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Chitosan Based Scaffolds and Their Applications in Wound Healing

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ABSTRACT

Over the last decade, much interest has been developed in biopolymer based materials due to their biocompatible, biodegradable, non-toxic and non-allergenic nature. Chitosan is a unique biopolymer that exhibits outstanding properties, besides biocompatibility and biodegradability. Most of these peculiar properties arise from the presence of primary amines along the chitosan backbone. Many works have been done to obtain chitosan based scaffolds, including surface modifications, the fabrication of chitosan based blends, chitosan based composite scaffolds, and drug-loaded scaffolds. This study provides an overview of the key features of inherent properties of chitosan, their modification, and its use in biomedical engineering particularly toward anti-inflammatory and wound healing.

Contents

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Introduction

In all biomedical applications today, polymers are playing an important role. Many notable advances in technology have followed exploitation of the properties offered by new polymeric materials like blends and composites (Kittur et al., 2002; Muzzarelli and Muzzarelli, 2005; Qin et al., 2012; Chee, 1990; Sun et al., 1992). Biopolymers include the polysaccharides such as cellulose, starch, the carbohydrate polymers produced by bacteria and fungi and animal protein based biopolymers such as chitin, chitosan, wool, silk, gelatin and collagen; biopolymers, especially the carbohydrate origin, have been found to have a very promising industrial application in various forms (Singh, 2011). Most commercial polysaccharides (e.g., cellulose, dextran, pectin, alginic acid, agar–agar, agarose, starch, carrageenan, and heparin) are either neutral or acidic, but chitosan is a basic polysaccharide. In neutral or basic pH, chitosan contains free amino groups and is insoluble in water, while in acidic pH, chitosan is soluble in water due to protonation of amino groups. The solubility depends on the distribution of free amino and *N*-acetyl groups (Wu and Bough, 1978; Costa-Pinto et al., 2011). Chitosan is a linear polyelectrolyte at acidic pH. It has high charge density, one charge per glucosamine. The pH and ionic strength have an important impact on the intrinsic viscosity of polyelectrolytes. Chitosan in aqueous acid solution has been surface reacted with polyanion aqueous solutions (heparin, sodium alginate, carboxymethyl chitin, polyacrylic acid) to give polyelectrolyte complexes (Rinaudo and Domard, 1989; Dutkiewicz and Tuora, 1992).

Chitosan

Chitosan is a copolymer which consists of β-(1 → 4)-linked 2-acetamido-2-deoxy-D-glucopyranose and 2-amino-2-deoxy-D-glucopyranose units. This is generally obtained by alkaline deacetylation of chitin, which is the main component of the exoskeleton of crustaceans, such as shrimps. Chitin is a naturally abundant mucopolysaccharide and is second to cellulose in terms of the amount produced annually by biosynthesis. Chitin is a common constituent of the exoskeleton in animals, particularly in crustaceans, mollusks and insects. The structure of chitosan (deacetylated chitin) is very similar to cellulose (Fig. 1); however less attention has been paid to chitin than cellulose, primarily due to its inertness. Hence, it remains an essentially unutilized resource. Deacetylation of chitin yields chitosan, which is a relatively reactive compound and is produced in numerous forms, such as powder, paste, film and fibre. Chitosan is soluble in dilute aqueous acetic, lactic, malic, formic and succinic acids (Skotak et al., 2011; Shakeel et al., 2014; Shakeel and Saiqa, 2015a; Mudasir et al., 2015a). Chitosan is polycationic at pH < 6 and it readily interacts with negatively charged molecules, such as proteins, anionic polysaccharides (e.g., alginate and carrageenan), fatty acids, bile acids and phospholipids. Nonetheless, chitosan may also selectively chelate metal ions such as iron, copper, cadmium and magnesium (Mudasir et al., 2015b). Much attention has been paid to chitosan based biomedical materials, because of its unique properties such as biodegradability, non-toxicity, anti-bacterial effect and biocompatibility. Chitosan is a benefit to wound healing because it stimulates haemostasis and accelerates tissue regeneration. For a material to be used for biomedical research, a natural product is preferred because these materials are more biocompatible than synthetic materials. Chitosan is metabolized by certain human enzymes, such as lysozyme, thus, it is biodegradable. Chitosan is an attractive material for a tissue engineering scaffold because it has structural similarities to glycosaminoglycans and is hydrophilic. Chitosan's monomeric unit, *N*-acetylglucosamine, occurs in hyaluronic acid, an extracellular macromolecule that is important in wound repair (Muzzarelli, 1996; Keong and Halim, 2009).

Modifications of Chitosan

An effective approach for developing a clinically applicable chitosan is to modify the surface of the material to provide excellent biofunctionality and bulk properties. Surface modification techniques to blend various compound derivatives include coating, oxidation by low-temperature plasma and surfactant addition in order to blend with various derivatives. Furthermore, chitosan can be fabricated into a stable, porous bioscaffold via surface modification and lyophilization. However, blending with various additives may affect its biocompatibility. Therefore, evaluation of the biocompatibility of various biomedical-grade chitosan derivatives is necessary to engineer material that is of high quality and biocompatible for human wound management. They offer the advantage of being easily processed into gels, membranes, nanofibres, nanofibrils, beads, microparticles, nanoparticles, scaffolds and sponge-like forms (Fig. 2). Due to these properties and its biocompatibility, it has versatile applications in tissue engineering, wound healing, as excipients for drug delivery and gene delivery (Anithaa et al., 2014).

