Chitosan based hydrogels and their applications for drug delivery in wound dressings: A review

Hamid Hamedi, Sara Moradi, Samuel M. Hudson, Alan E. Tonelli

Textile Engineering Chemistry and Science, Fiber & Polymer Science Program, College of Textiles, North Carolina State University, Raleigh, North Carolina 27606-8301, United States

ARTICLE INFO

Keywords:
Chitosan hydrogel
Wound dressing
Drug delivery
Growth factor
Nanoparticles

ABSTRACT

Advanced development of chitosan hydrogels has led to new drug delivery systems that can release their active ingredients in response to environmental stimuli. This review considers more recent investigation of chitosan hydrogel preparations and the application of these preparations for drug delivery in wound dressings. Applications and structural characteristics of different types of active ingredients, such as growth factors, nanoparticles, nanostructures, and drug loaded chitosan hydrogels are summarized.

1. Introduction

Hydrogels are three-dimensional, cross-linked networks which can absorb and retain significant amounts of water, without dissolving or losing their three dimensional structures (Ahmed, 2015; Kashyap, 2005; Y.B. et al., 2008). The gelation and biodegradation are two key factors affecting the fate of cells (Li et al., 2012). Moreover, some hydrogels have antibacterial and antifungal activities (Jaya kumar et al., 2011), and these properties could be useful for wound dressings and accelerating the wound healing process (Ueno et al., 2001). Polymers that form hydrogels may have hydrophilic or hydrophobic functional groups. Hydrophilic functional groups, such as hydroxyl (−OH), amine (NH2) and amide (−CONH−CONH2) enable the hydrogel to absorb water leading to hydrogel expansion, which is known as swelling. During swelling, the cross-linked structure of hydrogels prevents complete destruction of the hydrogel cross-links and dissolution. Hydrogels with hydrophobic chains such as poly lactic acid have lower water swelling capacity than hydrophilic lattices. Hydrogels can be prepared from synthetic or natural polymers, involving a wide range of chemical compositions and with different mechanical, physical and chemical properties. Natural polymers such as alginate (Balakrishnan et al., 2005; Kamoun et al., 2015; Kim et al., 2008; Murakami et al., 2010), chitosan (Milosavljević et al., 2010; Racine et al., 2017), hyaluronic acid (Luo et al., 2000; Segura et al., 2005; Silva et al., 2017) cellulose (Abd El-Mohdy, 2013; Chang et al., 2009; Maneerung et al., 2008; Sannino et al., 2009), starch (Pal et al., 2006; Pal et al., 2006; Zhai et al., 2002), ulvan (Alvesab et al., 2012; Morelli & Chiellini, 2010), gelatin (Draye, Delaey, Voorde, Bulcke, Reu et al., 1998; Wang et al., 2012), pullulan (Li et al., 2011; Wong et al., 2011) and/or synthetic polymers like polyvinyl alcohol (Kokabi et al., 2007; Razzaq, 2001; Yang et al., 2008), polyacrylamide (Ezra et al., 2009; Rishud & Bhonde, 2000; Rosiak et al., 1983) and polyethylene glycol (Aiji et al., 2005; Gupta et al., 2011; Lib et al., 2012) form hydrogels.

Hydrogels are classified into two categories: chemical or permanent gels that have covalently bonded cross-linked networks (replacing hydrogen bonds by strong and stable covalent bonds) and physical or reversible gels whose networks are held together by molecular entanglements, and/or secondary forces including ionic, hydrogen bonding or hydrophobic interactions (Ahmed, 2015). In physically cross-linked gels, dissolution is prevented by physical interactions, which exist between different polymer chains (Hennink & Nostrum, 2002). Hydrogels can be formulated in a variety of physical shapes, including slabs, microparticles, nanoparticles, coatings, and films (Hoare & Kohane, 2008).

The most important property of hydrogels is their biocompatibility, which can be defined as the ability of a material to be in contact with bodily organs with minimum damage to the surrounding tissues and without triggering undesirable immune responses (Caló & Khutoryanskiy, 2015). In some cases this property has been taken advantage of in wound dressings, because after healing it prevents excessive tissue granulation and scar formation (Chung et al., 1994), though they can be hard to handle, may be difficult to load with drugs and sterilize, and usually have low mechanical strength (Huffman, 2012). In spite of these disadvantages, hydrogels have many applications in different fields including tissue engineering, pharmaceuticals and biomedical engineering, in the forms of wound dressings (Benamer...
et al., 2006; Kokabi et al., 2007; Lu et al., 2010), drug delivery vehicles (Hoare & Kohane, 2008; Qiu & Park, 2001; Xinyin, 2003), implants (Refojo & Leong, 1981; Stasica et al., 2000; ZZZZZ, 2018), and injectable polymeric systems (J.P. & T.H., 2006; Macaya & Spector, 2001; Shibata et al., 2010; Yu & Ding, 2008).

Chitosan is one of the most widely used materials for making hydrogels, and has been considered for wound dressing applications. It has excellent biocompatibility, low toxicity and immune-stimulatory activities (Li, Rodrigues et al., 2012; Souza et al., 2009). Due to these properties, chitosan shows good biocompatibility and positive effects on wound healing. It can also accelerate repair of different tissues and facilitate contraction of wounds (Jaya kumar et al., 2011). The Cytocompatibility of chitosan has also been studied and shown that it does not have acute cytotoxic effect (Goncalves Ferreira et al., 2016). On the other hand, as a natural based compound, chitosan may be contaminated by organic and inorganic impurities, and presents broad polydispersity. Chitosan is poorly soluble, except in acidic medium, which makes analyses difficult to perform (Croiser & Jerome, 2013).

Chitosan also has been used in commercial wound dressings such as ChitoSam™ (sam medical, 2018), ChitoGauze XR pro (North American Rescue, 2018), ChitoFlex (H.M.T. Inc., 2007), and Axistost® (AXIO, 2018) which are high-performance hemostatic dressings.

This review emphasizes on chitosan hydrogels and their applications in wound dressing and drug delivery. Initially chitosan and its properties are described and different kinds of hydrogel preparation methods are discussed. In the second section the utilization of chitosan hydrogels are summarized. Finally, utilization of some drugs such as growth factors, nano particles and chemicals in chitosan hydrogels are described.

2. Chitosan

Due to its reactive amino groups, chitosan is the only natural cationic polymer and has many commercial applications: it accelerates wound healing, is antimicrobial, anticoagulant, antibacterial, antifungal, anti-tumor, and haemostatic [62,63]. The generic term chitosan describes a range of presumably pure poly-(beta-1-4) N-acetyl-D-glucosamine materials (shown in Fig. 1) whose properties are highly dependent on its degree of deacetylation; average molecular weight, polydispersity, and its structure.

(Zhou et al. (2008)) investigated the effect of molecular weight and degree of deacetylation on chitosan hydrogels and concluded that these two parameters affected the pH values, turbidity, viscosity, and thermosensitive characteristics of the product hydrogels. Degree of deacetylation affects chitosan antimicrobial activity (Chung et al., 2004; Liu et al., 2001); on the other hand chitosan microspheres with a high degree of deacetylation (97.5%) lead to higher positive charge density, which confers stronger antibacterial activity than moderate degree of deacetylation (83.7%) against Staphylococcus aureus at pH 5.5 (Kong et al., 2008b). It was reported that polydispersity is a useful quantity which can clarify the degradation mechanism (Chen et al., 1997). The decrease of polydispersity as degree of deacetylation increases could appear as surprising since the deacetylation reaction does not degrade chitosan chains (Schatz et al., 2003). Min et al. (Tsai et al., 2008) have studied factors that are effective on chitosan polydispersity, such as, solution temperature and solution concentration of chitosan. They also explained why the polydispersities of chitosan nanoparticles obtained by ultrasonic treatment or by mechanical shearing were similar. The structures of chitosan hydrogels can affect their swelling behaviors, because swelling behavior reflects the amount of network porosity and mesh size of the network, which may also indicate its drug release behavior (Berger et al., 2004). Porosity of chitosan membranes could be modified by crosslinking with some materials such as glutaraldehyde (Krajewska, 2001).

Chitosan under acidic condition also has a high capacity for entrapping various metal ions including (Ni^{2+}, Zn^{2+}, Cu^{2+}, Fe^{3+}, Mg^{2+} and Cu^{2+}) (Kurita, 1998). Rainaudo et al. (Mazeau et al., 2000) have studied chitosan chain stiffness, and concluded that chitin and chitosan are semi-rigid polymers characterized by a persistence length (asymptotic value obtained at high degree of polymerization) that depends moderately on the degree of acetylation of the molecule.

