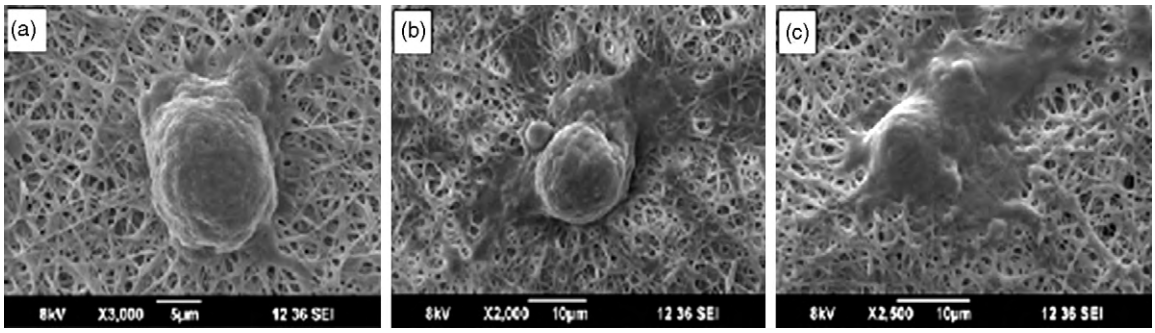

Fig. 2. SEM images of hMSCs attached on the surfaces of CMC/PVA scaffolds after (a) 12 h (b) 24 h and (c) 48 h of incubation.

assay, direct contact test and cell attachment studies (Fig. 3B). Results indicated no signs of toxicity and cells were found to be attached to the pore walls offered by the scaffolds. These results suggested that the developed composite scaffolds can be used for tissue-engineering applications.

Composite scaffolds of chitosan (CS)-gelatin (CG) with nBGC were prepared by blending of chitosan and gelatin with nBGC (Peter, Binulol, Nair, et al., 2010). The results showed macroporous internal morphology in the scaffold with pore size ranging from 150 to 300 μm. Degradation and swelling behavior of the nanocomposite scaffolds decreased, while protein adsorption increased with the addition of nBGC. Biomineralization studies showed higher amount of mineral deposits on the nanocomposite scaffold, which increased with increasing time of incubation. MTT assay, direct contact test, and cell attachment studies indicated that the nanocomposite scaffolds are better for cell attachment and spreading. So, these nanocomposite scaffolds can be used effectively for alveolar bone regeneration (Peter, Binulol, Nair, et al., 2010).

Similarly, Peter et al. also reported the preparation of chitosan-gelatin/nanophase hydroxyapatite (nHAp) composite scaffolds by blending chitosan and gelatin with nHAp (Peter, Ganesh, et al., 2010). The composites scaffolds were highly porous with a pore size of 150–300 μm. Composite scaffolds in the presence of nHAp showed a decreased degradation rate, controlled swelling and increased mineralization in SBF. The biological response of MG-63 cells on nanocomposite scaffolds was superior to chitosan-gelatin (CG) scaffold in terms of improved cell attachment, higher proliferation, and spreading (Peter, Ganesh, et al., 2010).

Silicon dioxide or silica (SiO_2) is a component of bioactive glass and is found to have apatite forming ability in SBF (Panjian, Ohtsuki, Kokubo, Nakanishi, & Soga, 1992). The ability of silica to induce apatite formation has already been examined. Hench proposed that a combination of high pH and repolymerization of SiO_2 from surface Si–OH groups is sufficient to accumulate CaO and P_2O_5 from the body fluids, thereby aiding the nucleation and growth of apatite layer (Hench, 1991). Karlsson, Froberg and Ringbom (1989) later proposed that silica chelates form an essential step in the formation and mineralization of hard tissues. The silica gel formed on the surface not only chelates but also provides sufficient atomic distance required by the crystal structure of bone apatite. Kokubo (1990) have proposed that hydrated silica formed on the surface of glass ceramics provides sites for favorable apatite nucleation. Panjian et al. (1992) studied the apatite forming ability of silica gel in SBF. They found that silanol groups (Si–OH) abundant on the surface of silica gel are responsible for apatite formation. Rhee, Lee, and Tanaka (2006) has developed chitosan membrane modified with silanol group and calcium ions and studied its bioactivity in SBF and found that they showed apatite forming ability within 1 day. Lee et al. (2009) has developed membrane of hybrid chitosan–silica xerogel for guided bone regeneration (GBR) and noted that these membranes showed high bioactivity *in vitro* compared to chitosan membranes. Addition of a material like silica can also improve the bioactivity and biocompatibility of chitin. Madhumathi, Sudheesh Kumar, et al. (2009) developed chitin composite scaffolds containing nanosilica using chitin hydrogel and their bioactivity, swelling ability and cytotoxicity were analyzed *in vitro*. These scaffolds were found to be bioactive and biocompatible when tested with MG63 cell line. Biocompatibility was observed to increase with increasing amount of powdered chitin/nanosilica scaffolds added to the media. These results suggest that chitin/nanosilica composite scaffolds can be useful for bone tissue engineering applications.

