Polymer-based Biomaterials as Dressings for Chronic Stagnating Wounds

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Summary: Chronic wounds, such as venous, pressure, and diabetic ulcers, are difficult to heal and represent a rising social and economical problem. Compared to acute wounds, non-healing wounds contain elevated levels of neutrophil elastase, pro-inflammatory cytokines (IL-1β, IL-6, IL-8), and matrix metalloproteases (MMP-2, MMP-9, MMP-13) as well as free radicals. Their overproduction perpetuates the inflammatory phase resulting in severe tissue damage and degradation of growth factors. Consequently, wound closure is prevented and the wound remains non-healing for month or even years. The increasing numbers of patients suffering from wounds that fail to heal are a significant challenge for health care professionals. Wound dressings play an important role in the entire management of these wounds. New materials and treatment strategies are needed to improve wound care. Recent advances in the field of biomaterials and their medical applications indicate the significance and potential of various natural polymers in the development of novel classes of wound dressings. Native polymers are an ideal source for bio-active wound dressings because of their availability and biocompatibility. Hence, several studies have been conducted to explore the influence of wound dressings consisting of collagen, oxidized regenerated cellulose, bacterial cellulose, chitosan, or alginate on the destructive milieu in chronic wounds.

Keywords: alginate; cellulose; chitosan; collagen; wound healing

Introduction

The current concepts of modern wound management are focused on a moist wound environment which is thought to promote healing by creating optimal conditions for skin regeneration.[1–4] Healing of skin wounds is a highly regulated, complex process, which involves different types of cells and matrix components and leads to the formation of new tissue after injury.[5] However, the repair process is not perfect and healing impairments can occur. This impaired wound healing derives from an imbalance between tissue degradation and remodeling which has been linked to the excessive production of proteolytic enzymes.[6,7] Moreover, wound infection can delay healing. The presence of bacteria most likely influences the balance of degradation and reconstructive processes during wound healing.[8] Hence, there is a demand for biologically active (bio-active) wound dressings that can intervene in the pathophysiological processes in chronic wounds establishing a physiological milieu and reducing the bacterial load to allow the wound to heal. Many different natural and synthetic materials have been developed for use as wound dressings. Consequently, a wide variety of occlusive dressings are on the market today in different forms including films, foams, and gels, and from diverse materials such as alginates, polyurethane, polyacrylate, cellulose, or collagen. Some of these have been quite successful; however the search for the ''ideal'' wound dressing

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material is ongoing. There are several requirements wound dressings have to meet, like creating a moist wound environment, promoting healing, and providing mechanical protection and impermeance to micro-organisms while allowing gaseous exchange. It should be capable of absorbing excess exudate and not adhere to the wound bed to be able to be removed without pain or trauma. Furthermore, it has to be accepted by the patient, easy to use, and cost effective. Biopolymers feature several of these properties. Furthermore, they have a variety of environmental benefits such as deriving from renewable sources and being biodegradable. Hence, the possibility of using proteins, carbohydrates, and others to meet these specific material requirements has received increasing attention. The strategy in the development of new, active wound dressings is to mimic the biological features of the extracellular matrix to enable cell migration, proliferation and organisation during tissue regeneration and wound healing in an environment that is as physiological as possible. Four biopolymers have received special attention for application as biomaterials in wound care: cellulose, collagen, alginate, and chitosan (Figure 1).

Physiological Wound Repair and Formation of Chronic Wounds

Physiological wound healing is a complex process following different phases: homeostasis (clotting), inflammatory reaction, granulation (cell proliferation, tissue formation), and re-epithelization (tissue remodeling), which are overlapping in time.[9,10] The healing mechanism involves various cell types, biochemical factors, and extracellular molecules.[5] After an injury, blood coagulation minimizes the loss of body fluid and re-establishes homeostasis. The fibrin clot serves as a temporary shield protecting the wounded tissue and provides a matrix scaffold for the recruitment of cells to the injured area.