2.1. Sources of chitosan

Chitin, poly (β-(1→4)-N-acetyl-D-glucosamine) is a natural polysaccharide, first identified in 1811 by French chemist Henri Braconnot (Arias et al., 2004). Chitin forms a part of the extracellular matrix of certain living organisms as a proteoglycan. It is the most abundant polymer after cellulose. This biopolymer is synthesized by an enormous number of living organisms among which are insects (cuticles) and crustaceans (skeletons), e.g., crab, shrimp and lobster. Cephalopod by-products are a valuable source of chitin, polyunsaturated acids, and collagen. (Koueta, 2014). Chitin is also extracted from the exoskeleton of cephalopod species, such as squid, cuttlefish and octopi (Arrouze et al., 2017; Jothi & Nachiyar, 2013; Kurita et al., 1993; Shanhugum et al., 2016). (See Fig. 1).

Chitosan, which was discovered and named in 1859 by Roget (Patrulea et al., 2015), is obtained by partial deacetylation of chitin under alkaline conditions and is the most important chitin derivative in terms of applications (Rainaudo, 2006b). (See Fig. 1). The physicochemical characteristics of the obtained chitosan are closely related to the taxonomy of the marine sources (Rhazi et al., 2000). Chitosan can
also be produced by enzymatic rather than alkali treatment at high
temperatures (Cai et al., 2006; No & Meyers, 1995). Chitosan can be
enzymatically degraded by chitinases and chitosanases but these en-
zymes are unavailable in bulk quantities for commercial applications.
It has been reported that β-glucosidase from almond emulsin can hydro-
lyze chitin substrates owing to an existing chitinase in the enzyme
preparation (Zhang & Neau, 2011). It has been reported that chitinases
are not responsible for the in vitro degradation of chitosans in human
serum (Vårum et al., 1997). Enzymatic degradation minimizes adverse
chemical modifications of products and promotes their biological ac-
tivities. However, higher costs of hydrolytic enzymes limit the appli-
cation of enzymatic methods. To reduce this production cost, reuse
of hydrolytic enzymes may be recommended (Kim & Rajapakse, 2005).

Chitosan preparation from fungal cell walls with fermentation
technology has become an alternative economical way for the pro-
duction of this polymer. It can be found in the cell wall of certain
groups of fungi, particularly zygomycetes (Nwe et al., 2002; Tayel et al.,
2010). There are several advantages of using these fungi to produce
chitosan. The most important is that the cell wall of zygomycetous fungi
contains a large quantity of chitosan and the physicochemical prop-
ties of this chitosan can be manipulated and standardized by controlling
the parameters of fermentation. For example, different molecular
weight chitosans can be produced when these fungi are grown on at
different pHs and on media with different compositions (Tan et al.,
1996). Sufficient amounts of chitosan have been identified in Mucor
rouxi (White et al., 1979), phycomycetes, and saccharomycetes (Arcidiaco-
no & Kaplan, 1992). In another study, chitosans with high degree of dea-
cetylation (above 97%) were extracted from R. miehei and M. racemosus
(Tajdini et al., 2010).

An investigation was performed to evaluate the chitosan char-
acterization produced by several species of fungi. Comparison of fungal
chitosan with a commercial one derived from crab shells showed lower
degree of deacetylation, viscosity and molecular weight of the fungal
chitosan. Chitosan with a low molecular weight reduces the tensile
strength and elongation of the chitosan membrane, but increase its
permeability. Fungal chitosan may have potential medical and agri-
cultural applications (Pochanavanich & Suntornsuk, 2002). This is in
general agreement with the findings of Yen et al. (Yen & Mau, 2007;
Yen, Yang, and Mau, (2009)).

2.2. Solubility of chitosan

Due to the high cohesive energy of chitin, which is related to strong
intermolecular interactions from hydrogen bonds, chitin is insoluble in
many typical solvents, such as water, dilute acids and alcohols (Zargar
et al., 2015). This is a major problem that limits the development of
processing and usage of chitin.

Chitosan, the most important derivative of chitin, is soluble in dilute
aqueous acidic media at a degree of deacetylation of 50% and higher
(depending on the origin of the polymer) due to its primary amino
groups that have a pKa value of 6.3. Solubilization occurs by protona-
tion of the −NH2 group of the D-glucosamine repeating unit, whereby
the polysaccharide is converted to a polyelectrolyte in acidic media.
Solubility of chitosan is usually examined in acetic acid by dissolving it
in 1% or 0.1 M acetic acid (Rinaudo, 2006a). Rinaudo et al. (Rinaudo,
Pavlov, and Desbrie`res, (1990)) demonstrated that the amount of acid
needed depends on the quantity of chitosan to be dissolved. The con-
centration of protons needed is at least equal to the concentration of
− NH2 units involved. As the degree of protonation is progressively
increased, so is the solubilization of chitosan. The effect of depoly-
merization on the solubility of the chitosans with different degree of
deacetylation at neutral pH-values was investigated by Vårum et al
(Varum et al., 1994). They emphasized that the solubility differences
between chitosans with different degree of deacetylation may have
profound influence on accessibility of chitosans to enzymes (many en-
zyme assays are performed at pH 6.5) and the biological effects of
chitosans (testing of chitosans in vivo and in vitro are often performed at
neutral pH-values).

Sorli et al. (Sorli et al., 2002) demonstrated that when the degree of dissociation (α) in solution increases, the role of the
cationicity of the amine groups, which depends on the degree of acet-
ylation, plays a more important role on the behavior of the polymer
chains. They showed that molecular weight and degree of deacetylation
have important effect on chitosan solubility.

Solubility of chitosan will be enhanced by decreasing its molecular
weight. Molecular weight changes the content of N-acetylglucosamines
units in chitosan, which will have intramolecular as well as an inter-
molecular influences, resulting in different chitosan conformations.
However, improving solubility by controlling deacetylation comes at
the cost of low yield (Kurita et al., 1991). Sogias et al. (Sogias,
Khutoryanskiy, and Williams, (2010)) showed that break down of chitosan
crystallinity will expand the range of chitosan solubility. They studied
two approaches for improving chitosan solubility, physical and che-
metrical methods. In their chemical approach, re-acetylation improved
chitosan solubility up to pH = 7.4. The physical approach included the
use of additives, such as urea, and guanidine hydrochloride, with the
abilities to disrupt intra- and inter macromolecular hydrogen bonding.
In the presence of 8 mol L\(^{-1}\) urea and 1.5 mol L\(^{-1}\) guanidine hydro-
chloride the solubility of chitosan shifted to pH = 8.0 and 6.5, respec-
tively.

Introducing small chemical groups to the chitosan structure, such as
alkyl (hydroxypropyl chitosan (Xie et al., 2002)) or carboxymethyl
groups can drastically increase the solubility of chitosan (Jayakumar
et al., 2010; Pillai et al., 2009). A simple and improved method of
preparing highly soluble chitosan (half N-acetylated chitosan) was de-
developed using a series of chitosan samples of low molecular weights
(Kubota et al., 2000).

2.3. Toxicity

There are various studies on chitosan toxicity. Scientists have done
some studies on chitosan toxicity in guinea pigs (Aspden et al., 1997),
frog, human nasal palate tissue (Aspden et al., 1994; Aspden et al.,
1995), and the nasal membranes of rats (Aspden et al., 1996). In all of
the tests, toxicity was negligible. Ribeiro et al. (Ribeiro et al. (2009))
studied the in vitro cytotoxicity of chitosan hydrogels tested with dermal
fibroblasts obtained from rat skin. Their cell viability study showed that
the hydrogel and its degradation by-products are non-cytotoxic. His-
tological analysis also revealed lack of a reactive or a granulomatous
inflammatory reaction in skin lesions with chitosan hydrogels, and the
absence of pathological abnormalities in the organs supported the local
and systemic histocompatibility of this biomaterial.

In vivo chronic toxicity studies reported by Carreño-Gómez et al.
(Carreño-Gómez & Duncan, 1997) have shown that intravenous ad-
ministration of chitosan (4.5 mg/kg/day for 11 days) to rabbits did not
lead to any abnormal changes. Data on chitosan toxicity from human
studies in general are quite limited. Gades et al. (Gades & Stern, 2003)
reported that quantities exceeding 4.5 g chitosan taken daily by human
volunteers did not result in toxic effects. Even higher oral levels of up to
6.75 g were reported as safe. It has been reported that short-term
human trials of up to 12 weeks have shown no clinically signi-
ficant symptoms, including no evidence of an allergic response (Tapola et al.,
2008). Chitosan mouthwash safety was evaluated through Ames, MTT
and V79 chromosomal aberration assays. The chitosan mouthwash
possessed lower toxicity and higher antimicrobial activity than the
commercial mouthwash tested. Furthermore, while it is capable of
completely inhibiting the two selected pathogenicity markers (strept-
tococci and enterococci), contrary to the commercial mouthwash, it did
not cause major reductions in the viability of the normal oral microflora
(Costa et al., 2014).

