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POTENTIAL WOUND HEALING MATERIALS FROM THE NATURAL POLYMERS -A review

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ABSTRACT

Wound healing represents a major health burden and the variety of wound types has resulted in a wide range of wound dressings with new products frequently introduced to target different aspects of the wound healing process. These wound dressing biomaterials play an active role in the healing process, thus accelerating the process. An ideal dressing should achieve rapid healing at reasonable cost with minimal inconvenience to the patient. The selection of an appropriate dressing plays an important role in both recovery and aesthetic appearance of the regenerated tissue. Among the various wound dressing materials, biomaterial or natural polymer based dressing are gaining importance because of their unique properties. This paper provides a review of the various biomaterials, bioactive polymers, polysaccharides, nano biocomposites, enzymes, growth factors that can be used as potential wound dressing material.

KEYWORDS: **Biomaterials, polysaccharides, nano biocomposites, wound healing materials**

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1.1 INTRODUCTION

In the last years, health care professionals faced with an increasing number of patients suffering from wounds and burns difficult to treat and heal. A wound is the disruption of the integrity of anatomical tissues caused by exposure to any Factor. Wound dressing materials, act as physical barriers permeable for moisture and oxygen, protect the wound mainly against microorganisms [1]. Wounds are examined under two groups:

1.1.1 Closed Wounds

In this type of wounds, tissue is injured but the skin is not broken. These include contusion, hematoma and abrasion. Contusion-type injuries involve damage to soft tissues, small blood vessels and deep tissue layers, resulting in their separation, but the anatomy of the skin remains intact. Oedema, and in later periods, atrophy and defective pigmentation are observed in wound and the healing is delayed. Vessel rupture or hyperaemia due to vessel damage is called hematoma and wounds such as scrapes are termed abrasions. The healing process is very painful because this type of wound involves damage to sensory nerves and the wound can easily become infected[2].

1.1.2 Open Wounds

Open wounds are injuries with a break in the skin or mucous membrane .This group includes lacerations, cutting-pricking tool wounds, gunshot wounds, surgical wounds, insect bites and stings, radio necrosis, vascular neurological and metabolic wounds. Wounds except for lacerations cause serious damage to tissues beneath the skin. In laceration type wounds, skin and subcutaneous tissue have been destroyed, but deep tissues remain healthy. The anatomical integrity of tissues is damaged in cutting pricking tool wounds without any tissue damage at the edges of the wound[3].

1.1.3 Wounds with Tissue Loss

These types of wounds involve damage or loss in some or all of the skin layers. Healing occurs via filling of the wound area by granulation tissue typically growing from the base of a wound. Wounds that involve tissue loss are collected in two groups in proportion to the loss. In superficial wounds, the entire epidermis and the papillar layer of the dermis are damaged. The epidermis, all the layers of the dermis and even subcutaneous tissue are damaged in full-thickness wounds covering second group [2,4].

1.1.4 Wounds without Tissue Loss

These kinds of wounds occur as a result of tissue crushing. The severity of bleeding occurring in tissue varies according to the condition of the wound. Tissues exposed to this kind of wound heal after granulation tissue formation in minimal level in first phase of the healing process[5].

1.1.5 Wound dressing materials

Dressings play an important role in the management of wounds. During the wound healing process, the dressing protects the injury and contributes to the recovery of dermal and epidermal tissues. The following characteristics are required for ideal wound dressing[6,15]. The wound dressings must possess ease of application, bioadhesiveness to the wound surface, sufficient water vapour permeability, easily sterilised, inhibition of bacterial invasion, elasticity and high mechanical strength, compatibility with topical therapeutic agents, optimum oxygen permeability, biodegradability and non-toxic and non-antigenic properties (ASTM F2458-05(2010).The use of biocompatible and biodegradable natural/synthetic polymers will substantially contribute to the development of novel types of wound dressings with large scale applications in the biomedical area and especially for the regenerative medicine. Because their biocompatibility, biodegradability and similarity to macromolecules recognized by the human body, some natural polymers such as polysaccharides (alginates, chitin, chitosan, heparin, chondroitin), proteoglycans and proteins (collagen, gelatin, fibrin, keratin, silk fibroin, eggshell membrane) are extensively used in wounds and burns management. The use of three-dimensional polymeric scaffolds for cell targeting is already a common strategy for tissue engineering. Obtained by electro

spinning technique, some synthetic polymers like bio mimetic extracellular matrix micro/nanoscale fibers based on polyglycolic acid[7], polylactic acid[8], poly-acrylic acid, poly-ε-caprolactone [9], polyvinylpyrrolidone, polyvinyl alcohol[10,11], polyethylene glycol[12], exhibit *in vivo* and *in vitro* wound healing properties and enhance re-epithelialisation. Wound and burn covering materials are classified as follows [13, 14].