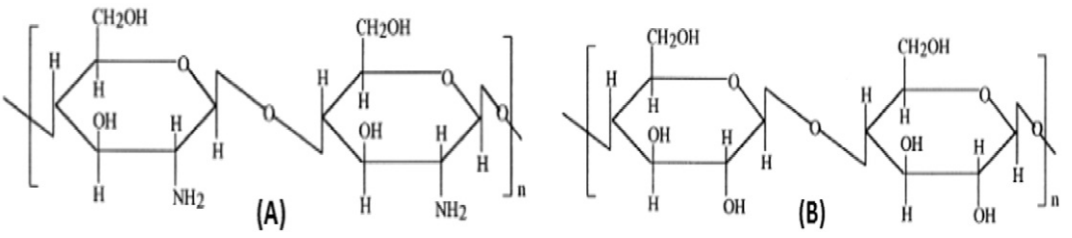

Fig. 1. Structure of (A) Chitosan and (B) Cellulose.

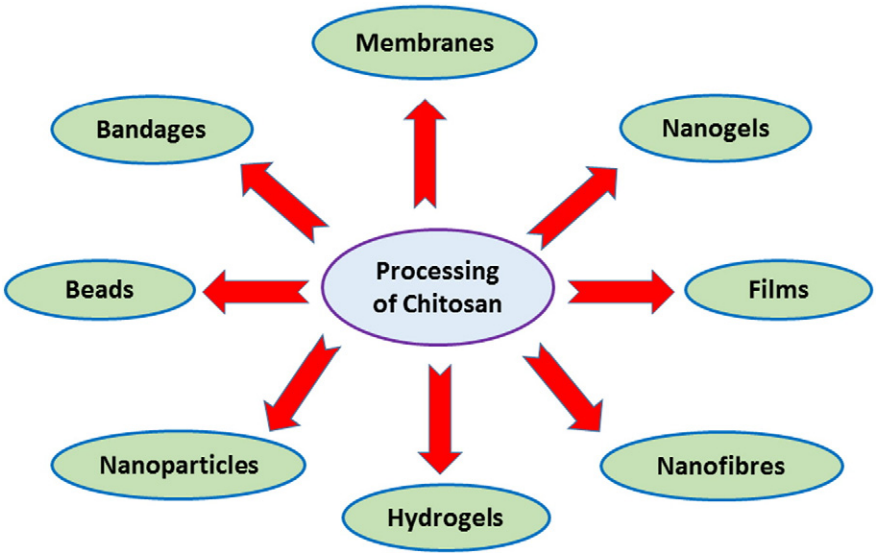

Fig. 2. Schematic representation of the possibilities of processing chitosan into different forms.

Cross-linking treatment has emerged as another important strategy to improve the performance of chitosan. The main parameters influencing the characteristics of chitosan are its molecular weight (MW) and degree of deacetylation (DD), representing the proportion of deacetylated units. These parameters are determined by the conditions set during preparation. Moreover, they can be further modified. For example, the DD can be lowered by deacetylation (Zhang and Cui, 2012) and MW can be lowered by acidic depolymerisation (Sorlier et al., 2001). In medical and pharmaceutical applications, chitosan is used as a component in hydrogels. In crosslinked hydrogels, polymeric chains are interconnected by cross linkers, leading to the formation of a 3D network. Cross linkers are molecules of MW much smaller than the MW of the chains between two consecutive crosslinks (Knapczyk et al., 1994). The properties of crosslinked hydrogels depend mainly on their crosslinking density, namely the ratio of moles of crosslinking agent to the moles of polymer repeating units. Moreover, a critical number of crosslinks per chain is required to allow the formation of a network. The structures of chitosan hydrogels formed are: (a) chitosan cross-linked with itself; (b) hybrid polymer network; (c) semi-interpenetrating network; and (d) ionic crosslinking (Berger et al., 2004). Depending on the nature of the cross linker, the main interactions forming the network are covalent or ionic bonds.