Ravindranathan et al. (Ravindranathan, Koppolu, Smith, and
Zaharoff, (2016)) Studied purified, low endotoxin chitosan, and
determined that viscosity/molecular weight and degree of deacetylation within the ranges of 20–600 CP and 80–97%, respectively, have no impact on chitosan’s immunoreactivity. Endotoxin contamination was expected to be a major factor influencing immunoreactivity. Their results indicated that only endotoxin content and not degree of deacetylation or viscosity influenced chitosan-induced immune responses. Their data also indicated that low endotoxin chitosan (< 0.01 EU/mg) with viscosities of 20–600 CP and 80%–97% degree of deacetylation are essentially inert. This study highlights the need for more complete characterization and purification of chitosan in preclinical studies before being used in clinical application.

Baldrick (Baldrick (2010)) implies that chitosan can be used as a safe pharmaceutical excipient for non-parenteral and non-blood contact. Based on the available data, safe use of chitosan as a parenteral excipient is not clear. Its use as a medical device to control bleeding relies on the haemostatic biological nature of the material and studies have demonstrated local findings, such as blood coagulation, thrombus formation and platelet adhesion/aggregation. In spite of some in vitro studies related to cytotoxicity findings (Baldrick (2010)), there are reports of non-toxic usages of chitosan in pharmaceuticals, such as to stop bleeding (Dowling et al., 2011; Horio et al., 2010; Valentine et al., 2010).

2.4. Biomedical properties

The degradation rate of chitosan can be controlled by changing its degree of deacetylation and/or its molecular weight (Tomihata & Ikada, 1997). Many researches have been done to prove the degraded products of chitosan are nontoxic, non-immunogenic, and non-carcinogenic (Muzzarelli, 1993). Risbud et al. (Risbud & Bhonde, 2000) reported characterization, biocompatibility, and release properties of polyacrylamide/chitosan hydrogels and showed that chitosan possess excellent biocompatibility and bioabsorbability. In this research, in vitro cytotoxicity studies using a colorimetric assay for assessing cell metabolic activity, membrane permeability, and lysosomal activity of cells (MTT and NR assays) showed no toxic effects on different cells in up to 40% hydrogel extract.

The antimicrobial properties of chitosan and its derivatives have attracted great attention from researchers (Kong et al., 2010). Antimicrobial activity of chitosan has been demonstrated against many bacteria, filamentous fungi, and yeasts. Chitosan has a wide spectrum of activity and high killing rates against gram-positive and gram-negative bacteria (Uno et al., 1997), with low toxicity toward mammalian cells.

Antibacterial effects of chitosan are reported to be dependent on its molecular weight (Jeon et al., 2001). To prove this idea, No et al. (No, Park, Lee, and Meyers, 2002) examined the antibacterial activity of six chitosans and six chitosan oligomers with widely different molecular weights against gram-negative and gram-positive bacteria. They showed that chitosan markedly inhibited the growth of most bacteria tested, although their inhibitory effects differed with molecular weight and the particular bacterium. Chitosan generally showed stronger bactericidal effects for gram-positive bacteria than for gram-negative bacteria. The reason for this difference is unclear, but Y. J. Jeon et al. (Jeon et al., 2001) showed the oligosaccharides as well as chitosan exhibited better inhibitory effects against Gram-positive compared with Gram-negative bacteria. Thus, it might be reasonable that chitosan showed remarkable inhibitory effect against the growth of lactic acid bacteria which are Gram-positive.

Sizes of chitosan particles play an important role in inflammamsome activation. The inflammamsome is a cytosolic complex which is responsible for the activation of inflammatory responses, such as IL-1β. Glycans passed through filters of defined size, showed that the smallest size fraction (< 20 μm) induced the most cleavage of pro-IL-1β and release of the mature cytokine. The larger-sized fractions also had some bioactivity, which may have been due to some smaller particles that failed to pass through the filters. Partial digestion of chitosan with pepsin, an enzyme that breaks down proteins into smaller peptides, enhanced the ability of the glycan to activate the inflammamsome (Bueter et al., 2011).

Biodegradability of chitosan has been studied by many researchers. It was found that lysozyme (Onishi & Machida, 1999), proteases (Rao & Sharma, 1997) and porcine pancreatic enzymes (Verheul et al., 2009) are able to degrade Chitosan under specific conditions. Pectinase isozyme from Aspergillus niger has also been shown to digest chitosan at low pH providing lower Mn, chitosans (Kittur et al., 2003; Kittur et al., 2005). Connell et al. (Connell et al., 2008) used human fecal preparations and showed significant degradation of chitosan films, glu taraldehyde crosslinked films, and tripolyphosphate crosslinked films. Brenner et al. (Brenner, Hostetter, and Humes, 1978)) demonstrated that many enzymes seem to show an activity against chitosan, at least in vitro, but derivatives could give indigestible molecules. To be clinically viable, these compound particles should remain small enough (< 42 Å radius for neutral molecules) to be renally excreted. The bio-distribution is controllable through the route of administration, dosage form and chitosan characteristics, i.e., a film/dense matrix will persist at the site of administration due to the physical characteristics. The degradation of the matrix is controllable through crosslinking modification of the amine groups. Intravenous distribution can be adjusted by particle size (< 100 nm), and molecular weight can determine the stability of the particle (Kean & Thanou, 2010).

2.5. pH sensitivity

The numerous amine groups on the chitosan backbone confer a pH-dependent solubility, which has been utilized to fabricate biosensors and drug delivery systems. Chitosan properties can be conveniently altered by derivatization of its hydroxyl and amino groups (Zhang et al., 2012). The amino groups of chitosan are ionized in acidic buffers, which contribute to the electrostatic repulsion between adjacent ionized –NH2 groups and lead to chain expansion and eventually increase the water absorption of the chitosan gels. The swelling degree of the ionic pH-sensitive hydrogel is mainly determined by the degree of ionization (Guanghua et al., 2008). Some attempts have been made for regulating chitosan pH-sensitivity by adding different additives or grafting or mixing with other polymers and preparing Polyelectrolyte complexes with anionic polymers (Krisha Rao et al., 2006; Meng et al., 2010; Qu et al., 1999b; Qu et al., 2000). The nature of the pH response of chitosan and its derivatives has been described by several researchers. Islam et al. (Islam & Yasin, 2012) synthesized a pH sensitive blend based on chitosan and poly vinyl alcohol (PVA) using a nontoxic cross linker for a drug delivery system. They concluded that the pH sensitivity and biocompatibility properties of this chitosan/PVA hydrogel made it suitable for medicinal and pharmaceutical applications. Antimicrobial activity of chitosan is also pH dependent, it has been reported that chitosan displayed antibacterial activity only in an acidic environment (Helander et al., 2001).

3. Chitosan hydrogel preparation methods

3.1. Chemical Cross linking (permanent hydrogels)

Chemically cross-linked hydrogels are formed by covalent linking of the chitosan macromers, where the bond formation is irreversible. The cross-linking of natural and synthetic polymers can be achieved through the reaction of their functional groups (such as OH, COOH, and NH2) with cross-linkers (Liu et al., 2014; Milosavljević et al., 2010; Singh et al., 2006; Zhang et al., 2005). There are a number of methods reported in the literature to obtain chemically cross-linked permanent hydrogels. The following section reviews the major chemical methods used to produce chitosan hydrogels.
3.1.1. Chemical cross-linkers

Cross-linkers are molecules with at least two reactive functional groups that allow the formation of bonds between polymeric chains. To date, the most common cross-linkers used to obtain chitosan hydrogels are di-aldehydes, such as glutaraldehyde (Milosavljević et al., 2010; Zhang et al., 2005), formaldehyde (Singh et al., 2006), or ethylene glycol di-glycidyl ether (EGDE) (Liu et al., 2014), genipin (Mi et al., 2000; Muzzarelli, 2009b) and others (Draye, Delaey, Voorde, Bulcke, Bogdanov et al., 1998; Hennink & Nostrum, 2002; Huffman, 2012) (See Fig. 2).

Among these mentioned cross-linkers, reaction of aldehydes with chitosan is well-documented; the aldehyde groups form covalent imine bonds with the amino groups of chitosan, due to the resonance established with adjacent double ethylenic bonds (Monteiro & Airoldi, 1999). Di-aldehydes allow direct reaction in aqueous media, under mild conditions and without the addition of auxiliary molecules, such as reducers, which is advantageous with respect to biocompatibility. However, the main drawback of di-aldehydes, such as glutaraldehyde, is that they are generally considered to be toxic. It is reported that glutaraldehyde is cytotoxic even at low doses and glutaraldehyde polymerization may release glutaraldehyde residues during storage or sterilization (Chang et al., 2004; MacDonald & Pepper, 1994). Glutaraldehyde is known to be neurotoxic, its fate in the human body is not fully understood. Therefore, even if hydrogels are purified before administration, the presence of free unreacted di-aldehydes in hydrogels could not be completely excluded and may induce toxic effects. This could impair the biocompatibility of the cross-linked products (Berger, Reist, Mayer, Felt, Peppas et al., 2004; Chen et al., 2004).