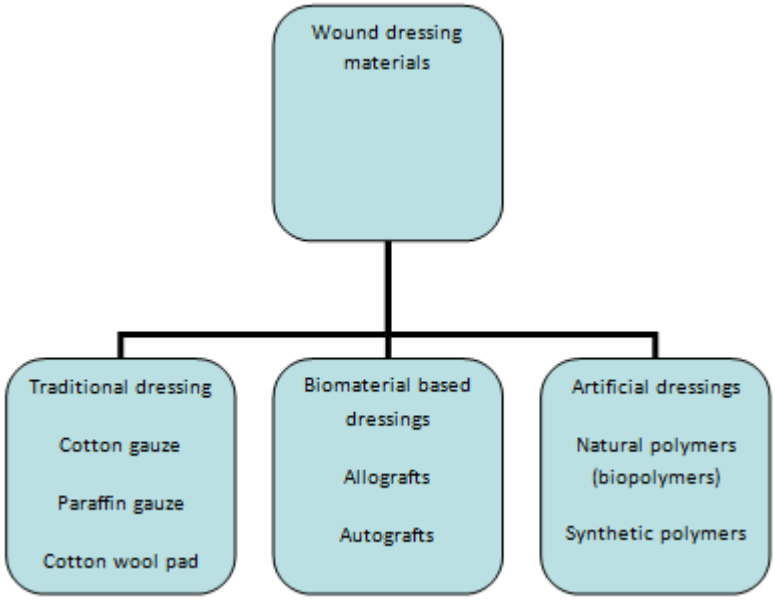

Figure1
Types of wound dressings

2.1 TRADITIONAL DRESSING

These are still the most commonly used materials for wound and burn dressings[15]. The traditional dressings, which are generally used during the first intervention in wound treatment, prevent wound's contact with outer environment and bleeding[14,16]. The best sample of this group is gauze and gauze-cotton composites which have very high absorption capacity. As they cause rapid dehydration whereas they are being removed from the wound surface, they can cause bleeding and damage of newly formed epithelium[14, 17].Therefore, gauze composites with a non-adhesive inner surface are prepared to reduce the pain and trauma which can occur when removing traditional wound dressings from the wound surface. Some of the dressings used are Paraffin gauze dressing containing0.5% chlorhexidine acetate, Paraffin gauze dressing ,petrolatum gauze, petrolatum gauze containing 3% bismuth tribromophenate, scarlet red dressing, sterile hydrogel dressing ,Highly absorbent cotton wool pad ,highly absorbent rayon/cellulose blend sandwiched with a layer of anti shear, high density polyethylene, and absorbent cotton pad. Exudates leaking from traditional dressing materials usually increases the risk of infection is one of the most significant problems of these type dressings. Antibacterial agents are added into the dressings to eliminate the infection. In addition, one of the most significant problems encountered in this material is a foreign body reaction in the wound caused by cotton fibres. The biggest advantage of these materials is their low cost[14, 18, 19].

2.1.1BIOMATERIAL-BASED DRESSINGS

Biological dressings are natural dressings with collagen-type structures, generally including elastin and lipid. Such dressings can mainly be categorised under the following groups[20, 21].

1. Allografts - (Scalp tissue, Amniotic)
2. Tissue derivatives- (Highly purified bovine collagen, Formaline fixed skin)
3. Xenografts- (Porcine tissue, Silver impregnated porcine Tissue).

The most common source for allograft dressing is fresh or freeze-dried skin fragments taken from the patient's relatives or cadavers. Immune reaction as a result of the

use of allograft can be seen and the body may reject the tissue. Infection risk also increases with suppression of the immune system to prevent the body's rejection of transplanted tissue. The other disadvantages of these dressings include the difficulty of preparation, lack of donors, high cost and limited shelf life[5]. .Amniotic membrane {AM), which is separated from chorion, generally uses in superficial partial thickness burns as a dressing material for many years. AM has been routinely used in several clinical studies for the treatment of burns on the skin, ulcers and, pre-dominantly in ophthalmology, for the treatment of eye-piece surface disorders. Its use is based on its ability to improve the process of epithelisation, as well as reducing the inflammatory processes, angiogenesis and scarring alopecia. Though it has advantages such as ease of preparation and use, it has disadvantages like causing cross infection and dehydration of the wound[13]. Xenografts are commercially available materials contrary to autografts and allografts. The most common of xenografts is the ones derived from pig skin[16], which have a long shelf-life and can be sterilized easily[20]. Although pig skin is not microscopically similar to human skin, it shows close similarity in terms of adhesion and collagen content. Its disadvantage is the risk of triggering an immune response due to the foreign tissue. Tissue derivatives materials, derived from different forms of collagen, have the advantages like ease of preparation, low contamination risk and weak antigenic features. The greatest disadvantage of these materials is the risk of infection, particularly in long term usage[6.14].

2.1.2 ARTIFICIAL DRESSING

These are used in the form of film/membrane, foam, gel, composite, spray. Two types of polymers are used for artificial dressings:

1. Natural polymers

Collagen, alginic acid & salts, hyaluronic acid derivatives, fucoidan, chitosan, poly N acetyl glucosamine**.**

2. Synthetic polymers

Polyurethane & derivatives, teflon, methyl methacrylate, silicon.

3.1 NATURAL POLYMERS

Natural polymers are widely used in the regenerative medicine field, for wounds dressing because of their biocompatibility, biodegradability and similarity to the ECM. Natural polymers are involved in the repair of damaged tissues and consequently in skin regeneration by inducing and stimulating the wound healing process[22]. These are typically composed of a polymeric network that can contain up to 99 per cent or higher water content. As a result, they are referred to as 'hydrogels', and their swelling capability in water allows them to exhibit an environment that resembles the highly hydrated state of natural tissues[23]. Although naturally derived polymers are characterized by having batch-to-batch variations and poor mechanical properties[24], they are also readily available, inexpensive and easy to fabricate into hydrogels, which makes them appealing choices for scaffolds. Due to their three dimensional cross-linked polymeric networks that are soaked with water or biological fluids, biomaterial hydrogels are employed in the pharmaceutical and biomedical area, especially for wound management, tissue engineering, drug delivery, and organ trans-plant[25]. Creamshas been formulated with honey and plant extract which can enhance wound healing[26]. In addition, novel biomaterials based on renewable, non-toxic, and biodegradable natural polymers are obtained through radiation processing. Therefore, hydrogels containing cross-linked natural polymers can be used for wounds and burns dressing[27].