2.2. Wound dressing

Antibacterial resistance of microorganisms is one of the major problems faced in the field of wound care and management resulting in complications such as infection and delayed wound healing. Currently a lot of research is focused on developing newer

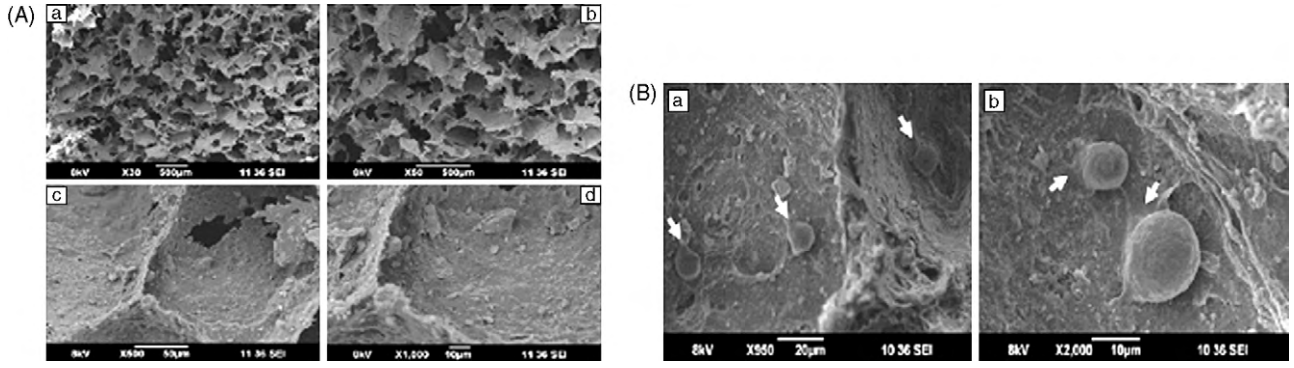

Fig. 3. (A) (a–d) SEM images of the macroporous structure of the composite scaffolds. Pore size ranged from 150 to 500 μm and (B) (a) SEM image of the cells attached to pore walls of composite scaffolds (white arrows) and (b) Higher magnification images showing initial signs of cell spreading (white arrows).

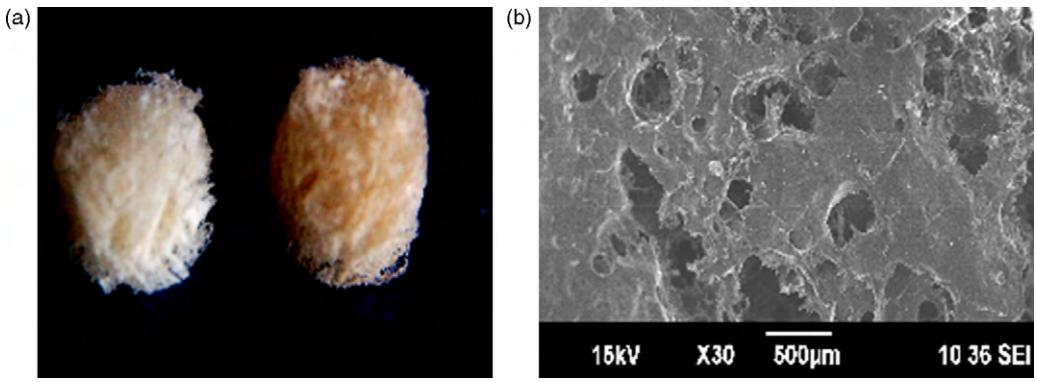

Fig. 4. (a) Picture showing chitin scaffold (left) and chitin/nanosilver composite scaffold (right) and (b) SEM image of chitin/nanosilver composite scaffold.