Antimicrobial Nature of Chitosan

Chitosan is a potent antimicrobial agent and its antimicrobial character is due to presence of its cationic nature. An antimicrobial is an agent that kills microorganisms or inhibits their growth (Antimicrobial-Definition from the Merriam-Webster Online Dictionary). Chitosan possesses unique properties that make it an ideal ingredient for development of antimicrobial edible film. Chitosan is an antimicrobial non-toxic biopolymer that has been proven to serve as a matrix to obtain edible films. The interaction (binding or chelation) of chitosan with endotoxins of Gram-negative bacteria decreased their acute toxicity. Because of the strong chelating ability of chitosan, external chelating agents such as EDTA may not be required, when antimicrobial agents such as nisin are added to chitosan to control Gram negative bacteria. Chitosan's ability to inhibit a wide variety of bacteria, fungi, yeasts, and viruses make its application in a broad range or variety of antimicrobial agents in experiments involving in vivo and in vitro interactions in different forms (solutions, films and composites). Chitosan-dependent antimicrobial activity has been observed against various microorganisms, such as fungi, algae and bacteria (Shakeel et al., 2015). These antimicrobial effects are controlled by intrinsic factors, including the type of chitosan, the degree of chitosan polymerization, the host, the natural nutrient constituency, the chemical or nutrient composition of the substrates and the environmental conditions (e.g., substrate water activity or moisture or both). The photo-cross-linked electrospun mats containing quartinized chitosan (QCS) were efficient in inhibiting growth of Gram-positive bacteria and Gram-negative bacteria and the results suggested that the cross-linked QCS/PVP electrospun mats were found to be promising materials for wound-dressing (Jayakumar et al., 2010). The antimicrobial activities of chitosan differs mainly in live host plants e.g. the fungicidal effects of *N*-carboxymethyl chitosan are different in vegetable and graminea hosts and is more immediate on fungi and algae than on bacteria (Ohshima et al., 1987). Furthermore, in the presence of more than 0.025% chitosan, the growth of *Excherichia coli*, *Fusarium*, *Alternaria* and *Helminthosporium* is inhibited (Savard et al., 2002). The inhibition of growth of these micro-organisms is due to cationic amino groups of chitosan which bind to anionic groups. During the infectious period of a burn wound, bacterial infection may delay the healing and probably cause serious complications, such as sepsis. Chitosan that is incorporated with minocycline hydrochloride was developed to achieve both wound healing enhancement and antibacterial effects (Joshua et al., 2008).

Anti-inflammatory Nature of Chitosan

The substance or treatment that reduces inflammation is known as anti-inflammatory. Anti-inflammatory drugs make up about half of analgesics that reduces inflammation thereby remedying pain in contrast to opioids, which affect the central nervous system. Chitosan has a variety of promising biomedical applications and at this time, is considered as a new innovative material in drug delivery systems, wound healing, antibacterial, fat binder, hemostatic agent, hypocholesterolemic effect as indicated by the large number of studies published over the last few years (Shakeel and Saiqa, 2015a). Due to intraperitoneal administration of acetic acid, chitosan treatment reduces inflammatory pain in a dose-dependent manner. Bradykinin is one of the main substances related to pain. It was reported that the bradykinin concentration during administration of a chitosan-acid acetic solution in the peritoneal lavage fluid was lower than during the administration of a 0.5% acetic acid solution, suggesting that chitosan has analgesic effects. Chitosan that is formulated for wound management may induce analgesia by providing a cool, pleasant and soothing effect when applied to an open wound. Excellent pain relief was conferred by chitosan when it was applied as a topical agent to open wounds, such as burns, skin abrasions, skin ulcers and skin grafted areas (Okamoto, 2002). Due to anti-inflammatory effects of chitosan, these are beneficial for the treatment of prolonged inflammation at the wound site. Water-soluble chitosan significantly suppresses the secretion and expression of proinflammatory cytokines (e.g., tumour necrosis factor-α and interleukin-6) and inducible nitric oxide synthase in astrocytes, the predominant neuroglial cells in the central nervous system, and is actively involved in cytokine-mediated inflammatory events (Aoyagi et al., 2007). Moreover, *N*-acetylglucosamine is an anti-inflammatory drug and is synthesized in the human body from glucose. It is incorporated into glycosaminoglycans and glycoproteins. Chito-oligosaccharides, which have a molecular weight of 5 kDa, showed better anti-inflammatory agents than nonsteroidal anti-inflammatory drug, indomethacin (Kim et al., 2002). Chitosan exerts anti-inflammatory effects by inhibiting prostaglandin E2 and cyclooxygenase-2 protein expression and attenuating the pro-inflammatory cytokines (e.g., tumour necrosis factor-α and interleukin-1β). However, chitosan treatment increases the expression of the anti-inflammatory cytokine, interleukin-10 (Spindola et al., 2009).

Biomedical Applications of Chitosan

In various disciplines of health care and hygienic applications, chitosan is used for contact disinfectants in many biomedical applications, including wound dressing, drug delivery carrier orthopaedic tissue engineering, gene delivery, and haemodialysis.

Several biomedical applications of chitosan have already been reported (Fig. 3) (Zuo, 2014; Rinaudo, 2006). Chitosan has the potential to be used as artificial kidney membrane, hypocholesterolemic agents, drug delivery systems, absorbable sutures and supports for immobilized enzymes. Chitosan has some advantages due to its nontoxicity and biodegradability without damaging the environment. It is a biocompatible material that breaks down slowly to harmless products that are absorbed completely in the body (Neethu et al., 2015).

Wound Healings

Skin is the largest body organ, functioning as a barrier to harmful mediums, preventing pathogens from entering into the body. A wound is result from physical, chemical, mechanical and/or thermal damages. The natural healing process of the skin is