3.1.1 Collagen

Collagen is a biodegradable and biocompatible protein mostly found in connective tissue. Collagen wound dressings can provide anti-infective, anti-inflammatory, anti-fibrotic, and analgesic properties, as well as promote angiogenesis, returning the body to its normal function and providing a foundation for wound healing. The first medical usage of collagen in humans was reported by[28] and was used to provide co-reaction of contour deformities. Later bovine collagen was used as suture and hemostatic agents. In 1980, Zyderm as released, a suspension form containing sterilised fibrillar bovine collagen was used for curing wound. Presently collagen is used in numerous biomedical applications These include collagen suspensions for dermal injection, as

topical haemostatic agents- collagen sponges for the haemostasis, wound dressing materials, collagen suture and catguts, collagen gels for periodontal reconstruction, and for coating of joint, [29, 30, 31,32]. Collagen serves as an excellent wound dressing material because of its negligible immunogenicity, excellent biocompatibility , and mechanical stability. It could be fabricated into 3-D scaffolds by chemical cross-linking techniques, forming a porous structure[33, 34]. However, such scaffolds usually have insufficient mechanical properties, swell substantially in water and are vulnerable to enzymatic digestion. Collagen dressings encourage the deposition and organization of newly formed collagen, creating an environment that fosters healing because of the chemo tactic properties of the dressings on wound fibroblasts. It can stimulate and recruit specific cells, such as macrophages and fibroblasts, along the healing cascade in order to enhance wound healing and also provide moisture or absorption, depending on the delivery system. Collagen dressings are easy to apply and remove and are conformable. Collagen dressings formulated from bovine, porcine or avian sources are recommended for the treatment of partial and full-thickness wounds with minimal to moderate exudates. It is contraindicated for third-degree skin burns and for sensitive/allergic patients[5, 36]. Numerous studies have been done concerning the application of different collagen dressings formulations for wounds and burns: collagen sponges in the healing of experimental deep skin wounds[37], collagen–glycosaminoglycan complex (Glycagen®) as a dressing for gingival wounds[38], collagen resorbable membrane for oral wound dressing[39], salmon milt DNA–salmon collagen composite films for wound dressing[40], biopolymer material prepared using type III collagen of avian intestine and anionic polysaccharides[41], collagen–minocycline based hydrogels potentially applicable for the treatment of cutaneous wound infections[42], denatured collagen microfiber scaffold seeded with human fibroblasts and keratinocytes for skin grafting[43] electrospun collagen nanofibrous scaffolds for artificial vascular grafts and wound repair[44], collagen-tocopherulate for topical applications[45], collagen–alginic acid cross-linked thermostable and biodegradable biopolymer as a wound dressing material[46]. Collagen is a natural substrate for cellular attachment, growth and differentiation, and promotes cellular proliferation and differentiation[47]. Effects of hyaluronic acid and fibronectin on wound healing were studied. These macromolecules play an important role in wound healing, embryonic development and cellular migration *in vitro*. The effects of addition of varying levels of fibronectin and hyaluronate to a collagen sponge were studied. Low levels of both hyaluronate and fibronectin modified the structure of implant and resulted in increased chemoattraction, replication and collagen deposition in an *in vivo* wound healing model[48, 49]. Repair of deep burn depends on angiogenesis. Dermal regeneration can be enhanced by VEGF/N,N,N-trimethyl chitosan chloride(TMC) loaded in bilayer porous collagen-chitosan/silicone membrane dermal equivalents, applied for the treatment of full thickness burn wounds[50]. Collagen has been widely employed in the construction of artificial skin substitutes used in the management of severe burns, and several collagen based commercial products have been marketed, such as collapat II® (Biomet Inc.), Healos® (Depoy Spine, Inc.), Collagraft® (Nuecoll Inc., Zimmer Inc.) and Biostite® (Vebas S.r.l.) [51]. Integra and Apligraf have been used for the clinical treatment of burn victims and other patients afflicted with skin disorders that require skin graft treatment[52, 53, 54]. There are two types natural and denatured collagen(also known as gelatin). Gelatin or gelatin-like, are highly processed as collagen appears to be unable to elicit some of the biological responses native collagen nature has programmed into cells involved in wound healing. New generations of collagen-based dressings combine properties of stimulation of wound healing with absorption of moderate to high levels of wound drainage. Some new products in the collagen dressing category seem to present a remarkably pure form of collagen, which could conceivably lead to less giant cell type of reaction in the wound bed due to the lack of non-biodegradable materials (such as plant-based or marine based cellulose derivatives). Denatured or degraded collagen (more accurately described as gelatin), especially when mixed with non

collagenous substances degrades faster than a native collagen dressing in a wound environment because of its inherently modified and processed nature. Crosslinking through the use of chemical crosslinking reagents is a conceivable method to improve the degradation\ rate of gelatin (denatured collagen) products. From a biochemical cellular response perspective, such artificial chemical methods do not improve, and may actually deteriorate, the nature of a material that is meant to be as close in chemical structure to its natural source as possible. The presence of a minimally degraded native collagen is expected to allow a collagen dressing to last longer (compared with denatured dressings) in the wound bed because of the longer time that wound proteinase takes in digesting a native protein compared to that of a denatured protein. Such a property leads to a higher longevity of the dressing in the wound bed, associated with a longer period of biological action in the wound bed and the resulting reduced frequency in dressing changes lessen the trauma associated and also be more cost-effective .Many pure collagen or denatured collagen dressings also combine collagen with silver to decrease the bio burden in the dressing[55] Gelatin is a natural polymer derived from collagen and is prepared from different animal by-products. In biomedical area, gelatin is used for the production of biocompatible and biodegradable drug delivery systems and wound dressings[55]. For wounds and burns dressing, it is administered in various formulations: cross-linked gelatin–alginate and gelatin–hyaluronate sponges with wound healing properties on the full-thickness dorsal skin defects of Wistar rat[56], EGF containing gelatin-based wound dressings in case of bulk loss of tissue or non-healing wounds such as burns, trauma, diabetic, decubitus and venous stasis ulcers[55], and also as biodegradable gelatin-based films in trauma and orthopedic surgery[57].