At the same time, the platelets in the clot initiate the healing cascade by releasing chemo-attractants and growth factors (e.g. PDGF, TGF-β, and EGF). In response to these mediators, inflammatory cells including granulocytes and macrophages infiltrate the wound. The inflammatory response regulates the cleaning of the wound from foreign particles as well as damaged tissue debris, which is crucial for tissue repair. Granulocytes release proteases such as PMN elastase[11] and collagenases[11,12] as well as reactive oxygen species (ROS), such as superoxide anions or hydroxyl

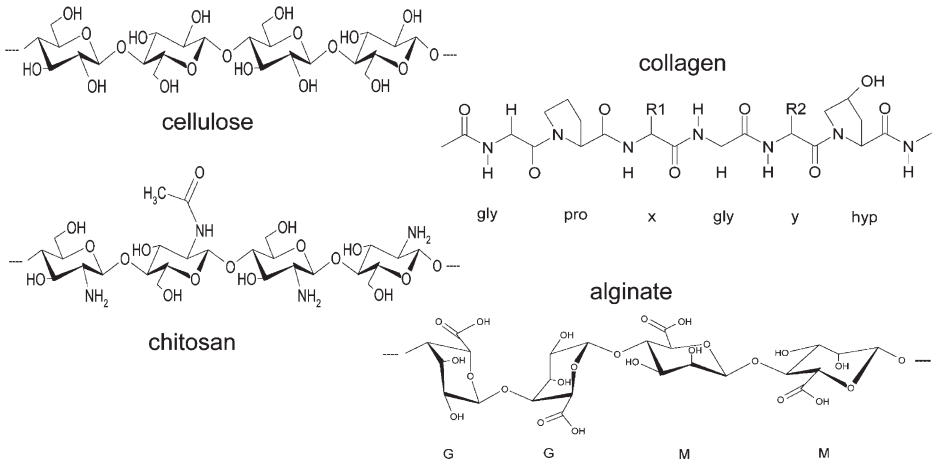

Figure 1.
Chemical structures of cellulose, collagen, alginate, and chitosan.

radicals.[13–15] Activated macrophages phagocytize pathogenic organisms, scavenge tissue debris, and destroy any remaining neutrophil. They also liberate pro-inflammatory cytokines such as TNF-α and IL-1β,[10] and release chemotactic factors, including PDGF, FGF, VEGF, TGF-β, and TGF-α.[9] These cytokines play an important role in inducing migration as well as proliferation of fibroblasts and keratinocytes and stimulate the synthesis of extracellular matrix components. Thus, macrophages regulate a crucial step in the transition between inflammation and repair.[9] In the proliferative phase granulation tissue is formed, the dermis is reconstituted and the epidermis is restored (re-epithelization).

However, the healing process is not perfect; the alteration or delay of any single process can lead to a failure in healing and the formation of a chronic wound. Wounds are considered chronic when the wounds persist for at least 8 to 12 weeks without signs of healing.[16,17] In addition, the quality of the formed tissue may be poor and functional closure is not achieved, so the wound may relapse.[7] There are various causes for the formation of a chronic wound such as defective arterial influx or venous and lymphatic efflux, neuropathological causes, heavy traumatic injuries (e.g. burns), possibly with a deep tissue infection, and even tumors. Hence, chronic wounds appear to be a heterogeneous group of disorders but there is a common biochemical base. Chronic wounds are stuck in the inflammatory phase and the destructive processes dominate (Figure 2). Under physiological conditions the inflammatory phase of the healing process extends only a few days and the number of neutrophils decreases after this period. In chronic-stagnating wounds a massive and constant infiltration of inflammatory cells has been observed,[18,19] which release pro-inflammatory cytokines[10] and a number of proteases.[11,12] The levels of protease such as PMN elastase, MMP-2, and MMP-13 are elevated in chronic compared to acute wound fluids (Figure 3). The excessive production of proteolytic enzymes found in chronic wounds leads to considerably reduced amounts of growth factors and proteinase inhibitors[19] as well as successive degradation of extracellular matrix.[17] Elastase has been held responsible for degrading essential growth factors such as PDGF and TGF-β.[19] The activity of the proteases decreases consistently in a large number of patients with venous ulcers that progress from a non-healing to a healing phase. This implies that there is an alteration in the normal control mechanisms regulating the levels of these enzymes.[20] The production of MMPs is regulated by pro-inflammatory cytokines. It has been shown that TNF-α and IL-1β stimulate the secretion of MMP-1, MMP-3, and MMP-9, and suppress the production of TIMP-1.[21] IL-1β, IL-6, and IL-8 concentrations were found to be significantly increased in wound fluid obtained from chronic as compared to acute wounds (Figure 3). This would act as a permanent pro-inflammatory stimulus shifting the physiological balance toward increased infiltration of inflammatory cells.[17,20] The pro-inflammatory cytokine quantities decrease significantly during healing, which has been postulated to directly modulate the observed reduction in protease activity.[20] Moreover, the increased liberation of reactive oxygen and nitrogen species, such as superoxide anion ($O_2^{\bullet -}$), hydroxyl radical ($OH^{\bullet}$) and nitric oxide ($NO^{\bullet}$), in chronic wounds has been shown to thoroughly impair the healing process. Hence, the inhibition of the activity of pro-inflammatory cytokines, the modulation of the concentration of proteases in the wound,[22,23] and the scavenging of ROS[24] were proposed to be a suitable way to stop the ongoing inflammatory processes, facilitate re-epithelization, and thus support the healing process. Furthermore, an increased bacterial load on the surface of a wound amplifies and/or perpetuates a pro-inflammatory environment. A study in a dermatological wound ambulance in Germany showed that 10 to 40% of the normal population is colonized with *Staphylococcus aureus*.