In order to obtain non-soluble hydrogels for controlling drug release and pharmaceutical applications, researchers have been attempting to find a cross-linking agent with low cytotoxicity (Chen et al., 2004; Cui et al., 2014; Delmar & Bianco-Peled, 2015). Genipin is a natural cross-linking agent extracted from geniposide, an iodoid glucoside isolated from the Genipa fruits (Tsai et al., 1994). Genipin has been reported to bind with biological tissues (Sung et al., 2001) and biopolymers, such as chitosan and gelatin (Bigi et al., 2003; Mi et al., 2005), leading to covalent coupling. It works as an effective cross-linker for polymers containing amino groups (mostly used for chitosan crosslinking) (Sung et al., 1999). It has been found that the reaction rate of genipin with amino group containing biomaterials is significantly slower than that of glutaraldehyde (Moura et al., 2007; Sung et al., 2000). The reaction between chitosan and genipin (See Fig. 3) is well understood for a variety of experimental conditions and has no cytotoxicity for human and animal cells (Muzzarelli et al., 2015). Biocompatibility, biodegradability and pharmaceutical effects of the genipin-crosslinked hydrogels were investigated, and it was concluded that genipin may be well suited for clinical usage (Kitano, 2006; Muzzarelli, 2009a; Yaoa et al., 2005; Zhang et al., 2006). Effects of genipin cross-linking of chitosan hydrogels on cellular adhesion and viability were investigated. It was found that the cross-linked hydrogels did not induce cytotoxic effects and significantly improved the cell adhesion and viability on hydrogel surfaces (Gao et al., 2014). Genipin has also been shown to act significantly as an anti-inflammatory and anti-angiogenesis agent, and protected the hippocampal neurons from the toxicity of Alzheimer’s amyloid beta protein (Kooa, 2004).

3.1.2. Photo crosslinking

Photo-crosslinking, a type of chemical crosslinking, is performed in the presence of ultraviolet (UV) light and a chemical photo initiator (PI). A variety of natural and synthetic macromers have been modified to form hydrogels by photo-activation of the coexisting photo initiators, such as methacrylated or aryl azide modified macromers (Rickett et al., 2011). The degree of crosslinking reaction depends on UV irradiation time and increases with the increase of exposure time. This lead to a hydrogel with higher mechanical properties and lower swelling ratio (Ik et al., 2016). The polymerization reaction can also be controlled by adjusting the distance from the UV light source (Ahmadi, Oveis, Mohammadmi Samani, & Amoozgar, 2015). Obara et al.. (Obara et al. (2003)) prepared a photo cross-linkable chitosan aqueous solution including fibroblast growth factor-2 (FGF-2) by using ultraviolet light (UV) irradiation to result in an insoluble, flexible hydrogel. A viscous azide-chitosan-lactose aqueous solution was converted into an insoluble hydrogel within 10 s upon UV-irradiation at a lamp distance of 2 cm through crosslinking of the azide and amino groups of the azide-chitosan-lactose molecules. The results showed that the growth factor FGF-2 molecules remained biologically active, and were released from the chitosan hydrogel upon in vivo biodegradation. Application of the chitosan hydrogel significantly induced wound contraction and accelerated wound closure.

Among the photo initiators, Irganacure2959 caused minimum cytotoxicity (cell death) over a broad range of mammalian cell types and species (Hu & Gao, 2008). Pluronic-chitosan hydrogels used as a dressing for ulcers wounds were fabricated, and the release of growth factor was investigated. Photo-irradiation was applied to chemically cross-link the hydrogel. Before irradiation by UV, a photo-initiator, Irganacure2959 (0.1%, w/w), was added to the hydrogel mixture to form physical hydrogels. Release profiles of rEGF growth factor from hydrogel networks were evaluated with modification of the chitosan blend ratios and photo-irradiation time. At the same photo-irradiation time, chitosan accelerated rEGF release due to Pluronic chains (b-PPO-b-PEO-b-PPO) inhibiting the chitosan from being tightly crosslinked because of hydrophobic interactions. Increasing exposure time led to a tight network which can control growth factor release (Choi & Yoo, 2010b).

3.1.3. Graft polymerization

Graft Polymers are segmented copolymers with a linear backbone made of one polymer and randomly distributed branches made of another polymer. Grafting of natural polymers such as chitosan containing two types of reactive groups is of considerable interest for modification of polymer structure. Chitosan has two types of reactive groups that can be grafted; the free amine groups on deacetylated units and the
hydroxyl groups on the C3 and C6 carbons of acetylated or deacetylated units. Recently researchers have shown that chitosan derivatives, such as hydroxypropyl chitosan (HPCT) and carboxymethyl chitosan sodium (CMCTS) functionalized by improved water solubility, antibacterial, and antioxidant properties (Alves & Manoa, 2008; Xie et al., 2002; Xie et al., 2001).

3.1.3.1. Chemical grafting. Chemical grafting is initiated by generating one or more free radicals on the chitosan chain and allowing these radicals to react with polymerizable monomers that build the grafted chain (Girin et al., 2012). In recent years, a number of initiators such as Ferrous Ammonium Sulfate (FAS), Potassium PerSulfate (PPS), Ceric Ammonium Nitrate (CAN), and Ammonium PerSulfate (APS) have been developed to initiate grafting copolymerization.

Yazdani-Pedram et al. (Yazdani-Pedram, Retuert, and Quijada, 2000) modified chitosan by grafting with polyacrylic acid, a well-known hydrogel forming polymer. The reaction was carried out in a homogeneous aqueous phase by using PPS and FAS as the redox initiators. It was observed that the efficiency of grafting depended on monomer, initiator, and ferrous ion concentrations, as well as reaction time and temperature. Also the level of grafting could be controlled by varying the amount of ferrous ions used as a co-catalyst in the reaction.

Jung et al. (Jung, Chung, and Lee, 2006) prepared a chitosan and eugenol hydrogel to enhance and maintain antioxidant activities by using CAN as initiator. Eugenol is generally recognized as a safe material by the Food and Drug Administration, so it can be used in biological applications.

The graft yield increased with the concentration of CAN. Thermal stabilities of chitosan alone and eugenol-chitosan hydrogels were measured using TGA analysis and it was observed that the eugenol-chitosan hydrogels have a faster thermal decomposition in comparison with that of chitosan alone. This is likely because the introduction of the bulky eugenol side chains inside the hydrogel decreased the crystalline regions of chitosan. They observed that the equilibrium swelling ratio of eugenol-grafted chitosan hydrogels is smaller than that of chitosan gels and decreased with increased graft yield, because of the hydrophobicity of the attached eugenol molecules. Because chitosan is a cationic polymer, the swelling ratio of chitosan hydrogels decreased with increasing pH values, but the swelling ratios of the more hydrophobic eugenol-grafted chitosan hydrogels were not affected by the pH of the media, because chitosan amino groups, which were pH sensitive, were grafted with eugenol.

The pH responsive property of chitosan hydrogels can be explained as indicated in Fig. 4 (Zou et al., 2015). At low pH, the amide groups on the chitosan can become protonated and the resulting electrostatic repulsion between the protonated amino groups weaken the intermolecular and intramolecular hydrogen bonding interaction of chitosan molecules. Consequently, buffer solution can easily diffuse into the network and lead to increased swelling ratios. In neutral and basic conditions, no protonation occurs and the swelling ratio is low.

Mahdavinia et al. (Mahdavinia, Pourjavadi, Hossein zadeh, and Zohuriaan, 2004) introduced graft copolymerization of mixtures of acrylic acid (AA) and acrylamide (AAm) onto chitosan by using PPS as a free radical initiator and methylene bis-acrylamide (MBA) as a cross linker. It was concluded that the swelling capacity of the hydrogels is affected by the MBA cross linker concentration and monomer ratio, so that the swelling decreases with increased MBA concentration and AAm/AA ratio. They also concluded that this pH-sensitive super-absorbent poly-ampholytic network may be considered as an excellent candidate to design influential drug delivery systems.

3.1.3.2. Radiation grafting. Grafting can also be initiated by the use of high energy radiation from gamma rays and from electron beams. Radiation graft copolymerization is a well-established technique for producing polymeric materials which combine chemical and physical properties of both base polymer and grafted monomer. Grafting copolymerization of thermal and pH-sensitive chitosan and N-isopropyl acrylamide hydrogels were investigated by γ-radiation (Cai et al., 2005). In this research the effects of monomer concentration and irradiation dose on grafting percentage and efficiency were examined.

Grafting percentage and efficiency increased with increased monomer concentration and total irradiation dose. However, γ-radiation degrades chitosan. Sterilization of chitosan by γ-radiation leads to discoloration and loss of amino groups and lowering of molecular weight.