3.1.2 Chitin and Chitosan

Chitosan is derived from chitin, which is found in the exoskeleton of marine crustaceans such as shrimps and crabs, as well as insects and the cell walls of fungi[58, 59]. It is derived from chitin through a deacetylation process to obtain a linear structure of glucosamine and N-acetyl glucosamine linked in a b-1,4 manners[60]. Chitin is the most abundant natural amino polysaccharide (poly-N-acetyl-glucosamine) . Chitosan is soluble at pH lower than 5.5, thus solvents such as acetic acid and hydrochloric acid are often used so as to dissolve chitosan. Chitosan forms gels either by raising the pH to 6 or higher[61], or by interacting with a variety of divalent and polyvalent anions[57] and is biocompatible, biodegradable, non-toxic and biofunctional[58]. The applications of chitosan include drug delivery[58, 59], growth factor encapsulation[62] and gene delivery by forming complexes between the cationic chitosan and negatively charged DNA[63, 64].This cationic natural polymer, has been widely used as a topical dressing in wound management owing to its hemostatic, stimulation of healing, antimicrobial, nontoxic, biocompatible and biodegradable properties. Chitosan preparations are classified into native chitosan, chitosan formulations, complexes and derivatives with other substances. Chitosan possess properties of binding with red blood cells allow it to rapidly clot blood, and it has recently gained regulatory approval in the USA for use in bandages and other hemostatic agents[65,66]. In addition, chitosan modulates the functions of inflammatory cells and promotes granulation[67]. As a semi permeable biological dressing, it maintains a sterile wound exudate beneath a dry scab, which prevents dehydration and contamination of the wound, thus helps to optimize conditions for healing. The antimicrobial effects of chitosan are due to destabilization of the outer membrane of Gram-negative bacteria[68] and permeabilization of the microbial plasma membrane. Apart from this many factors present in the chitosan molecule or its environment can influence the antimicrobial properties, such as the molecular weight, DDA and the ionic strength and pH of the dissolving medium, the physical state of the chitosan, such as whether the it is present in the form of films, hydrogels, coatings, in solutions or in combinations with other materials. Its use in the treatment of wounds and burns is due to its hemostatic effect[69, 70, 71]. It is thought that chitosan accelerates the formation of fibroblasts and increases early phase reactions related to healing[72]. The applications of chitosan in commercial and

biomedical fields have increased due to its low toxicity and biodegradation products, and its biocompatibility with blood and tissues [73, 74, 75]. Chitosan can be prepared in a variety of forms, namely films, hydrogels, fibres, powders and micro-nanoparticles. Chitosan was evaluated to determine the efficacy to act as a wound healing accelerator. Open skin wounds were made on the dorsal side of 3 beagles and cotton fiber chitosan was applied for 15 days. Wound healing process was evaluated histologically and immunochemically. Results showed that the chitosan treated wounds had a severe infiltration of polymorphonuclear cells and increase in effusion compared with that in control. Granulation was more pronounced by the chitosan treatment on day 9 and15 post wounding[76]. Composite nanofibrous membrane of chitosan/collagen is known for their beneficial effects on wound healing. The membrane was found to promote wound healing and induce cell migration and proliferation. Animal studies have proved that nanofibrous membranes are better than gauze and commercial collagen sponge in wound healing[77]. The chitin powder was found to be more efficient than chitin or chitosan as a wound healing accelerator: wounds treated with chitin hydrogel were completely re-epithelialized, granulation tissues were nearly replaced by fibrosis and hair follicles were almost healed in 7 days after initial wounding. Also, the chitin hydrogel treated skin had the highest tensile strength and the arrangement of collagen fibers in the skin was similar to normal skins[78]. Dibutyrylchitin (DBC) is a water soluble chitin derivative. DBC fibrous materials were used for wound healing applications[79]. In a study done on 9 patients with different indications satisfactory results were obtained especially in case of burn wounds and post operative/ post traumatic wounds and various other conditions causing skin/epidermis loss[79]. Treatment of full thickness cutaneous wounds in a diabetic mouse model with chitin-containing membranes results in a increased wound closure rate correlated with the impressive rise of angiogenesis. Serum starved endothelial cells were treated with either VEGF or different concentration of chitin. The results obtained after 48 hrs and compared with the control plate. In control plates a two fold reduction in cells occurred whereas this effect is compensated by VEGF and different concentration of chitin in the test plates. Chitin treated plated does not show any increase in cell number indicating no proliferation occurred but chitin helped to rescue the cells from dying due to serum deprivation[80,81], developed a non adherent wound dressing with sustained antimicrobial capability to treat mustard burns. It contains two layers: upper layer is a carboxymethyl chitin hydrogel material, and lower layer is an antimicrobial impregnated biomaterial. Carboxymethyl chitin hydrogel provides mechanical and microbial barrier along with the capability of adsorbing wound exudates. This property makes it ideal for use in second degree burns as hydrogels can swell considerably holding upto 4 times its own weight of water thus preventing accumulation of fluids in highly exudating wounds. Chitosan acetate foam impregnated with chlorhexin gluconate forms the lower layer. In the *in vitro* release studies, to obtain sustained antimicrobial activity for 24 h loading concentrations were optimized which in turn provide sufficient anti-microbial drug into the wound area.For treatment of full thickness burn injuries a bio-inspired bilayered physical hydrogel only constituted of chitosan and water were processed and applied. To ensure good mechanical properties and gas exchange first layer is made of rigid protective gel and a soft and flexible second layer allow the material to follow the geometry of the wound and ensure good superficial contact. In order to compare highly viscous solutions of chitosan were also considered. Only one chitosan material is used for each time [82].Studies were done to determine effects of sterilization methods on morphology, mechanical properties, and cytotoxicity of chitosan membranes used as wound dressing. In the study effects induced by two different sterilization methods(exposed to gamma radiation and ethylene oxide) and an antiseptic technique (immersion in 70% ethanol aqueous solution) on the morphology, tensile strength, percentage of strain at break, and *in vitro* cytotoxicity to Vero cells on chitosan membranes designed for wound healing was done. With chitosan, glycerol, and chitin as components four different membrane compositions were evaluated. Gamma radiation, in spite of being one of the most