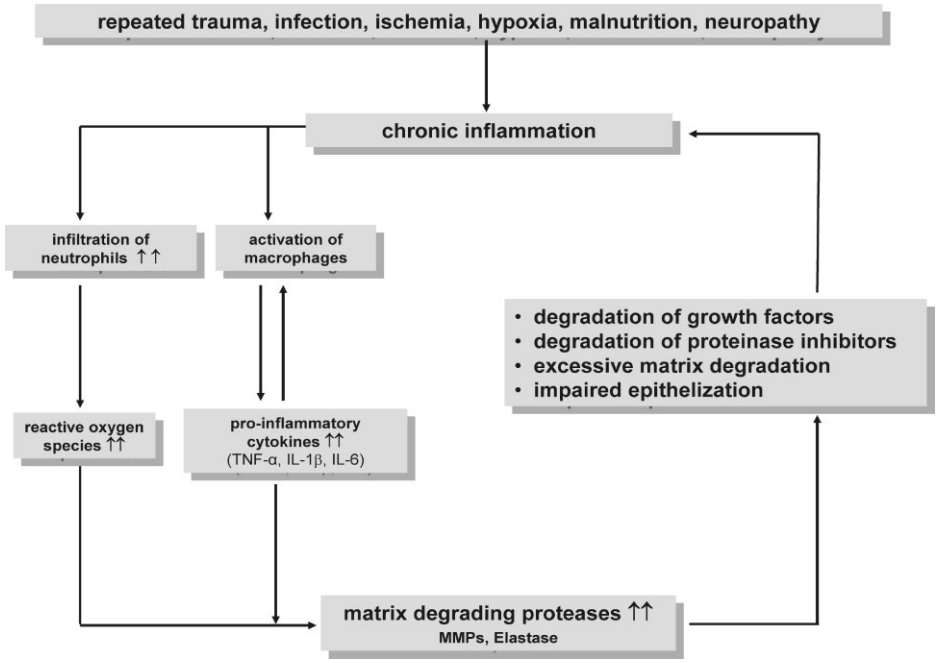

Figure 2.
The pathophysiology of chronic wounds is thought to be based on a prolonged inflammatory phase and the destructive processes dominate (modified after Ref. [7]).