Compared to other methods, radiation crosslinking doesn’t require any additives to start the process, so the final product contains only polymer. Moreover, ionizing radiation usually provides combination of the synthesis and sterilization of polymeric materials in a single technological step, which leads to reduced cost and production time. Therefore, ionizing radiation or electron beam radiation methods are an excellent tool in the fabrication of materials for biomedical applications (Zhao & Mitomo, 2008). A series of hydrogels were prepared from polyvinyl alcohol (PVA) and carboxy methylated chitosan with electron beam irradiation at room temperature. The mechanical properties and degree of swelling improved substantially after adding chitosan into the PVA hydrogels. These blend hydrogels, even at low concentration of chitosan (3 wt%), exhibited satisfactory antibacterial activity against E. coli (Zhao, 2003). In another study, N-maleoyl-chitosan was synthesized by reaction of chitosan with maleic anhydride (MAH) in N,N-dimethyl formamide (DMF) via electron beam irradiation. Grafting yield and grafting efficiency increased with increasing absorbed radiation dose and monomer amount, and then decreased. Usually, the free radicals in

![Fig. 4. Mechanism of pH-responsive swelling behavior of chitosan (Zou et al., 2015).](image-url)
the reaction system increase with dose and then eventually decay. The swelling ratio of the copolymer hydrogel was low at pH 4–5 (Fan et al., 2009).

### 3.2. Physical Cross-linking (reversible hydrogels)

Physically cross linked gels have attracted much interest recently due to the absence of chemical crosslinking agents and reagents. Many chemical crosslinking agents are toxic compounds which can be detached or isolated frequently from prepared gels before application. They can also affect the nature of the substances when entrapped (e.g., proteins, drugs, and cells) (Kamoun et al., 2015). The main disadvantages of physical gels are their instability (uncontrolled dissolution may occur), low mechanical resistance, and difficult control of pore size (Croisier & Jérôme, 2013). Also free chain ends or chain loops are also present as transient network defects in physical gels (Huffman, 2012). Some of the most common physical cross-linking methods for chitosan hydrogel preparation are now described.

#### 3.2.1. Hydrophobic interaction

Polymers with hydrophobic domains can crosslink in aqueous environments via reverse thermal gelation, also known as ‘sol-gel’ chemistry. The hydrophobic segment is coupled to a hydrophilic polymer segment by post-polymerization grafting or by directly synthesizing a block copolymer to create a polymer amphiphile. These amphiphiles are water soluble at low temperature. But by increasing the temperature, hydrophobic domains aggregate to minimize the hydrophobic surface area contacting the bulk water, reducing the amount of structured water surrounding the hydrophobic domains, and maximizing the solvent entropy. The temperature at which gelation occurs depends on the concentration of the polymer, the length of the hydrophobic block, and the chemical structure of the polymer (Hoare & Kohane, 2008).

Tien et al. (Tien et al., 2003) studied the N-acylation of chitosan with fatty acyl chlorides to introduce hydrophobicity for use as a matrix for drug delivery. They concluded that hydrophobic interactions enhance the stability of substituted chitosan via hydrophobic self-assembly. Also release of drugs depended on the acyl chain length and the degree of acylation and could be managed by diffusion, or by swelling followed by diffusion. An injectable in situ thermosensitive chitosan–β-glycerophosphate (C–GP) gel formulation has been proposed for tissue repair and drug delivery (Ruel-Gariépya et al., 2002). The sol/gel transition can be summarized as a competition between several intermolecular interactions: 1) the electrostatic repulsion between positively charged chitosan molecules, 2) a screening effect on this repulsion, induced by the negatively charged β-glycerophosphate, and 3) attractive interactions due to hydrophobicity and hydrogen bonding between the chitosan molecules (Supper et al., 2013). This system can release macromolecules over a period of several hours to a few days. The results presented in this work indicated that the liposome–C–GP system could be used for the delivery of small molecular weight hydrophilic compounds. The release profile of the incorporated compound can be controlled by adjusting liposome characteristics, such as size and composition. This formulation can be a good candidate for local release of drugs and biomedical applications which require the local and controlled delivery of pharmaceuticals, such as growth factors in tissue repair.

#### 3.2.2. Polyelectrolyte complexation

Formation of chitosan hydrogels by polyelectrolyte complexation (PEC) has become an alternative for cross-linking. In the last decade there has been an increasing interest in the use of PEC gels formed by chitosan and polyanions as carriers for drug delivery systems. PECs are...
generally biocompatible networks exhibiting interesting swelling characteristics. PEC gels formed by the electrostatic attractions between two oppositely charged polyelectrolytes in aqueous solution are known to exhibit unique physical and chemical properties. For instance, the electrostatic interactions within the PEC gels are considerably stronger than most secondary binding interactions. The electrostatic attraction between the cationic amino groups of chitosan and the anionic groups of the other polyelectrolyte is the main interaction leading to the formation of PECs (Argin-Soyal et al., 2009; Berger, Reist, Mayer, Felt, Gurny et al., 2004). However, the main drawback of these systems is their difficult preparation, especially in large-scale processes (Berger, Reist, Mayer, Felt, Gurny et al., 2004; Long & Luyen, 1996).

Archana et al. (Archana, Dutta, and Dutta, 2013) have studied a ternary nano-dressing consisting of titanium dioxide (TiO2) nanoparticles loaded into a chitosan pectin polyelectrolyte complex that was prepared to evaluate biocompatibility, and antimicrobial and particles loaded into a chitosan pectin polyelectrolyte complex that was ionized amino groups of chitosan (NH2) and the ionized carboxyl acid groups (COO−) of pectin are the main interactions leading to the formation of the pectin/chitosan PECs illustrated in Fig. 5. The tensile strength of the blended dressing material increased with decreasing pectin content (1:1) and incorporation of TiO2 nanoparticles. This nano-dressing exhibited superior antimicrobial activity against five pathogens (E. coli, S. aereus, P. aeruginosa, B. subtilis and A. niger) and good blood-compatible properties. The chitosan–pectin–TiO2 dressing material could control evaporative water loss from wound beds at an optimal level and absorb more exudates, keeping the wound beds moist without risking dehydration or accumulation of exudates. Also the nano-TiO2 particles in the chitosan pectin matrix have been shown to decrease cytotoxicity.

Structurally stable chitosan and γ-poly glutamic acid (γ-PGA) PEC hydrogel formed through simple ionic complex interaction, between the amine groups of chitosan and the carboxylic acid groups of γ-PGA, was confirmed by FTIR spectroscopy (Tsao et al., 2010). It was found that the chitosan–γ-PGA PEC hydrogels show antibacterial activity and promoted cell proliferation. Therefore, the chitosan–γ-PGA polyelectrolyte hydrogel appears to have potential as a new material for biomedical applications.

3.2.3 Freeze-Thaw processing

The mechanical properties of chitosan hydrogels are not adequate for some biomedical applications and many researchers have tried to improve them. In order to achieve good mechanical and biological performance, a combination of a synthetic and a natural polymer seems to be a good choice. These materials are known in the literature as ‘bioartificial’ polymeric materials. Among synthetic polymers, polyvinyl alcohol (PVA) has been widely used in biomedical and biochemical applications because of its excellent chemical and physical properties, biodegradability, easy preparation and low cost. Blends of chitosan with PVA have been reported to have good mechanical and chemical properties (Miloslavčević et al., 2010).

Hydrogels prepared by freeze–thaw processing of poly vinyl alcohol (PVA) solutions have drawn extensive attention. Some advantages of physically cross-linked hydrogels obtained by freeze thawing are the nontoxic, noncarcinogenic, and biocompatibility of the resulting polymers, good mechanical strength and the absence of crosslinking agents or initiators in the hydrogel synthesis (Ricciardi et al., 2004; Smith et al., 2009; Xiaomin et al., 2008). During the freezing step, a liquid–liquid phase separation and formation of ice crystals in the polymer-poor phase occurs. Polymer chains in the polymer-rich phase lead to the formation of hydrogen bonding and PVA crystallites (See Fig. 6). In addition, the thawing procedure facilitates the interactions and formation of crystalline regions between the remaining polymers, leading to formation of hydrogel networks (Holloway et al., 2013; Zhang et al., 2013). The ice crystals act as crosslinkers during the formation of hydrogels and leave porous structures in the hydrogels, because of the space left from the melting ice crystals during the thawing stages (Guan et al., 2014).

The size of ice crystals formed in the structure increases with the number of repeated freeze/thaw cycles (Guan et al., 2014). Ricciardi et al. (Ricciardi et al., 2004) studied the structure of physical PVA hydrogels prepared by freeze-thaw cycling with a systematic X-ray powder diffraction technique, as a function of the number of cycles and aging time. Their results showed that both the degree of crystallinity of the gels and the size of PVA crystallites increased with increasing number of freeze/thaw cycles as illustrated in Fig. 7. The increased degree of crystallinity is mostly due to the increase in the crystallite size, although formation of new crystalline aggregates could not be excluded.