commonly employed sterilizing agent, negatively affected the morphology of membranes composed solely by chitosan as well as the percentage of strain break of the chitosan-membranes containing glycerol on their composition. Its use also affected the colour of chitosan membranes. The use of 70% ethanol aqueous solution does not change the chitosan membrane characteristics significantly, but its use has limitation concerning process scale up. With ethylene oxide(EtO), chitosan morphology, percentage of strain break, and *in vitro* cytotoxicity to Vero cells were not significantly affected. The tensile strength of membranes containing chitin were reduced after the treatment with ethylene oxide; however, the obtained values were comprised in the range verified for normal human skin. Therefore, when considering their use as a wound healing devices, and because this sterilization process is easily adjusted to use as an industrial scale, EtO can be considered the most adequate sterilization agent for chitosan membranes. However, it should be considered that this chemical is associated with toxicity, flammability, and environmental risks, as well as with possible material contamination with ethylene oxide residues[83]. Effect of chitosan and linear polyvinyl amine were studied to explore and compare its antibacterial properties of the prepared dressing based on cotton. Using butane tetracarboxylic acid biopolymer molecules were covalently fixed on the cotton. They are characterised using amine groups created on the surface of the fabric. *Escerichia coli (E. coli)* DSMZ 498 was used to evaluate bacteriostatic effect. From the results obtained it showed a synergistic bacteriostatic effect of treated cotton samples by using chitosan/polyvinylamine finishing system. Thus produced cotton can be used to treat wounds, ulcers as well as diabetic ulcers in addition to some kind of burns[84]. Using Tegaderm as backing a novel wound dressing was made with chitosan(CH) and minocycline hydrochloride(MH).CHs with different deacetylation degrees were used. CH with 67% and 83% showed sustained release of minocycline *in vitro*. During *in vivo* studies a negative effect was shown in wounds applied with 10mg minocycline and sealed completely with Tegaderm. CH83 with 2mg minocycline showed an excellent effect along with CH 83 alone. Pus removal was better for CH83 with minocycline thus it is considered as better than CH83 alone[85]. In recent years, new forms of chemically modified chitosan have been developed in order to improve the properties of chitosan for various biological activities, and these substances have gained increasing attention. Representative members of these novel polymers include ammonium chitosan, carboxymethyl chitosan and derivatives[86].

Table 1
Natural polymers as wound dressing materials

Polymer		Type of wound dressing material	in vitro/in vivo models used	Results	Reference
chitosan and its derivatives	Photo crosslinked chitosan	Ointment	Mouse	accelerated wound closure and healing	[87]
	Chitosan-heparin complex	Hydrogel	Mouse	complete regeneration of apendage structure similar to dermis	[88]
	Carboxyethyl chitosan/polyvinyl alcohol (PVA)	Nanofibres	L929 fibroblast	promote cell attachmet and proliferation	[89]
	dibutyryl chitin	Non-woven	Mouse	doesnt show any adverse effect in wound healing	[90]
	Chitin-poly(acrylic acid)	Hydrogel	L929 fibroblast	Enhanced cell proliferation and attachment of cells to film	[91]
	Chitosan lactate		Mouse	accelerated healing. Will not result in scar formation	[92]
	Collagen-chitosan-polyethylene oxide	Composite nanofibrous membranes	3T3 fibroblast, Mouse	no cytotoxicity. Better wound healing rate	[93]

	Heparin linked chitosan	Gel	Human skin	stimulates re-epithelialisation	[94]
	Chitosan	Hydrogel	Wistar rats	non cytotoxic, no pathological abnormalities, better wound healing rate compared to control	[95]
	Alginate/chitosan/fucoidan	Powder	Rats	stimulated wound healing in mitomycin treated impaired wounds, granulation tissue and capillary formation	[96]
	Chitosan/nanosilver		Sprague dawley rats	increase in wound healing rate, low silver level in blood and tissue than control	[97]
	Electrospinned chitosan	Nanofibres	human dermal fibroblast	blood clotting efficiency is increased, cell proliferation also increased	[98]
	Chitosan/Honey/Gelatin	Hydrogel	Rats	non toxic or irritant, significant contraction and intact dermis formation	[99]
	Silver loaded chitosan polyphosphate		Rats	greater bactericidal activity, even though severe cytotoxicity found, wound healing was not inhibited	[100]
	Assymmetric chitosan with silver and sulfadiazine		3T3 fibroblast	prolonged antibacterial activity, decreased silver toxicity	[101]
	Chitosan with neurotensin		Raw 264.7, HaCaT cells, Wistar rats	non toxic, decrease expression of inflammatory cytokine and decreased amount of inflammatory infiltrate. decrease in MMP9 in diabetic skin and increased collagen expression , deposition and fibroblast migration	[102]
Collagen	Type I collagen in 1,1,1,3,3,3-hexafluoro-2-propanol(HFIP)	Nanofibres	Rats	high cell adhesion and it acts as wound healing accelerators	[49]
	Collagen with chitosan	Porous scaffold	Human Fibroblast, rats	no cytotoxicity., improved biostability, accelerate cell infiltration and proliferation. Good biocompatibility	[103]
	Curcumin incorporated collagen matrix	Matrix	Rats	quenches free radicals more efficiently, increased hydrothermal stability. Curcumin bound to collagen without affecting its triple helicity	[104]
	Quercetin incorporated collagen	Matrix	Rats	better wound healing, hydroxyproline and uronic acid content showed increase in proliferation of cells. Increase in wound contraction and more efficient radical quenching	[105]
	Triphala incorporated collagen	Sponge	Albino rats	increase in thermal stability, water uptake capability, faster wound closure, improved tissue regeneration, collagen content at the wound site. MMPs expression was correlated well with reduction in the reduction in the inflammatory phase	[106]
	Pullulan,collagen composite	Hydrogel	Mouse	improved wound closure, formation of vascularised granulation tissue. Increased recruitment of stromal cells	[107]