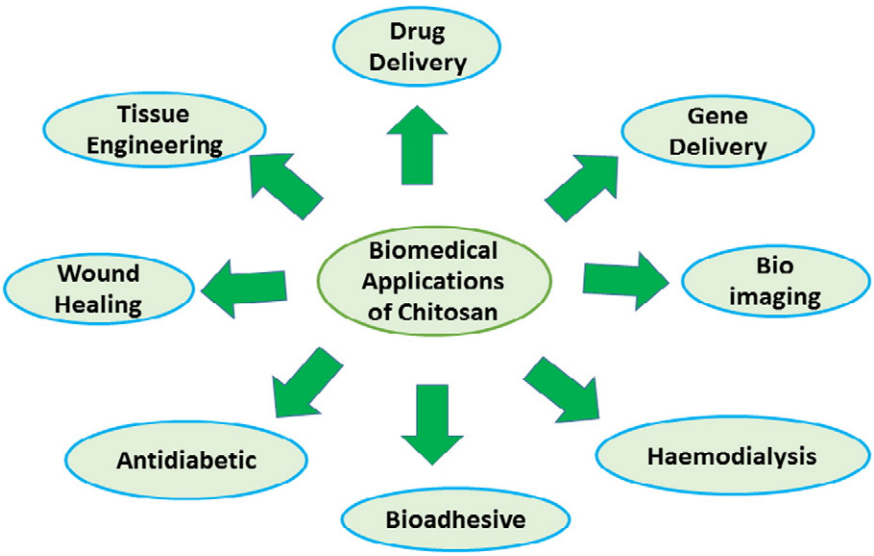

Fig. 3. Various biomedical applications of chitosan.

complex and continuous. The treatment of skin lesions is a critical issue in healthcare. Usually, the treatment for skin loss is traditional autografts and allografts. As the tissue engineering is rising, it has emerged as an alternative treatment for excessive skin loss. Wound dressing has attracted great interest in this area. Wound dressing materials have changed continuously and significantly during the past years. Nowadays many polymers, including natural materials, synthetic materials and combinations of both types, nanoparticles immobilized polymer materials have been used for the application of skin regeneration (Wen et al., 2015).

Chitosan in Wound Healings

Wound healing is a particular biological route allied to the general phenomenon of growth and tissue regeneration. It progresses through a series of inter-reliant and corresponding stages in which a variety of cellular and matrix components act together to regenerate the integrity of damaged tissue and replacement of lost tissue (Boateng et al., 2008). Wound healing is a complex and dynamic regenerative process that is divided into four phases — haemostasis, inflammation, proliferation, and remodelling (Hani and Satya, 2013). The development of bacterial-resistant prosthetic counterparts through binding of an antibiotic to the materials have been focussed. For example, chitosan hydrogel coated grafts, crosslinked upon ultraviolet light irradiation, exhibited a resistance against *E. coli* in vitro and in vivo. The chitosan hydrogel directly acted as antibacterial biomaterial on a Dacron graft, and was effective to inhibit the local infection (Fujita et al., 2004). A number of studies showed that chitosan showed wound healing properties. The biocompatible carboxyethyl chitosan/poly(vinyl alcohol) (CECS/PVA) nanofibres were prepared by electrospinning of aqueous solution as wound dressing material. Cell culture results showed that fibrous mats were good in promoting the L929 cell attachment and proliferation (Zhou et al., 2008). The wound dressing should be non-allergenic and non-toxic, keep moist environment, allows gas exchanges, protect the wound against microbial organisms and absorb wound exudates (Croisier and Jérôme, 2013).

Haemorrhage remains a leading cause of early death after trauma, and infectious complications in combat wounds continue to challenge caregivers. Although chitosan dressings have been developed to address these problems, they are not always effective in controlling bleeding or killing bacteria. Immobilized onto poly(N-isopropylacrylamide) gel/polypropylene nonwoven composites surface using the cross-linking agent (glutaraldehyde), chitosan hydrogels displayed antibacterial ability to *E. coli* and *Staphylococcus aureus*. Meanwhile, the product showed easily stripped-off property without damaging the new regenerated tissue as it should be removed from the wound (Chen et al., 2005a, 2005b). Composite sponge of curcumin/chitosan/gelatin was prepared at the various ratios of chitosan and gelatin showing improved water uptake ability, antibacterial activity, and wound closure. The higher content of gelatin in composite sponge exhibited a faster release behaviour up to 240 min. These composite sponges were also found to enhance the formation of collagen and wound closure in vivo and therefore improved the wound healing activity (Van et al., 2013).

Chitosan with Synthetic/Natural Polymer Blend scaffolds in Wound Healings

Chitin grafted poly(acrylic acid) (PAA) was prepared with the aim of obtaining a hydrogel suitable for wound dressing application. The morphology and behaviour of the cells on the chitin-PAA film were found to be normal after 14 days of culture (Tanodekaew et al., 2004). Ribeiro et al. (2009) developed chitosan hydrogel for wound dressing. In their study, fibroblast cells isolated from rat skin were used to assess the cytotoxicity of the hydrogel. The results showed that chitosan hydrogel was able to promote cell adhesion and proliferation. Cell viability studies showed that the hydrogel and its degradation by-products are non-cytotoxic. This study suggested that chitosan hydrogel may aid the reinstatement of skin architecture (Ribeiro et al., 2009).