Guanghua et al. (Guanghua et al. (2008)) prepared physically cross-linked PVA/chitosan hydrogels by the freeze-thaw technique. The swelling results indicated that the hydrogels have good pH-sensitive properties in acidic environments and maximum swelling rate rises with the increase of chitosan content in the blend, but leads to more dissolution at pH 1.0. Longer freeze/thaw cycle times result in a lower swelling rate. They concluded that the swelling behavior of the composite hydrogels could be adjusted by changing the chitosan contents and the freezing/thawing cycle times.

PVA hydrogels including chitosan were synthesized by combined irradiation and freeze-thaw cycling and their properties were compared with those prepared by irradiation or freeze-thaw cycling alone. It was found that hydrogels made by irradiation alone have large swelling capacity and are transparent, but too weak to be used as a wound dressing. Hydrogels made by irradiation followed by freeze-thaw have large swelling capacity, good thermal stability, high mechanical strength, and translucent appearance. All the hydrogels containing chitosan showed pH sensitivity and antibacterial activity (Yang, Liu et al., 2008).

Fig. 8 shows the effects of the hydrogel preparation method on mechanical compression ratio (Afshari et al., 2015). The mechanical strengths of the hydrogels prepared by the combinational method are higher than that of the hydrogels prepared only by radiation. This is due to the presence of physical crosslinks in addition to chemical crosslinks. This fact suggests that in order to increase the mechanical strength of hydrogels prepared by radiation, the freeze-thawing treatment after irradiation is very effective (Yang et al., 2008, 2010; Boyneğri et al., 2009).

4. Clinical studies

There are few prospective clinical trials in the area of chitosan hydrogels for wound repair. Some clinical studies have been reported for using chitosan in various medical fields (Diamond et al., 2003; Méthot et al., 2016; Mason & Clarke, 2015). A research has been done on a chitosan gelling fiber dressing (Kytocel®) in Staffordshire community care to examine improved healing of patients with chronic non-healing wounds. KytoCel® (Aspen Medical) is a highly absorbent dressing composed of chitosan fibers which bond with wound exudate to form a clear gel absorbing pathogens and is conformable to the wound bed. Over a 4-week period or until complete wound healing, leaking, strikethrough, pain, time, and malodour were examined. This new advanced wound dressing has the ability to gel when in contact with wound fluid, making it less painful to remove, suitable for moderate to high exudate, reduced bioburden, and maintain haemostasis (Mason & Clarke, 2015). Another clinical study was conducted at three medical centers in China. A total of 90 patients with unhealed chronic wounds including pressure ulcers, vascular ulcers, diabetic foot ulcers, and wounds with minor infections, or at risk of infection, were treated with chitosan wound dressing or traditional Vaseline gauze as a control (Foshan United Medical Technologies Ltd, Guangdong, China). After 4 weeks of treatment, the wound area reduction was significantly greater in the test group than the control group. The average pain level in the
The mean duration of the test group was 27.31 ± 5.37 days and the control group 27.09 ± 6.44 days. Consequently, the new chitosan wound dressing can enhance the wound healing process and reduce the patient’s pain level. Furthermore, the dressing was shown to be clinically safe and effective in the management of chronic wounds (Mo et al., 2015).

5. Chitosan application for drug delivery in wound dressings

Wound healing is a complicated process including four overlapping phases: coagulation, inflammation, migration-proliferation (including matrix deposition), and remodeling (Falanga, 2005; Kirsner & Eaglstein, 1993; Velnar et al., 2009). The human body can provide all the requirements for the healing process in normal wounds, unless there is any kind of deficiency of skin function or massive fluid losses in large wounds. However, providing appropriate pH, moisture, oxygen pressure, and preventing microbial invasion can accelerate the wound healing process (Field & Kerstein, 1994; Guo & DiPietro, 2010). Wound care for non-healing wounds has recently been a growing concern of many wound dressing applications involving various mechanisms, such as coagulation, inflammation, angiogenesis, epithelization, contraction and remodeling. An ideal wound dressing should protect the wound from bacterial infection, provide a moist and healing environment, and be biocompatible (Boateng, Matthews, Stevens, & Eccleston, 2008; Bhuvanesh, 2010; Paul & Sharma, 2004). Because of chitosan’s previously mentioned significant characteristics, such as biocompatibility, biodegradability, hemostatic and anti-infection activity, and the ability to accelerate wound healing, it has attracted many researchers to be used as a wound dressing material. Applications of chitosan in different types of wound dressing are explained in the following sections.

5.1. Application of growth factors in chitosan-based wound dressings

Growth factors are polypeptides which control the growth, differentiation, and metabolism of cells and regulate the process of tissue repair. Despite their small amounts, they exert a powerful influence on the process of wound repair. Use of growth factors for accelerating the healing of wounds presents tremendous promise as a therapeutic approach in treating chronic wounds. Several peptide growth factors, such as epidermal growth factor (EGF), platelet derived growth factor (PDGF), fibroblast growth factor (FGF) and transforming growth factor-beta (TGF-β), have been shown to accelerate cellular proliferation and synthesis of the extracellular matrix (Alemdaroğlu et al., 2006; Paul &
Table 1
The average stained cell number in Chitosan gel with and without EGF (Yenilmez et al., 2015).

<table>
<thead>
<tr>
<th>Groups</th>
<th>The average stained cell number</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3rd day</td>
</tr>
<tr>
<td>EGF</td>
<td>1.20 ± 0.17</td>
</tr>
<tr>
<td>EGF+</td>
<td>1.63 ± 0.35</td>
</tr>
</tbody>
</table>

* Burn wounds treated by chitosan gel without (E) and with (EJ) with EGF.

Regarding EGF and chitosan properties for wound healing, Alemdaroğlu et al. (Alemdaroğlu et al. (2006)) developed an effective chitosan hydrogel formulation containing EGF to determine its effect on healing of second-degree burn wounds in rats. Burns are classified according to the depth of the injury. In superficial second-degree burns, the epidermis and the superficial dermis are mainly affected. And these kinds of burns are very painful. According to the in vitro release studies, the release of EGF from the chitosan gel was found to be 97.3% after 24 h. The release kinetics from the gel formulation was found to be of first order, and the release rate of EGF from the gel varied with time. The in vitro study exhibited significant increase in cell proliferation in the EGF containing gel applied group. The average numbers of proliferating cells observed on the 3rd, 7th and 14th days are presented in Table 1. Evaluation of these immunehisto-chemically observed results showed that the rate of healing in the EJ group was increased. Finally it has concluded that this formulation can play an important role in burn wound treatment.

Park et al. (Park, Clark, Lichtensteiger, Jamison, and Wagoner Johnson, (2009)) prepared chitosan scaffolds loaded with basic fibroblast growth factor (bFGF) contained in gelatin micro-particles and tested for clinical relevance in an aged mouse model. The application of this kind of wound dressing was for accelerating the healing process of pressure ulcers. bFGF was successfully delivered to the wound and increased angiogenesis compared to chitosan alone. They showed that levels of bFGF in wound tissue were significantly higher, indicating successful and prolonged growth factor delivery without burst effects. Finally they concluded that chitosan loaded with bFGF is a good candidate for treatment of chronic wounds and reduces the proteolytic environment of the wound and potentiates bFGF activity, leading to accelerated wound closure.

Table 2 Summarizes some reported chitosan hydrogels with incorporated growth factors for wound dressing applications.

5.2. Nanoparticle and nanostructure incorporated hydrogels

Nanotechnology is developing as a new growing field with applications in Science and Technology for the purpose of manufacturing new materials at the nanoscale level. Nano-particles are used not only in fundamental chemistry and physics investigations, but also widely in biomedical fields. Due to their good antibacterial properties, their large surface area to volume ratios, and high microbial resistance, nanoscale materials have emerged as novel antimicrobial agents (Sung et al., 2010; Radhakumary et al., 2011). Different types of metal materials have been known to be used as antibacterial and/or antimicrobial agents in wound healing such as, silver, zinc oxide, titanium dioxide, copper oxide, and graphene. However, there could be problems with long term exposure.

Sudheesh Kumar et al. (Kumbar et al., 2003) developed a flexible and microporous chitosan/nano zinc oxide composite hydrogel. At neutral pH, chitosan does not show much antibacterial activity; in order to impart that activity, it is necessary to incorporate some antibacterial agents into it. Zinc oxide nanoparticles were used in this research due to its antibacterial activity. In vitro cytocompatibility studies revealed that the dressing showed enhanced cell viability and infiltration. In vivo wound healing evaluation proved the healing ability and antibacterial potential of the prepared hydrogel without causing toxicity to cells. They found that these advanced dressings can be used to prevent infections in burns and chronic and diabetic wounds. It is also possible to synthesize chitosan quaternary salts that have a permanent cationic site, at all pH.

Silver and silver ions have been known as effective antimicrobial agents for a long time. Owing to this ability, they have been applied to a wide range of healthcare products, such as burn dressings, scaffolds, skin replacements and medical devices (Altiok et al., 2010).