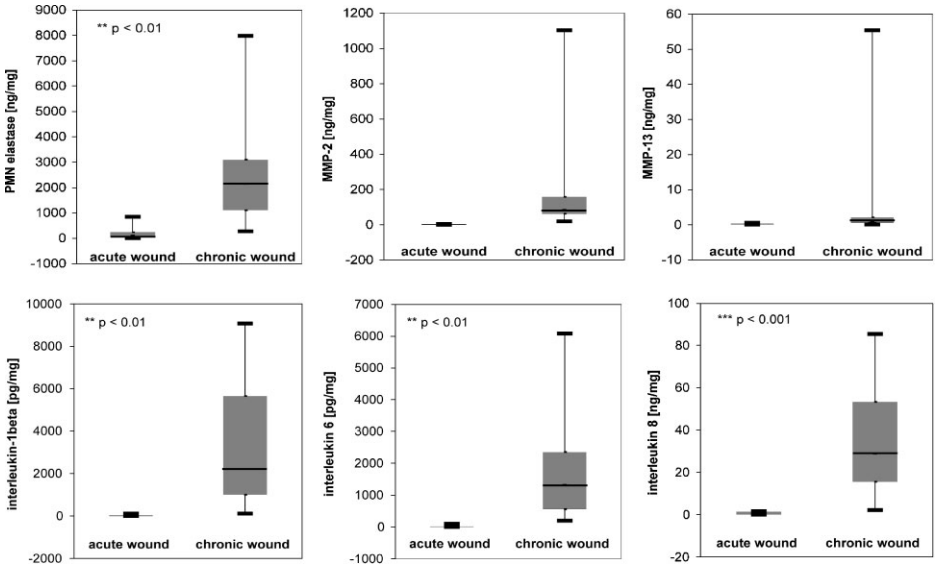

Figure 3.
Comparison of the parameters measured in acute and chronic wound exudates based on total protein content. Levels of the proteases PMN elastase, MMP-2, and MMP-13 are elevated and concentrations of the pro-inflammatory cytokines IL-1β, IL-6, and IL-8 are increased in chronic compared to acute wounds. Data presented are median (---), upper and lower quartile (grey box), and 95%-confidence interval (whiskers).

Furthermore, 71% of the patients with chronic wounds were colonized with *S. aureus* and 30% of these carried MRSA.[25] Other micro-organisms such as *Pseudomonas aeruginosa*, *Escherichia coli*, and *Klebsiella pneumoniae* may also play a role in chronic wound infection.[26] The presence of bacteria induces the immigration of monocytes, macrophages and leukocytes, whose inflammatory response exaggerates the tissue damaging processes.[27,28] Most chronic wounds are polymicrobial, and infections generally involve mixed populations of aerobic and anaerobic bacteria. It is suggested, that a lower probability of healing is seen when four or more pathogens are present, based on their synergistic relationship. Microbial synergy may increase the net effect of pathogens and the severity of infection by increasing local oxygen consumption.[27] Hence, it has been postulated that in order to improve the opportunity for wound healing, it is necessary to create conditions that are unfavorable to micro-organisms and favorable for the host repair mechanisms.[29]

Cellulose

Cellulose is the most abundant component of biomass and serves as an important material feedstock for many industries, e.g. paper production, textile manufacture, health care etc. It is traditionally extracted from plants where it is the principal component of the cell wall. Cotton gauze is still a standard of care in the management of chronic wounds. However, cotton gauze is not able to reduce protease activities but gauze dressings can be modified to selectively inhibit for instance elastase activity in solution.[30,31] Furthermore, bio-active wound dressings contain cellulose in the form of oxidized regenerated cellulose (ORC).[32,33] ORC possesses antimicrobial activity due to its ability to reduce the pH.[34] Furthermore, it is able to effectively reduce elastase activity *in vitro* and possesses a high antioxidant potential. Cellulose can also be produced by certain bacterial species by fermentation, e.g. *Gluconacetobacter*, *Agrobacterium*, *Pseudomonas*, *Rhizobium* and *Sarcina* [35], yielding a very pure and highly crystalline cellulose product.[36] The unique properties of bacterial cellulose are due to the reticulate network of fine fibers, the diameter of which (0.1 μm) is about 100 times smaller than that of plant-derived fibers.[37,38] This combination of nano- and micro-cellulose fibers creates a large surface area that can retain a large amount of liquid.[38] Bacterial cellulose as wound dressing grants several demands, like close adhesion to the wound bed and effective barrier to invading micro organisms[39,40] as well as absorbing high amounts of liquid, clearing the wound of exudate, and keeping a humid and warm wound climate that should be beneficial for healing. The fluid absorption and donation profile can be afterwards adjusted to optimize the product for specific applications where a dual-fluid-handling capability is needed.[41] This means, the wound dressing is able to adapt to the needs of the wound ('HydroBalance' effect); the dry wound margins receive moisture from the dressing to hydrate the skin and provide a moist wound environment while at the centre excess exudate is absorbed and locked into the dressing. However, it could be shown that bacterial cellulose itself exhibits no binding affinity for proteases such as elastase or inflammatory cytokines like IL-6 and IL-8 and shows a low antioxidant potential *in vitro.* [42,43] The function of bacterial cellulose can be improved by inclusion of active substances into the biopolymer. The ability of *A. xylinum* to incorporate insoluble substances in the forming cellulose pellicle[44] can be used to generate a composite of bacterial cellulose and collagen type I, designated CollagenBC. The composite achieved a significant reduction of protease amount, interleukin concentration and ROS activity *in vitro.* [43] Consequently, it should combine the ability of collagen to alter the milieu parameters in chronic wounds with the positive physical properties of bacterial cellulose.