The biocompatible chitosan/polyethylene glycol diacrylate blend films were successfully prepared by Michael addition reaction with different weight ratios as wound dressing. Indirect cytotoxicity assessment of films with mouse fibroblasts (L929) indicated that the material showed nocytotoxicity toward growth of L929 cell and had good in vitro biocompatibility and have the potential to be used as wound dressing material (Zhang et al., 2008). Porous microspheres of chitosan and its derivatives were prepared in order to deliver antigens in a controlled way (Mi et al., 1999). Mucoadhesivity of chitosan and its cationic derivatives is recognized and proved to enhance the adsorption of drugs especially at neutral pH. *N*-trimethyl chitosan chloride interacts with the negatively charged cell membranes thus, inhibit the growth of microorganisms (Hamman and Kotzé, 2002). Heterogeneous chemical modification of the chitosan film can tune the surface properties of the film; for instance, when a stearoyl group was attached to the chitosan films, they became more hydrophobic and promoted proteins adsorption. When chitosan films were reacted with succinic anhydride or phthalic anhydride, it resulted in more hydrophilic films which promoted lysozyme adsorption (Tangpasuthadol et al., 2003). The PVA/bentonite, PVA/Ag nanoparticles, PVA/clove extract, and PVA/cellulose nanocomposite hydrogel was reported by Gonzalez et al. PVA/clove hydrogels did not exhibit a homogenous aspect, while PVA/bentonite and PVA/Ag nanocomposite hydrogels showed significant antimicrobial activity against growth of *E. coli*, good water vapour transmission rate, and adequate water absorbing capacity due to addition of clay and Ag nanoparticles as filler (Gonzalez et al., 2011). For effective wound healing accelerator, water-soluble chitosan/heparin complex was prepared by Kweon et al. using water-soluble chitosan with wound healing ability and heparin with ability to attract or bind growth factor related to wound healing process (Kweon et al., 2003).

Chitosan Based Composite Scaffolds in Wound Healings

The biocompatible chitosan/sericin composite nanofibres were fabricated by electrospinning having good morphology with diameter between 240 nm and 380 nm. Furthermore, the composite nanofibres showed good bactericidal activity against both of Gram-positive and Gram-negative bacteria, could promote cell proliferation and are promising for wound dressing applications. The cell viabilities for 24 h were over 90% at all test concentrations up to 250 g/mL, revealing the non-toxicities and good biocompatibilities of composite nanofibres toward cells as shown in Fig. 4. Interestingly, we found that all of the cell viabilities were greater than 100% compared to control group after 72 h incubation (Rui et al., 2014).

The selective sulfation at O-2 and/or O-3 of chitosan may generate potent antiretroviral agents that display a much higher inhibitory effect on the infection of AIDS virus (Nishimura et al., 1998). The controlled silver sulfadiazine release was shown by silver sulfadiazine incorporated CS membranes with controlled water vapour evaporation, adequate swelling ability, cytocompatibility and prolonged antibacterial activity (Fwu et al., 2003). Silver sulfadiazine incorporated CS-alginate composite membranes with excellent tensile strength and adequate elongation at break were developed in another report. The release was enhanced when the alginate concentration was about 50%, thus suggesting release kinetics dependant on alginate (Meng et al., 2010). Wang et al. evaluated the wound healing ability of PEC membrane of CS and alginate suspensions developed through solvent casting method. The prepared membrane was nontoxic toward mouse and human fibroblast cells. In vivo evaluation in incisional wounds in rat proved the faster healing potential of this composite membrane (Wang et al., 2002). Silver sulfadiazine incorporated bilayer wound dressing membrane composed of a soft upper layer and dense; a lower layer showing sustained release of silver sulfadiazine showed excellent antibacterial activity against *Pseudomonas aeruginosa* and *S. aureus* in the in vivo system as well (Mi et al., 2002). Pang et al. developed a composite membrane which showed adequate mechanical strength, excellent antibacterial activity against *E. coli* and tremendous haemostatic potential. This developed material was cytocompatible toward human skin fibroblast cells (Pang et al., 2008). Interestingly, a thermo-sensitive film was prepared by combining thiolated chitosan, poly(N-isopropyl acrylamide) and ciprofloxacin, so that by decreasing the temperature, the film can easily be removed from wound (Radhakumary et al., 2011).

A biocomposite coating containing chitosan, silver, and hydroxyapatite was developed on anodized titanium substrate by electrochemical deposition and it exhibited antibacterial activity because of the synergistic effect of silver and chitosan. The needle-like HAp crystal is observed in both HAp and CSAgHAp coatings; CSAgHAp crystal is denser and shorter from that of the high magnification image (Fig. 5). The coatings show a uniform and porous surface, which is beneficial to cell adhesion. These composite coatings could minimize the risk of infection and are promising materials with biomedical applications (Yajing et al., 2015).

The use of silver nanoparticles have exhibited improved antibacterial properties due to high surface area and high fraction of surface atoms, leading to incorporating more nanoparticles inside the bacteria and promoting its efficacy in a sustained manner (Vimala et al., 2010). Silver nanoparticles exhibit significantly novel and distinct physical, chemical, and biological properties and functionality due to their nanoscale size, have elicited much interest, over the past few decades. Due to antimicrobial nature of silver nanoparticles, it has potential to heal the wounds (Shakeel and Saiqa, 2015b, 2015c; Shakeel et al., 2016a, 2016b). The antibacterial potential of nanosilver incorporated and chitosan hydrogel composite bandage the developed scaffolds showing high swelling ratio, controlled biodegradation and excellent blood clotting ability. The composite bandages showed excellent antibacterial potential against *S. aureus* and *E. coli* and found to be suitable for wound healing applications. Biocompatibility of the prepared scaffolds toward Vero cells, proved the non-toxic nature of the composite bandages (Madhumathi et al., 2010; Chen et al., 2011).