Nano silver/gelatin/carboxymethyl (CM)-Chitosan hydrogels were synthesized by radiation-induced reduction and crosslinking at ambient temperature (Altiok et al., 2010). After incubation at 37 °C for 12 h, these hydrogels clearly showed antibacterial activity against gram-negative E. coli. The hydrogel with 10 mM nano silver content inhibited more than 99% of the E. coli. When the nano silver content decreased to 5 mM, the maximum inhibition ratio was around 50%, and when the nano silver content was further decreased to 2 mM, the ratio decreased to 26%. The results of this study suggest that nano silver/gelatin/CM-Chitosan hydrogels have potential as an antibacterial and anti-inflammation wound dressing.

Nano-curcumin was incorporated in N,O-carboxymethyl chitosan/oxidized alginate hydrogel to develop a novel nano-composite hydrogel wound dressing (Gayinsky et al., 2008). Curcumin, a natural product of the rhizomes of Curcuma longa, has been widely used as coloring
agent and spice in food. But studies have shown that curcumin could significantly accelerate the healing of wounds and enhance wound repair in diabetic impaired healing (Gaysinsky, 2007). Nano-curcumin could be released slowly from hydrogel to stimulate fibroblast proliferation, capillary formation and collagen production which significantly has effect on the healing phases. So, the nano-curcumin/chitosan/alginate hydrogel might have potential application in wound healing.

Tigecycline nanoparticles were loaded into chitosan–platelet-rich plasma hydrogel for wound infection treatment. The tigecycline nanoparticle incorporated chitosan hydrogel showed a significant anti-bacterial activity against S. aureus. This system can be an effective medium for antibiotic delivery and applied on the infection sites to effectively prevent various skin infections caused by S. aureus (Galotto et al., 2016).

Table 3 Summarizes some reported Chitosan hydrogels containing nanoparticles used for wound dressing applications.

### Table 3
Application of nanoparticles Chitosan hydrogels.

<table>
<thead>
<tr>
<th>Drug Preparation Technique</th>
<th>Potential Application</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zinc oxide / Silver</td>
<td>Genipin-crosslinking</td>
<td>Burn dressings (Yang et al., 2017)</td>
</tr>
<tr>
<td>Silver sulfadiazine</td>
<td>Genipin-crosslinking</td>
<td>As a carrier dressing (Choi &amp; Yoo, 2010a)</td>
</tr>
<tr>
<td>Silver Oxide</td>
<td>Casting/solvent evaporation</td>
<td>Wound dressing (Pulat et al., 2013)</td>
</tr>
<tr>
<td>Silver</td>
<td>UV radiation</td>
<td>Biomedical fields (Ishihara et al., 2003)</td>
</tr>
<tr>
<td></td>
<td>Glutaraldehyde-crosslinking</td>
<td>Anti-infectious wound dressing (Ho et al., 2009)</td>
</tr>
<tr>
<td>Copper oxide</td>
<td>Epichlorohydrin-crosslinking</td>
<td>Biomedical fields (Tyli szczak et al., 2017)</td>
</tr>
<tr>
<td>Zinc oxide</td>
<td>Casting/solvent evaporation</td>
<td>Wound dressing (Haji et al., 2017)</td>
</tr>
<tr>
<td>Zinc oxide</td>
<td>Genipin-crosslinking</td>
<td>Burn dressings (Wahid et al., 2017)</td>
</tr>
<tr>
<td>Titanium dioxide</td>
<td>Casting/solvent evaporation</td>
<td>Wound healing dressing (Begin et al., 2011)</td>
</tr>
<tr>
<td>Graphene oxide</td>
<td>Schiff-base reaction</td>
<td>Wound dressing (Vasile et al., 2014)</td>
</tr>
<tr>
<td>Curcumin nanoformulation</td>
<td>Casting/solvent evaporation</td>
<td>Wound healing dressing (Archana et al., 2015)</td>
</tr>
<tr>
<td>Nano Curcumin</td>
<td>Mixing</td>
<td>wound dressing (Gaysinsky et al., 2008)</td>
</tr>
<tr>
<td>Tigecycline nanoparticles</td>
<td>Mixing</td>
<td>Infectious wound treatment (Galotto et al., 2016)</td>
</tr>
</tbody>
</table>

*Reaction between aldehyde and the amino group of two materials.

agent and spice in food. But studies have shown that curcumin could significantly accelerate the healing of wounds and enhance wound repair in diabetic impaired healing (Gaysinsky, 2007). Nano-curcumin could be released slowly from hydrogel to stimulate fibroblast proliferation, capillary formation and collagen production which significantly has effect on the healing phases. So, the nano-curcumin/chitosan/alginate hydrogel might have potential application in wound healing.

Tigecycline nanoparticles were loaded into chitosan–platelet-rich plasma hydrogel for wound infection treatment. The tigecycline nanoparticle incorporated chitosan hydrogel showed a significant anti-bacterial activity against S. aureus. This system can be an effective medium for antibiotic delivery and applied on the infection sites to effectively prevent various skin infections caused by S. aureus (Galotto et al., 2016).

Table 3 Summarizes some reported Chitosan hydrogels containing nanoparticles used for wound dressing applications.

#### 5.3. Drug incorporated hydrogels

Controlled delivery systems provide an alternative approach to regulate bioavailability of therapeutic agents. In controlled drug delivery systems, an active therapeutic is incorporated into a polymeric network structure, and the drug is released from the structure in a predefined manner. Depending on the drug delivery formulation and application, the drug release time may be from a few hours to several months (Vimala et al., 2011). Different approaches have been developed for drug incorporation. The method of drugs loading directly impacts the availability of the drugs during release.

According to the application of wound dressings and the types of wounds, various kinds of drugs can be loaded in wound dressings. Sung et al. (Zhang et al., 2015) developed a minocycline-loaded wound dressing made with a PVA and Chitosan blended hydrogel using the freeze-thaw method. Minocycline was used in this study because it has been used in the treatment of superficial infections of the skin. The minocycline-loaded wound dressing composed of 5% PVA, 0.75% chitosan and 0.25% drug was more swellable, flexible and elastic than the PVA gel alone, because of the relatively weak cross-linking interaction of chitosan with PVA. According to their histologically examined data presented in Table 4, hydrogels prepared with drug resulted in less inflammatory cells, more numerous collagen proliferations, and microvessels in rats than the conventional product or the control (sterile gauze). The healing effect of minocycline-loaded hydrogel was great, indicating the potential healing effect of minocycline. Finally they concluded that the aforementioned minocycline-loaded wound dressing is a potential wound dressing with excellent forming and enhanced wound healing characteristics.

A thermo responsive and cytocompatible chitosan based hydrogel consisting of thiolated chitosan with poly (N-isopropyl acrylamide) loaded with ciprofloxacin was introduced by Radhakumary et al (Mishra et al., 2017). They reported that polymers with thiol groups provide enhanced adhesive properties in comparison with many mucoadhesive polymers, and the strong cohesive properties of thiolated chitosan make it highly suitable for controlled drug release. Ciprofloxacin is a quinolone antibiotic drug recognized for treatment of skin and skin structure infections. Their prepared film exhibited appropriate mechanical properties for a wound/burn dressing and was found to be cytocompatible and antibacterial as they released the entrapped antibiotic in a controlled manner for a period of more than 48 h.

Hydrogel microspheres of polyacrylamide-grafted-chitosan crosslinked with glutaraldehyde were prepared to encapsulate indomethacin, a non-steroidal anti-inflammatory drug. The microspheres were produced by the water/oil emulsion technique and encapsulation of indomethacin was carried out before crosslinking of the matrix. The initial release of indomethacin is due to the polymer chain relaxation process, but at longer times, the release occurs from the fully swollen polymer and is controlled mainly by the molecular diffusion phenomenon. This study suggests that the hydrogel microspheres prepared from chitosan-based polymers can be useful in the delivery of indomethacin-like drugs (Zhang et al., 2018).

Additional drug-loaded chitosan hydrogels reported for wound dressing applications are presented in Table 5.

Essential oils and herbal extracts are natural complex mixtures of volatile, lipophilic substances obtained from different parts of plants by steam distillation and solvent extraction. In recent decades, interest in essential oils for use in pharmaceutical and biomedical field has risen. Essential oils have been shown to possess antibacterial, antifungal, interaction of chitosan with PVA. According to their histologically examined data presented in Table 4, hydrogels prepared with drug resulted in less inflammatory cells, more numerous collagen proliferations, and microvessels in rats than the conventional product or the control (sterile gauze). The healing effect of minocycline-loaded hydrogel was great, indicating the potential healing effect of minocycline. Finally they concluded that the aforementioned minocycline-loaded wound dressing is a potential wound dressing with excellent forming and enhanced wound healing characteristics.