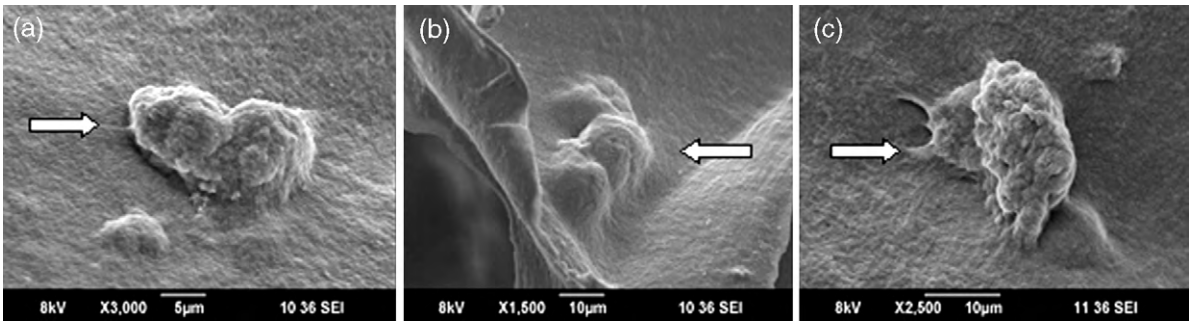

Fig. 5. SEM images of Vero cell attachments of (a) β-chitin control, (b) β-chitin + 0.001 and (c) β-chitin + 0.003% nanosilver composite scaffolds.

antibacterials to treat wounds infected with antibacterial resistant microorganisms. Silver has been used as an antibacterial agent for a long time in the form of metallic silver and silver sulphadiazine ointments. Recently silver nanoparticles have come up as a potent antibacterial agent and are finding diverse medical applications ranging from silver based dressings to silver coated medical devices (Rai, Yadav, & Gade, 2009).

A number of studies have reported the use of chitosan scaffolds and membranes to treat patients with deep burns, wounds, etc. Recently, Madhumathi et al. (2010) developed novel α-chitin/nanosilver composite scaffolds (Fig. 4a and b) for wound healing applications. These α-chitin/nanosilver composite scaffolds were found to possess excellent antibacterial activity against *S. aureus* and *E. coli*, combined with good blood clotting ability. These *in vitro* results suggested that α-chitin/nanosilver composite scaffolds could be used for wound healing applications.

Similarly, Sudheesh Kumar et al. (2010) developed and characterized β-chitin/nanosilver composite scaffolds for wound healing applications using β-chitin hydrogel containing silver nanoparticles. The antibacterial, whole blood clotting, swelling and cytotoxicity of the prepared composite scaffolds were studied. These β-chitin/nanosilver composite scaffolds were found to be antibactericidal against *E. coli* and *S. aureus* and showed good blood-clotting ability as well. In addition, β-chitin/nanosilver composite scaffolds were evaluated for their cell adhesion properties using epithelial cells (Vero cells). The attachment of the Vero cells to the surface of the β-chitin/nanosilver composite scaffolds (Fig. 5) suggests that nanosilver incorporated scaffolds are promising matrices providing good cell attachment apart from their antibacterial activity, which is ideal for wound healing applications.