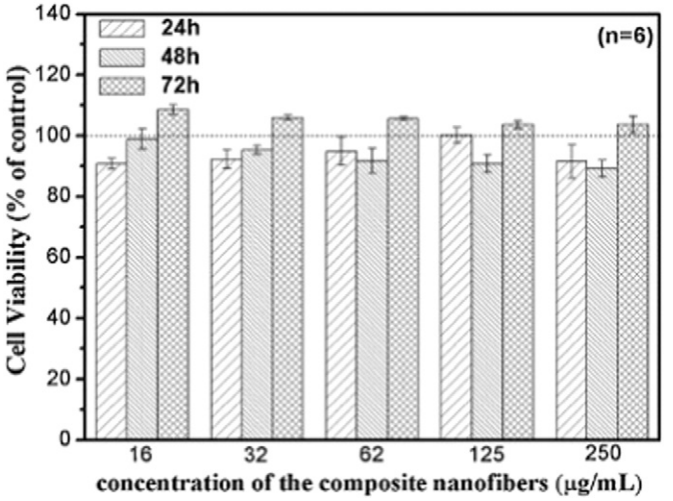

Fig. 4. The cell viability of L929 fibroblasts in different concentrations of nanofibres culture medium for 24, 48, and 72 h (Rui et al., 2014) (Reproduced with permission from Elsevier).

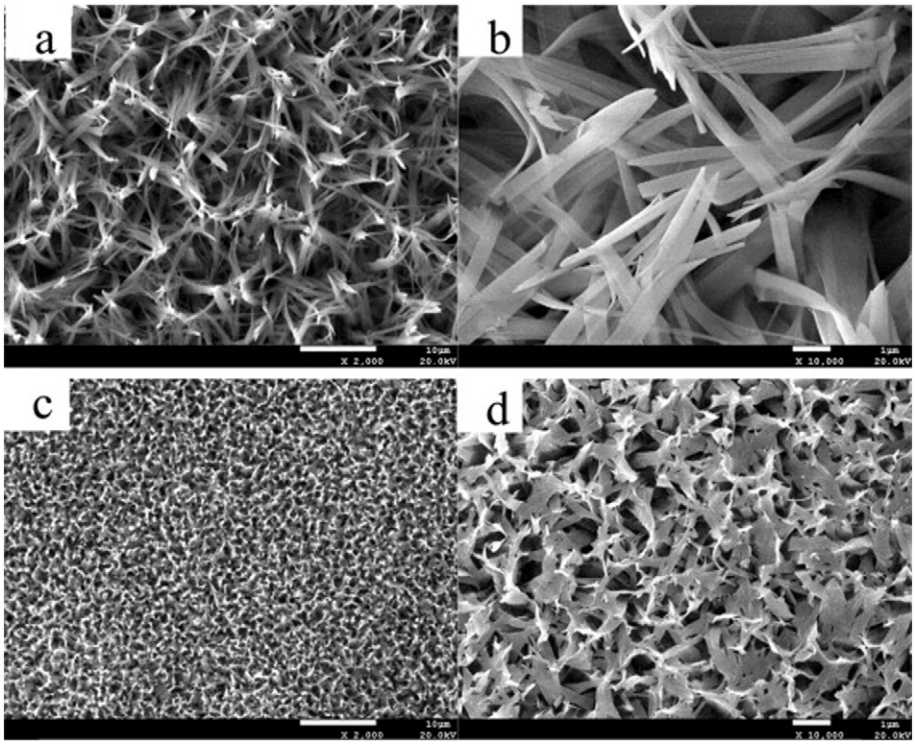

Fig. 5. FESEM surface morphology images of the prepared coatings on TNs: (a and b) HAp, (c and d) CSAgHAp (Yajing et al., 2015) (Reproduced with permission from Elsevier).

Chitosan and gamma-polyglutamic acid (PGA) polyelectrolyte complexes increases the degree of the complex formation lowered the water uptake, reduced the pore size of the porous structure, decreased the in vitro degradation, but increased the compressive modulus of the chitosan/PGA PECs. Furthermore, the chitosan/PGA PECs provided adequate moisture, and thus, reduced the risk of dehydration in the presence of PGA. The wounds treated with the chitosan/PGA PECs healed significantly faster than wounds given no treatment (Ching et al., 2011).

The chitosan (CS)/gelatin (GE) composite nanofiber membranes were electrospun. The excellent mechanical enhancement can be explained by the effective dispersion of fillers and the filler-matrix interactions, which ensures the efficient load transfer from CS/GE matrix to Fe_3O_4 nanofillers. Moreover, zones of inhibition for *E. coli* and *S. aureus* expanded markedly with the supplement of Fe_3O_4 NPs. In all, nanofiber membranes made of Fe_3O_4/CS/GE composite with tailored mechanical and antibacterial properties appear a promising wound dressing material (Ning et al., 2016). The poly(vinyl alcohol)/chitosan/montmorillonite nanocomposite hydrogels were prepared by freezing–thawing method, as a biocompatible wound dressing. Improved mechanical properties of this system along with the other characteristics such as biocompatibility, antibacterial activity and good swelling behaviour, made it desirable candidate for wound dressing applications (Nooria et al., 2015).