A thermo responsive and cytocompatible chitosan based hydrogel consisting of thiolated chitosan with poly (N-isopropyl acrylamide) loaded with ciprofloxacin was introduced by Radhakumary et al (Mishra et al., 2017). They reported that polymers with thiol groups provide enhanced adhesive properties in comparison with many mucoadhesive polymers, and the strong cohesive properties of thiolated chitosan make it highly suitable for controlled drug release. Ciprofloxacin is a quinolone antibiotic drug recognized for treatment of skin and skin structure infections. Their prepared film exhibited appropriate mechanical properties for a wound/burn dressing and was found to be cytocompatible and antibacterial as they released the entrapped antibiotic in a controlled manner for a period of more than 48 h.

Hydrogel microspheres of polyacrylamide-grafted-chitosan crosslinked with glutaraldehyde were prepared to encapsulate indomethacin, a non-steroidal anti-inflammatory drug. The microspheres were produced by the water/oil emulsion technique and encapsulation of indomethacin was carried out before crosslinking of the matrix. The initial release of indomethacin is due to the polymer chain relaxation process, but at longer times, the release occurs from the fully swollen polymer and is controlled mainly by the molecular diffusion phenomenon. This study suggests that the hydrogel microspheres prepared from chitosan-based polymers can be useful in the delivery of indomethacin-like drugs (Zhang et al., 2018).

Additional drug-loaded chitosan hydrogels reported for wound dressing applications are presented in Table 5.

Essential oils and herbal extracts are natural complex mixtures of volatile, lipophilic substances obtained from different parts of plants by steam distillation and solvent extraction. In recent decades, interest in essential oils for use in pharmaceutical and biomedical field has risen. Essential oils have been shown to possess antibacterial, antifungal, interaction of chitosan with PVA. According to their histologically examined data presented in Table 4, hydrogels prepared with drug resulted in less inflammatory cells, more numerous collagen proliferations, and microvessels in rats than the conventional product or the control (sterile gauze). The healing effect of minocycline-loaded hydrogel was great, indicating the potential healing effect of minocycline. Finally they concluded that the aforementioned minocycline-loaded wound dressing is a potential wound dressing with excellent forming and enhanced wound healing characteristics.

A thermo responsive and cytocompatible chitosan based hydrogel consisting of thiolated chitosan with poly (N-isopropyl acrylamide) loaded with ciprofloxacin was introduced by Radhakumary et al (Mishra et al., 2017). They reported that polymers with thiol groups provide enhanced adhesive properties in comparison with many mucoadhesive polymers, and the strong cohesive properties of thiolated chitosan make it highly suitable for controlled drug release. Ciprofloxacin is a quinolone antibiotic drug recognized for treatment of skin and skin structure infections. Their prepared film exhibited appropriate mechanical properties for a wound/burn dressing and was found to be cytocompatible and antibacterial as they released the entrapped antibiotic in a controlled manner for a period of more than 48 h.

Hydrogel microspheres of polyacrylamide-grafted-chitosan crosslinked with glutaraldehyde were prepared to encapsulate indomethacin, a non-steroidal anti-inflammatory drug. The microspheres were produced by the water/oil emulsion technique and encapsulation of indomethacin was carried out before crosslinking of the matrix. The initial release of indomethacin is due to the polymer chain relaxation process, but at longer times, the release occurs from the fully swollen polymer and is controlled mainly by the molecular diffusion phenomenon. This study suggests that the hydrogel microspheres prepared from chitosan-based polymers can be useful in the delivery of indomethacin-like drugs (Zhang et al., 2018).

Additional drug-loaded chitosan hydrogels reported for wound dressing applications are presented in Table 5.

Essential oils and herbal extracts are natural complex mixtures of volatile, lipophilic substances obtained from different parts of plants by steam distillation and solvent extraction. In recent decades, interest in essential oils for use in pharmaceutical and biomedical field has risen. Essential oils have been shown to possess antibacterial, antifungal,
Table 5
Application of drug-loaded Chitosan hydrogels.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Preparation Technique</th>
<th>Potential Application</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gentamicin sulfate</td>
<td>EDC/NHS-ε-crosslinking</td>
<td>Anti-bactericidal wound dressing</td>
<td>Chen et al., 2017</td>
</tr>
<tr>
<td>Gentamicin sulfate</td>
<td>EDC/NHS-ε-crosslinking</td>
<td>Anti-bactericidal wound dressing</td>
<td>Patel et al., 2017</td>
</tr>
<tr>
<td>Tetracycline hydrochloride</td>
<td>Emulsion-crosslinking</td>
<td>Anti-bacterial wound dressing</td>
<td>Yang et al., 2018</td>
</tr>
<tr>
<td>Tetracycline hydrochloride</td>
<td>Mixing</td>
<td>Scar preventive wound healing</td>
<td>Fan et al., 2016</td>
</tr>
<tr>
<td>Tetracycline hydrochloride / silver sulfadiazine</td>
<td>Casting/solvent evaporation</td>
<td>Anti-infection wound dressing</td>
<td>Gao et al., 2016</td>
</tr>
<tr>
<td>Naproxen</td>
<td>Casting/solvent evaporation</td>
<td>Anti-infection wound dressing</td>
<td>Ma et al., 2017</td>
</tr>
<tr>
<td>Superoxide dismutase</td>
<td>Polyelectrolyte complexes</td>
<td>Antioxidant wound dressing</td>
<td>Rocasalbas et al., 2013</td>
</tr>
<tr>
<td>Lignin</td>
<td>Freeze-thaw</td>
<td>Wound dressings</td>
<td>Mogado et al., 2017</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>Freeze-thaw</td>
<td>Antibiotic dressing</td>
<td>Akincibay et al., 2007</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>Not mentioned</td>
<td>Wound dressings</td>
<td>Perchynook et al., 2014</td>
</tr>
<tr>
<td>Metronidazole benzoate</td>
<td>Mixing</td>
<td>Treatment of chronic periodontitis</td>
<td>Ribeiro et al., 2013</td>
</tr>
<tr>
<td>Clarithromycin, Tramadol and Heparin</td>
<td>Genipin-crosslinking</td>
<td>Pharmaceutical applications</td>
<td>Archana et al., 2013</td>
</tr>
<tr>
<td>Nystatin</td>
<td>Mixing</td>
<td>Wound healing application</td>
<td>Liu &amp; Kim, 2012</td>
</tr>
<tr>
<td>Acidic oxyphenbutazone &amp; glafenine</td>
<td>Coacervation techniques</td>
<td>Essential oils or herbal extracts</td>
<td>Harris et al., 2008</td>
</tr>
<tr>
<td>Nerolidol</td>
<td>–</td>
<td>Wound healing dressing</td>
<td>Goncalves Ferreira et al., 2016</td>
</tr>
<tr>
<td>Thymol</td>
<td>NVP-crosslinking</td>
<td>Wound dressing</td>
<td>Qin et al., 2006</td>
</tr>
<tr>
<td>Apigenin</td>
<td>PEG-crosslinking</td>
<td>Diabetic wound dressing</td>
<td>Kim et al., 2015</td>
</tr>
<tr>
<td>Lupon</td>
<td>Glutaraldehyde-crosslinking</td>
<td>Wound dressing</td>
<td>Ishida et al., 2000</td>
</tr>
<tr>
<td>Polyphenolic</td>
<td>Laccase- crosslinking</td>
<td>Chronic wound dressing</td>
<td>Li et al., 2012</td>
</tr>
</tbody>
</table>

Note: NVP: 1-vinyl-2-pyrrolidone.

antiviral, insecticidal and antioxidant properties due to their biologically active compounds (See Table 5 for their applications in wound dressings). Chitosan films loaded with thyme oil were prepared by a solvent casting method (Jiang et al., 2016). Thyme oil has shown to have inhibitory activities against various bacteria and yeasts (Shukla et al., 2016). Thymol, the major component of thyme oil (Anjum et al., 2016), has also been shown to exhibit antimicrobial activity against several bacteria and fungi (Koosegoli et al., 2017). The prepared films showed both antibacterial and antioxidant activities. Results of this research revealed that the thyme oil has a good potential to be incorporated into chitosan to make antibacterial and permeable films for wound healing applications.

6. Conclusions

Wound healing is a complex and dynamic process of replacing denuded and missing cellular structures and tissue layers. Many efforts have been focused on wound care with an emphasis on new therapeutic approaches and development of technologies for wound management. In recent decades, smart wound dressings with accelerated wound healing properties have attracted much interest.

This review summarized the potential applications of chitosan hydrogels for pharmaceutical and biomedical uses, particularly with regard to drug delivery in wound dressings. Chitosan is an excellent candidate due to its non-toxicity, stability, biodegradability, and biocompatibility. Also chitosan hydrogel structures may be loaded with various types of drugs in different physical forms, such as nanoparticles, essential oils, and solubilized drugs. These properties have made chitosan a very versatile material with extensive applications in controlled release formulations for treatment of acute and chronic wounds like burns and pressure and diabetic ulcers. Therefore it can be concluded that chitosan-based hydrogels are expected to become a promising matrix for use in regenerative medicine, drug delivery, and wound dressing applications.

References