	Collagen loaded with neurotensin		Diabetic induced mice, Raw 264.7, HaCaT cells	Faster wound healing in early phase of wound healing, non-cytotoxic, MMP-9 reduction diabetic kin which increased fibroblastmigration and collagen deposition	[108]
	Collagen with fibrin incorporated with macrotyloma uniflorum	Sponge	Fibroblast, keratinocytes, rats	biocompatible. Supress cyclooxygenase-2(COX-2) and inducible nitric oxide syntahses(iNOS) thereby reducing inflammation	[109]
	Sericin/collagen	Membrane	Keratinocytes, fibroblasts	a fibrous capsule was formed around with an acute inflammation. Increase in sericin content increased stability and water uptake	[110]
	Collagen	Bioengineered skin equivalents		Scaffolds for cardiovascular, musculoskeletal & nervous tissue engineering	[111]

3.2.1.1 Ammonium chitosan

Native chitosan is not soluble in neutral and alkaline aqueous solutions. As a result, chitosan derivatives containing quaternary ammonium salts, such as N,N,N-trimethyl chitosan, N-propyl-N,N-dimethyl chitosan and N furfuryl- N,N-dimethyl chitosan have been investigated for improved solubility in water and subsequently improved biological activities. Studies have shown that all ammonium chitosan derivatives were highly water-soluble at acidic, basic and neutral pH[110,111,112,113]. Compared with native chitosan, ammonium chitosan demonstrated enhanced antimicrobial properties[110,111] and drug-delivery abilities[114].

3.2.1.2 Carboxymethyl chitosan

Carboxymethyl chitosan (CMC) is another modification of chitosan formed by attaching carboxymethyl groups to the chitosan backbone. Depending on the location of the carboxymethyl group attachment, CMC can be referred to as 'N' when the carboxymethyl group attaches to the amine, 'O' when it attaches to the primary hydroxyl group or N,O,- carboxymethyl chitosan when attached to both[115]. CMC has the advantage of a greater solubility range than native chitosan and has now been extensively studied for its activities for drug delivery[116, 117], hemostasis, antimicrobial action[118,119,120] and the stimulation of wound healing[121]. Different formulations of chitin and chitosan have been studied: water-soluble chitin (WSC) ointment as a wound healing assistant[122], microcrystalline partially deacetylated chitin hydrochloride as promising hemostatic material[123], bactericidal films based on chitin and silver nanoparticles for wound dressing applications[124], chitin-based wound dressing containing silver sulfadiazine[125], chitin hydrogel–nano zinc oxide composite bandage[126], phosphorylated chitin and chitosan, bioactive chitin/chitosan hydrogel membranes and scaffolds co cultured with keratinocyte and fibroblast cells[127, 128]. Films, sponges and hydrogels of microcrystalline chitosan with antimicrobial and wound-healing effects for wounds and burns[129, 130], chitosan mesh membrane with a 75% degree of deacetylation as a wound-healing dressing [131], antibacterial chitosan–nanosilver film[132], chitosan film enriched with an antioxidant agent, taurine, in fenestration defects[133],chitosan film/gel containing fucoidan as a wound dressing for dermal burn healing[134], chitosan–aloevera membranes for wound dressing[135, 136, 137], chitosan films incorporated with thyme oil for potential wound healing applications[138], chitosan–cellulose–silver nanoparticle composite films[139], chitosan–alginate polyelectrolyte complex membranes for wound dressing applications[140], chitosan–gelatin sponge-like/composite films for wound-healing dressing[141], ciprofloxacin loaded chitosan–gelatin composite films[142], chitosan gel formulation containing epidermal growth factor (EGF) for burn wound healing[143], graft copolymerized chitosan by choosing various types of side chains for drug delivery and tissue engineering[144], novel chitosan–Ca3V10O28complex membrane with

sustained antimicrobial capability for wound dressing[145], Tencel–chitosan–pectin composite[146], chitosan–silk fibroin composite nanofibers for wound-dressing applications[147], chitosan–fibrin micro beads for cell delivery in tissue repair applications [148], chitosan–fibrin–sodium alginate composite for wound dressing[149], microporous chitosan hydrogel–nanofibrin composite bandage for skin tissue regeneration[150]. Polysaccharides are also administered in the form of hydrogels, some polysaccharides are extensively used for the management of wounds and burns: neutral (glucans, dextrans, cellulose), acidic (alginic acid, hyaluronic acid), basic (chitin, chitosan) or sulfated polysaccharides (heparin, chondroitin, dermatan sulfate, keratan sulfate)[147,148].

3.3 Homoglycans

These are naturally occurring cellular response modulators that actively participate in the wound healing process[151]. Among these materials, electro-spun dextran, cellulose or starch have been found to be useful materials for obtaining nanofiber matrices which are made use in wound dressings and tissue engineering[152].

3.3.1 Glucans

Pullulan is a (1→4),(1→6)-D-glucan biosynthesized from starch by the ubiquitous yeast-like fungus Aureoba-sidium pullulans (Dothioraceae).A hydrogel based on a derivative of pullan has been prepared by chemical crosslinking method. This hydrogel has been found to be a super absorbent because of its high water absorption capacity with a swelling ratio upto 4000%. This property prevents wound bed dehydration and also helps in fast haemostasis and further incorporation of antimicrobial drugs into this hydrogel protects wounds and burns from microbial invasion. The antimicrobial property is imparted to the hydrogel by incorporation of antibacterial or antimycotic drugs. ([153]. It has also been reported that a purified yeast –derived (1→3)-D-glucan supports wound healing activity, does not support adipogenic differentiation and also has the ability to lower skin irritation[154].

3.3.2 Beta -Glucan

Recent studies highlight that highly purified yeast-derived insoluble (1→3)-D-glucan (Glucan 300) strongly inhibits adipogenic differentiation, supports wound healing and significantly lowers skin irritation and crosslinked poly(vinyl alcohol) hydrogels for wound dressing applications[153,154, 155].