Chitosan Based Sponges in Wound Healing

Sponges are nothing else than foams with an open porosity. These solid structures are able to absorb high amount of fluids (more than 20 times their dry weight), due to their micro-porosity. They typically offer good cell interaction, still being soft and flexible. Chitosan sponges are mainly used as wound healing materials, as they can soak up the wound exudates, while helping the tissue regeneration. Chitosan sponges also find application in bone tissue engineering, as a filling material (Jayakumar et al., 2011). Mei Dai et al. reported a biodegradable sponge which is composed of chitosan and sodium alginate loaded with curcumin. Histological results of three different kinds of wound dressings applied on the dorsal skin wound of SD rat. The gauze-treated wounds showed immature granulation tissue with congested vessels and numerous inflammatory cells. The dermis is still under the process of remodelling on 12th day of dressing. However, in the C2A2 sponge and C2A2-curcumin sponge-treated wounds, the granulation tissue and collagen alignment were more advanced in comparison to the gauze-treated wounds. These two kinds of sponges-treated wounds demonstrated compact and well-aligned collagen, showing that the wounds were under a better healing environment. In wound healing testing, full-thickness wounds were made on the back of each rat using C2A2 sponge as wound dressing (Mei et al., 2009).

The sponge-like dressings based on chitosan glutamate (high molecular weight) and sericin was developed for the treatment of chronic skin ulcers. The amount of sericin in the optimized dressing is suitable to exert a protective effect on human fibroblasts against oxidative damage. Moreover, the optimized dressing is able to improve fibroblast proliferation, that is, to promote wound

healing (Michela et al., 2016). Fibrillar structure was obtained and those sponges showed promising use for wound dressing application. In 2009, efforts were dedicated to the use of supercritical carbon dioxide (scCO2) as a green medium to induce porosity to chitosan scaffolds (Kumari et al., 2009). Cross-linked chitosan sponges loaded with a model of antibiotic drug — the norfloxacin were prepared by solvent evaporation technique (Emiir et al., 2004). Absorbable and non-absorbable dressings have been fabricated into sponges via a modified thermally induced phase separation method, using a grafted derivative of chitosan with 2-hydroxyethylacrylate. The In vitro release studies in simulated body fluids give a burst effect due to the surface entrapment of the drug and its amorphization in the first 3 h as well as a low release rate until complete dissolution time that reaches 24 h. Results encouraged that loaded sponge with Levo even at low concentrations, offers a promising solution in wound infections management, providing increased tolerability, safety and antibacterial protection against common susceptible and resistant wound pathogens (Panoraia et al., 2016).

Chitosan Based Oil Immobilized Scaffolds in Wound Healings

The castor oil-based films reinforced with different amounts of CS-modified ZnO nanoparticles were successfully prepared via solution mixing and casting technique. The nanocomposites exhibited antimicrobial activity against both Gram-negative and Gram-positive bacteria in the presence and the absence of UV light, and the effect increased with the CS-ZnO concentration. From the morphology, water absorption, thermal stability, biodegradability, cytocompatibility, barrier, mechanical, viscoelastic, wound healing, and antibacterial studies of the these bionanocomposites, these are found to be promising for use in biomedical applications, particularly as wound healing materials (Ana and Angel, 2015).

Chitosan Based Extract Immobilized Scaffolds in Wound Healings

Hydrogel films are very soft and cause no disturbance during its applications in wound dressings. Plant extract is natural antimicrobials exhibiting low compatibility in the hydrogel polymeric solution and their release and effectiveness in hydrogel films are low as compared to its pure form. Plants are more potent healers and heal the wounds in a naturel way. Hydrogel film encapsulated with gentamycin sulphate, *Salix alba* and *Juglena regia* leaves extract showed both antibacterial and antifungal activities in disc diffusion method and in MIC. Since these properties are the most important properties for ideal wound healing materials which provides a future site to treat wound care system (Mohammad and Fehmeeda, 2014, 2015a, 2015b; Mohammad et al., 2015).

Wounds are frequently contaminated with a variety of bacteria, so the potential for infection is always present. This has encouraged the development of improved wound dressings that show an antimicrobial effect through the incorporation or not of antimicrobial agents. The use of the therapeutic properties of aloe-vera could be very useful in the creation of active wound dressing materials. The chitosan/aloe-vera based membranes were developed as wound dressing materials. The addition of aloe vera to chitosan enhanced the antimicrobial potential of the resulting membranes. CAV1 (CS:AV:1:1, v/w) showed the highest antibacterial potential compared with the other formulations (Fig. 6) indicating the greater effectiveness of CAV1 as a bactericide which could be due to greater content of AV in the membrane, which consequently increases its inhibitory potency. On the chitosan membrane (Fig. 7), hDFs exhibited a round morphology with cell agglomeration throughout the study period. Cells seeded on the CAV membrane showed better spreading and a higher number of cells attached to the surface. From days 1 to 7 the cells were uniformly distributed on the surface of the CAV membrane, exhibiting a spindle-like shape typical of fibroblasts, suggesting good adherence to this surface. Moreover, on day 7 hDFs were well spread on and were able to adhere to both sides of the CAV membrane surface. Despite the good cell adhesion and proliferation observed on the CAV membrane, the higher AV content of CAV1 did not result in the expected increase in cell spreading and proliferation. The chitosan/aloe vera based membranes might be promising wound dressing materials (Silva et al., 2013).