The mechanism of action of silver nanoparticles has been proposed by Rai et al. (2009) to be the following. Silver nanoparticles

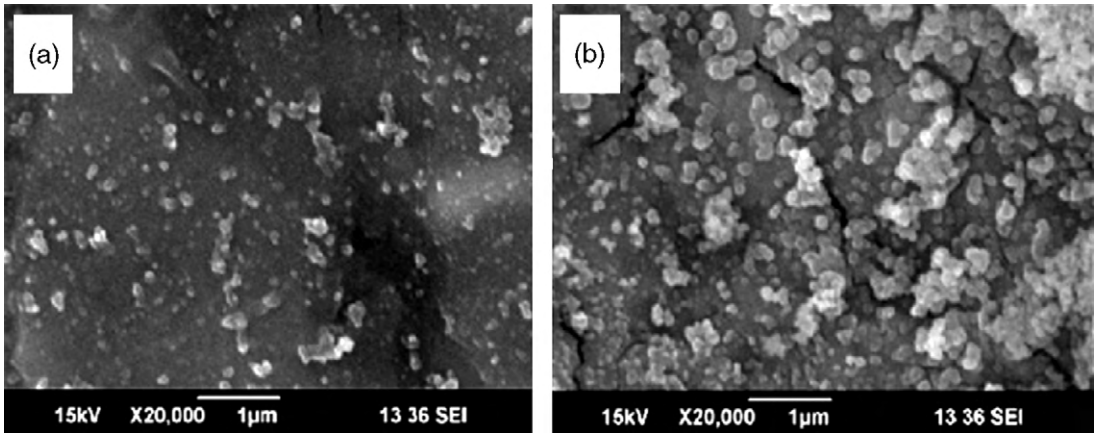

Fig. 6. SEM images of (a) CMC nanoparticles and (b) CMC nanoparticles loaded with 5-FU drug.

show efficient antimicrobial property compared to other salts due to their extremely large surface area, which provides better contact with microorganisms. The nanoparticles get attached to the cell membrane and also penetrate inside the bacteria. The cell membrane contains sulphur-containing proteins and the silver nanoparticles interact with these proteins in the cell membrane as well as with the phosphorus containing compounds like DNA. When silver nanoparticles enter the bacterial cell it forms a low molecular weight region in the center of the bacteria to which the bacteria conglomerates thus, protecting the DNA from the silver ions. The nanoparticles preferably attack the respiratory chain, cell division finally leading to cell death. The nanoparticles release silver ions in the bacterial cells, which enhance their bactericidal activity (Rai et al., 2009). The antibacterial response displayed by the chitin/nanosilver-based scaffolds can also be attributed to the above mechanism.

2.3. Drug delivery

Water-soluble carboxymethyl chitin (CMC) was used for drug delivery applications (Jayakumar, Prabaharan, Nair, Tokura, et al., 2010). CMC nanoparticles (Fig. 6) were prepared through cross-linking approach using $CaCl_2$ and $FeCl_3$ (Dev, Mohan, et al., 2010). The SEM images of CMC nanoparticles showed a spherical morphology with diameters in the range of 200–250 nm (Fig. 6a). The SEM images of 5-fluorouracil (5-Fu) drug-loaded nanoparticles also showed similar morphology (Fig. 6b). Cytotoxicity of CMC nanoparticles was evaluated with MTT assay and they were found to be non-toxic to normal fibroblast L929 mouse cells. The hydrophobic anticancer drug 5-Fu was loaded into CMC nanoparticles via emulsion cross-linking method. Drug release studies showed that the CMC nanoparticles provided a controlled and sustained drug release at pH-6.8. Moreover, the prepared nanoparticles were also found to be antibacterial and its ferromagnetic behavior allow for its potential use in drug tracking systems. These results indicated that the CMC nanoparticles are a promising carrier system for cancer drug delivery.

Dev, Binulal, et al. (2010) prepared poly(lactic acid) (PLA)/CS nanoparticles by emulsion method for anti HIV drug delivery applications. The hydrophilic antiretroviral drug lamivudine was loaded into PLA/CS nanoparticles. The encapsulation efficiency and *in vitro* drug release behavior of drug loaded PLA/CS nanoparticles were studied using absorption spectrophotometry. In addition, the cytotoxicity of PLA/CS nanoparticles using MTT assay was also studied. The *in vitro* drug release studies showed that the drug release rate from PLA/CS nanoparticles decreased when the pH of the medium changed from alkaline to acidic to neutral. The drug release rate was lower in the acidic pH when compared to alkaline pH. This may due to the repulsion between H^+ ions and cationic groups present in the polymeric nanoparticles. These results indicated that the PLA/CS nanoparticles are a promising carrier system for controlled delivery of anti HIV and cancer drugs (Dev, Binulal, et al., 2010).