3.3.3 Dextrans

Dextran, which consists of a-1,6-linked D-glucopyranose residues, is a bacterial-derived polysaccharide generally produced by enzymes from certain strains of Leuconostoc or Streptococcus. More recently, dextran hydrogels have been considered for biomaterials applications and investigated as drug delivery vehicles. They are particularly compelling as scaffolds for soft tissue-engineering applications because dextran is resistant to both protein adsorption and cell-adhesion allowing cell adhesion to be achieved by specific derivatization with extracellular matrix (ECM)-based peptides. CMDBS (Carboxymethyl benzylamide sulfonate dextran) which is a soluble polymer has been reported to stimulate wound healing, affect proliferation and metabolism of different cells[156]. Hydrated cyclodextrin possess the ability to capture lipophilic odor molecules and have been used in odor control dressings. These are found to be active for a longer time and further serum proteins helps in enhancing the property of odor absorption.

3.4 Cellulose

Cellulose is a naturally occurring polysaccharide having glucose-based repeat units connected by b-1,4-glucosidic linkages[157]. Various properties of the polysaccharide which makes it suitable for wound dressing application are biocompatibility[158], high tensile strength, ease of mouldability, water absorption ability and also forms fine fibrous network[159]. In case of chronic wounds, it has been found that use of scaffold /matrix made from bioengineered cellulose as wound dressing reduces the healing time as well as pain. In case of partial or full thickness wounds these materials has been found to support the process of epithelialization and granulation. The cellulose based wound dressing materials can be modified by incorporation of active molecules

like antimicrobial drugs, antioxidants, hormones, enzymes and vitamins[160]. .Microbial cellulose synthesized by *Acetobacter xylinum* (*Acetobacteraceae*), also can be used as wound dressing and as tissue engineered skin[161].It has been used in regenerative medicine as wound healing scaffold for damaged skin because of its excellent physico-chemical properties, nanostructure ,biodegradability, mechanical strength ,anti-microbial property and biocompatibility. It has also been reported to be at an alternate dressing material for superficial partial-thickness burn wounds[162, 163]. Some of the commonly used cellulose derivatives include carboxymethyl cellulose (CMC),hydroxyl ethylcellulose (HEC), hydroxyl propyl cellulose (HPC), hydroxyl propyl methyl cellulose (HPMC), cellulose acetate and bacterial cellulose[157,164,165]. Bacterial nanocellulose has been reported to be a good wound dressing material in biomedical applications. Porous nanofibrous BNC has been used for large area skin transplantation, tissue repairing and remodelling[166]. These materials possess special properties like biocompatibility, biodegradability, chirality, hydrophilicity, broad chemical modifying capacity, ability to form various semi crystalline fiber types making it suitable for such applications[167]. Cellulose derivatives have also been used as film coatings, gel base , bioadhesives, as base material for controlled drug release and also for tissue regeneration applications[157,159,165].

3.4.1 Heteroglycans

Some heteroglycans, exhibit important applications in biomedical area due to their biocompatibility, biodegradability and peculiar physico-chemical features: electrostatic complexes, film or gel-forming, thickening properties such as alginates, agarose, carrageenans, pectins, gums, glycosaminoglycans[168].

3.5 Alginates

Alginate is a polysaccharide derived from brown algae, certain seaweeds or bacteria. It is a linear polysaccharide copolymer of (1,4)-linked b-D-mannuronic acid (M) and a-L-guluronic acid (G) monomers[169], and its structure consists of blocks of M or G monomers. Alginate gels readily dissolves in the presence of divalent ions such as Ca^{2+}, Ba^{2+} and Sr^{2+} [170] via ionic cross-linking. Alginate-based wound dressings are commonly used for their haemostatic properties in exudation/bleeding wounds and burns. Calcium alginate, calcium–sodium alginate, collagen–alginate and gelatin–alginate obtained from processed algae are highly absorbent natural fiber dressings[154, 171,172,174]. Alginate can absorb water/body fluids up to 20 times its weight and the resulting hydrophilic gel provides a moist wound-healing environment. As alginate dressings are very porous and have no adhesive properties, a secondary dressing is needed to protect and fix them[173]. Different alginate-based materials due to their antimicrobial properties and wounds/burns healing applications have been obtained: zinc alginate, silver alginate[174], asiaticoside-loaded alginate films, sodium alginate–chitosan two ply composite membranes[175], sodium alginate–chitosan-based films combined with laser therapy. Alginate scaffolds have been used in conjunction with another weaker material to reinforce the mechanical properties[176, 177, 178]. Alginic acid and its salts are used for the treatment of wound and burn due to their haemostatic properties. Their first applications were in the form of a gel, but sponges produced from calcium alginate are also used effectively in the treatment of wounds. It is also indicated that calcium alginate increases cellular activity properties such as adhesion and proliferation[179].

3.5.1 Agar and agarose

Agarose is a polysaccharide with alternating copolymers of (1,4)-linked 3,6- anhydro-a-L-galactose and (1,3)-linked b-D-galactose[180]. Most studies conducted to date using agarose as a scaffold involve cartilage repair[181,182,183]. Agar polysaccharides and natural agarose fibers obtained through wet-spinning process are promising bio-materials for wound dressing applications. Although the agar fibers exhibit a better water swelling capacity compared to agarose fibers nevertheless the agaro pectin has been proved to cause flexibility flaws[184].

3.6 Pectins and gums

Hydrocolloids such as pectins and gums are used as occlusive and semi -occlusive moist

dressings (gels) for wounds management. Pectin as it is a biocompatible and biodegradable natural polymer, it is widely used in the food industry, targeted drug delivery, wound healing, tissue engineering[185,186]. Tamarind seed polysaccharide (TSP), obtained from the seeds of Tamarindus indica, tamarind (Fabaceae), shows utility as wound dressing[187]. Pectin–gelatin (PEGE) hydrogel membranes, cyto compatible with B16 melanoma cells, are used for wound dressing (moist wound care) applications. The increase in gelatin ratio significantly improves the microporous nature of the membranes with highly interconnected honeycomb type architecture and enhanced thermal stability as compared to reference pectin alone[185].