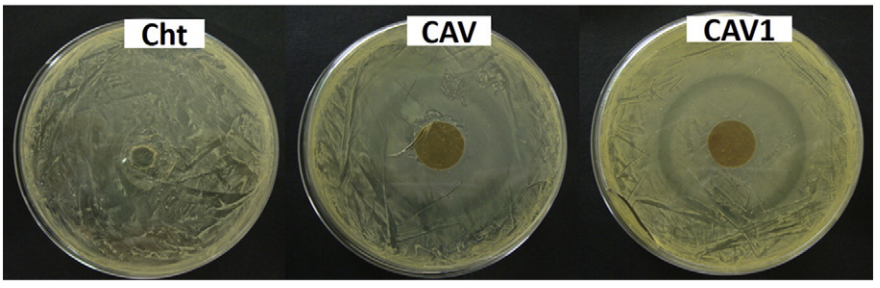

Fig. 6. Photographic image of the developed membranes (Cht, CAV and CAV1) on culture plates inoculated with *S. aureus* after 72 h (Silva et al., 2013) (Reproduced with permission from Elsevier).

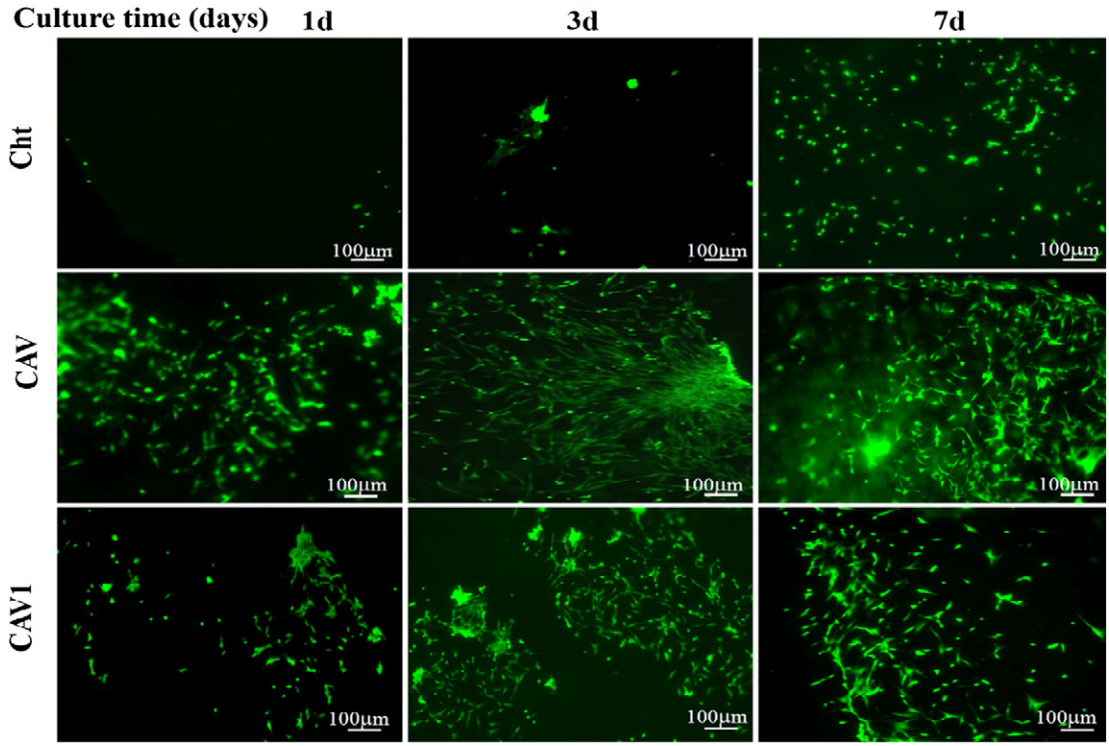

Fig. 7. Calcein-AM staining of hDFb cells cultured for 1, 3 and 7 days on the membranes (Cht, CAV and CAV1) (Silva et al., 2013) (Reproduced with permission from Elsevier).

Chitosan Based Drug Loaded Scaffolds in Wound Healings

Researcher have also tried to use drug loaded chitosan based scaffolds. The thiolated chitosan with poly(N-isopropyl acrylamide) loaded with ciprofloxacin was cytocompatible and found to modulate the release of the incorporated ciprofloxacin in a sustained fashion reflecting its suitability to protect a wound for a prolonged period. The combination of thiolated chitosan with poly(N-isopropyl acrylamide) and ciprofloxacin showed antibacterial properties to the virulent bacteria *E. coli* supporting its potential as a wound dressing (Radhakumary et al., 2011).

Conclusions and Future Prospective

In this review, the various properties such as antimicrobial, anti-inflammatory and biomedical applications of chitosan based materials especially wound dressings have been covered. Chitosan has been shown to be a potential biomaterial to be used in wound healing due to its antimicrobial and anti-inflammatory nature. Effective dressings should have characteristics optimized for particular type of wound at a reasonable low cost and with minimum inconvenience to patients. Many results have been reported, but complete and standard characterization of these materials is needed. Therefore, there is need to manipulate the physical properties of identified systems to achieve such objectives.

Although a lot of literature is available on chitosan and modified chitosan (blends/composites/derivatives) for wound healing, there are many challenges which need to be explored in process of wound healing. We expect that this article will provide insights on the use of these important chitosan based materials for researchers working in the field of biomedical and pharmaceutical sciences.

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