2.4. Cancer diagnosis

Semiconductor nanocrystals (or quantum dots) are the most promising fluorescent probes for many biomedical applications. By appropriate bioconjugation, such nanocrystals can replace the conventional organic fluorescent dyes in immunostaining and bioimaging of tissues and cancerous cells. However, many of the quantum dots investigated for this purpose including cadmium sulphide, cadmium selenide, zinc selenide, etc. are cytotoxic owing to their heavy metal composition (Derfus, Chan, & Bhatia, 2004). A heavy-metal-free luminescent quantum dot (QD) based on doped zinc sulphide (ZnS), conjugated with a cancer-targeting ligand, folic acid (FA) has been developed as a promising bio-friendly system for targeted cancer imaging (Manzoor et al., 2009). Folate receptors are over-expressed on cancer cells and they provide a receptor-based endocytosis upon interaction with conjugated nanoparticles providing cellular uptake. Similarly, mannose receptors, which are also studied for their functional applications, targeted cancer diagnosis (Higuchi, Oka, Kawakami, & Hashida, 2008). A novel nanomaterial system based on mannosylated zinc sulphide (ZnS) exhibiting strong fluorescence emission and long stability has been synthesized using an aqueous chemistry route at room temperature (Sasidharan et al., submitted for publication). In this study, chitosan encapsulated ZnS nanoparticles were further functionalized with D-Mannose to yield mannosylated ZnS of size ~120 nm. *In vitro* cytotoxicity of the synthesized nanomaterials assessed using MTT assay suggests low cytotoxicity of the mannosylated ZnS nanoparticles towards both normal and cancer cell lines. Active targeting of cancer cells was attempted using the mannosylated nanoparticles. Fluorescence microscopic observations revealed the targeting specificity of mannosylated ZnS nanocrystals towards the mannose bearing KB cells, with no specific attachment on the normal cells. Our investigations highlight the role of nanomedicine in cancer through receptor mediated imaging via nanoparticles.

The targeted anticancer drug delivery as well as tracking the path of the drug carrier with a biofriendly heavy metal free quantum dot is a great contribution to cancer therapy. Manjusha et al. (2010) developed a novel folic acid (FA) conjugated carboxymethyl chitosan (CMCS) coordinated to manganese doped zinc sulphide (ZnS:Mn) quantum dot (FA-CMCS-ZnS:Mn) nanoparticles. The system can be used for targeting, controlled drug delivery and also imaging of cancer cells. The above multifunctional system was prepared by a simple and environment friendly aqueous route. The size range of 5-FU encapsulated FA-CMCS-ZnS:Mn nanoparticles ranged from 130 to 150 nm. The anticancer drug selected in this study was 5-Fluorouracil which can be used for the breast cancer treatment. The non-toxicity of FA-CMCS-ZnS:Mn nanoparticles were studied using L929 cells. Breast cancer cell line MCF-7 was used to study the imaging, specific targeting and cytotoxicity of the drug loaded nanoparticles. The *in vitro* imaging of cancer cells with the nanoparticles was studied using fluorescent microscopy. The bright and stable luminescence of quantum dots can be used to image the drug carrier in cancer cells without affecting their metabolic activity and morphology (Manjusha et al., 2010).

3. Conclusions

This review summarizes the biomedical applications of chitin and chitosan based nanomaterials in tissue engineering, wound dressing, drug delivery and cancer diagnosis. In addition, this review also opens up the novel applications for which these natural biopolymers can be put to use in a variety of nanostructural forms and sizes. Nanostructured composite scaffolds can be developed as promising tissue engineered constructs or for wound healing. Multifunctional use of chitin and chitosan based nanomaterials have been proved to aid simultaneous cancer targeting and drug delivery. We expect that this review will provide insights on the use of these important of chitin and chitosan nanomaterials for researchers working in nanobiotechnology.

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