3.7 Glycosaminoglycans

Glycosaminoglycans (hyaluronic acid, heparin and chondroitin sulfate) are the most important components of ECM, and is essential to bone and skin regeneration. According to the structure (polymer length, degree of sulfation), they modulate the attraction of skin and bone precursor cells and have potential in tissue engineering for wounds and burns[188].

3.8 Hyaluronic acid

Hyaluronic acid is a natural biopolymer that alternately consists of D-glucuronic acid and 2- acetamido-2-deoxy-D-glucose and is generally found in mammal's bond tissues and synovial fluids[189,190]. It has been reported that hyaluronic acid interacts with proteins, proteoglycans, growth factors and tissue components called biomolecules which has vital importance in healing of various types of wounds[191]. This interaction plays an important role in acceleration of tissue repair and wound healing. Due to their bacteriostatic activity hyaluronic acid and its derivatives also play a role in the protection of the injured area against microorganisms[192]. Directing tissue regeneration has been achieved using hyaluronic acid (HA) hydrogel scaffolds. HA is a naturally occurring non-immunogenic linear polysaccharide made from N-acetyl-d-glucosamine and glucuronic acid. It has remarkable effects in scar-free wound healing, supporting angiogenesis and neuritis outgrowth/repair[193,194]. HA and silver sulfadiazine-impregnated polyurethane foams are also used for wound dressing applications. After one week of foams application in an experimental model, the wound size decreased around 77% at the rat skin level without any inflammation or yellow crust[195]. Biomimetic hydrogels specifically designed to promote tissue repair (wound-healing applications) contain chemically modified HA cross linked by photo polymerization with glycidyl methacrylate groups[194] functionalized with thiol cross-linking sites [196] or cross-linked with DNA for gene delivery[197]. Heparin-coated aligned nano fibers are shown to increase endothelial cell infiltration in three-dimensional scaffolds and tissue remodeling *in vitro* and *in vivo*, in a full-thickness dermal wound model[198]. Heparan sulfate glycosamino glycans (HS-GAGs) and OTR4120, a polymer engineered to mimic HS-GAGs properties, has shown to decrease inflammation and stimulate neovascularization (angiogenesis) and collagen maturation in wounds and burns healing process[199].

6.0 Nanomaterials Nanofiber

Biodegradable nanofiber-based antibiotic delivery system is developed to treat wounds with antibiotics. This system can work as both biodegradable gauze and as an antibiotic delivery system. Nanofibers of poly (lactide-co-glycolide) (PLAGA) was fabricated using electro spinning process of the polymer PLAGA. This study was done to determine the effect of fabrication parameters such as orifice diameter (needle gauge), polymer solution concentration, and voltage per unit length, on the morphology and diameter of electrospun nanofibers. Different orifice diameter tested were 16 (1.19mm), 18(0.84mm), and 20(0.58mm). 0.375kV/cm to 1.5kV/cm was the range of voltage per unit electrospinning distance used to determine the effect of voltage. Polymer solution concentration in range of 10g/mL to 0.30 g/mL was used for the study. Mass per unit area of the electrospun nanofiber as a function of time and feasibility of loading antibiotic (cefazolin) into the nanofibers were also determined. Results indicated that with increase in needle gauge (decrease in orifice diameter) diameter of nanofiber decreased and with the increase in the concentration of polymer solution nanofiber diameter increased. From the

voltage studies it was demonstrated that average diameter of the nanofiber decreased with an increase in voltage. Effect of voltage on fiber diameter was less pronounced as compared to polymer solution concentration. Areal studies indicated that the mass per unit area of electrospun nanofibers increased linearly with time. Feasibility of drug incorporation into the nanofiber was demonstrated with the use of cefazolin, a broad spectrum antibiotic. Therefore, PLAGA nanofibers show potential as antibiotic delivery systems for the treatment of wounds[200]. Biodegradable polymers were electrospun and recombinant human epidermal growth factor (EGF) was immobilized on the electospun nanofibers for the purpose of treating diabetic ulcers. Amine-terminated block copolymers composed of poly(ε-caprolactone) [PCL] and poly(ethyleneglycol)[PEG] and PCL were electrospun to biocompatible nanofibers with functional amine groups on the surface via PEG linkers. EGF was chemically conjugated to the surface of the nanofibers. The conjugation amount of EGF on the nanofibers was quantitated by X-ray photoelectron scattering. Human primary keratinocytes were cultivated on EGF-conjugated nanofibers on the differentiation of keratinocytes. Wound healing effects of the EGF nanofibers were confirmed in diabetic animals with dorsal wounds. The expression of keratinocytes-specific genes significantly increased with application of EGF-conjugated nanofibers. The EGF-nanofibers exerted superior *in vivo* wound healing activities compared to control groups or EGF solutions. Immuno histochemical staining results showed that EGF-receptor (EGFR) was highly expressed in the EGF nanofiber group. Studies have shown that EGF-conjugated nanofiber could potentially be employed as a novel wound healing material by increasing proliferation and phenotypic expression of keratinocytes[201].

CONCLUSION

This review has considered many classes of wound dressings that can be used for wound healing purpose. The use of three-dimensional polymeric scaffolds for cell targeting is a common strategy for tissue engineering. Recent studies about biocompatible and biodegradable natural/synthetic polymers led to a substantial development of novel types of wound dressings and to outstanding applications in the biomedical area particularly for regenerative medicine. The effectiveness of these can be improved further by incorporating wound healing accelerating molecules like growth factors, peptides or various natural substances like honey, aloe vera and various plants and peel extracts. Various polysaccharides have been used either alone or in combination or in derivative forms for wound healing applications. Most of these are biodegradable in human body which makes it more attractive. Effective dressings should have properties and delivery characteristics that are optimised for specific wound types with minimum or no inconvenience to the patient and at reasonable cost. To achieve such objectives, manipulation of the physical characteristics of the identified systems is necessary so the use of composite dressings which combine the different characteristics of various polymers is good. This will be helpful for targeting many aspects of the complex wound healing process, and to ensure effective, complete wound healing and shorter healing times for chronic wounds and other difficult to heal wounds.

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