Collagen

Collagen is the major protein in the extracellular matrix and in the connective tissue. It accounts for approximately 30% of all vertebrate body protein. The triple helical confirmation is the defining structural element of all collagens.[45] At least 80% of the collagen in the body consists of fibrous collagen type I-III, V, and IX that have a non-intermittent triple helical domain of about 1000 amino acids. In contrast, the other non-fibrillar collagens contain triple helical domains of various lengths and build up other higher order structures, e.g. type IV collagen which creates a two-dimensional network of loose fibrils in the basement membrane.[46] Collagen has found many biomedical applications such as implant scaffolds for artificial organs[47], drug carrier systems[48], and bone tissue reconstruction.[49] It also is used in wound healing due to its ability to absorb large quantities of fluids and forming a soft gel that keeps its environment moist.[50] A variety of wound dressings containing collagen of different type and origin are commercially available (Figure 4). Bovine, porcine, and equine collagen containing wound dressings were tested for their binding capacity for neutrophil elastase[51] as well as IL-1β and TNF-α.[52] A significant affinity for the inflammatory mediators was observed in all cases. However, porcine collagen exhibits a lower binding of elastase compared to bovine and equine collagen[51], and they show distinct binding rates for IL-1β and TNF-α.[52] Hence, differences in the amino acid composition observed for collagen from different sources[53,54] might directly affect the binding capacity of collagen from different origin by modifying the ability to form electrostatic interactions. For example, equine collagen has a higher amount of lysine and hydroxylysine residues compared to bovine collagen. These amino acids confer intra- and intermolecular cross-linking; therefore equine collagen might exhibit a higher thermal stability.[53] These residues are then, however, not available for additional interactions with other molecules, resulting in a lower binding capacity for proteases, cytokines, and growth factors. Thus, the results obtained for a collagen of a certain species may not be transferable to wound dressings consisting of collagen from a different source. Furthermore, sterilization methods affect the physical properties of collagen. Treatment with β- and γ-radiation was found to have a doses dependent effect on

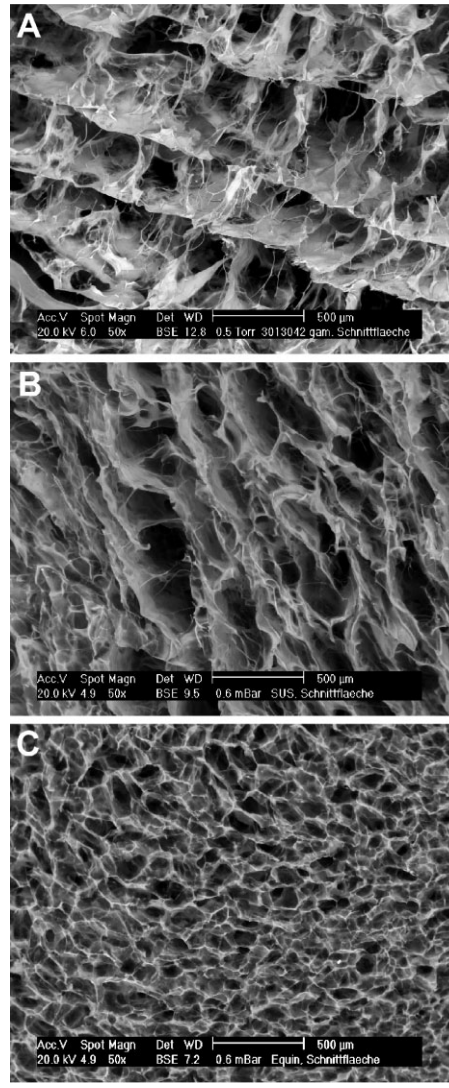

Figure 4.
SEM of collagen from different origin: A- bovine collagen, B – porcine collagen, C – equine collagen.

the mechanical strength of the collagen sponge.[55] The fibrillar collagen molecules within the foam product build a supercoiled triple helical structure, which is based upon non-covalent interactions between vicinal polypeptide strains.[56] Ionizing radiation resulted in a loss of fibril stability. It could be observed that irradiated collagen shows a dramatic decrease in viscosity caused by chain scissions in collagen leading to a fraction of lower molecular weight material.[57,58] In contrast, ethylene oxide (EtO) treatment increases collagen helix stability.[55] The fragile molecular bonds of EtO allow it to react quickly with a wide variety of compounds. The resulting chemical reaction is called alkylation. For collagen the most susceptible amino acids to this reaction are lysine and hydroxylysine.[59] As a result additional cross-links may be formed, tightening the collagen sponge against outer influences. Regarding the influence on physical properties EtO sterilization appears to be advantageous for collagen-based wound dressings compared to radiation sterilization. However, mechanical strength is just one side of the medal. It has also been reported that EtO gas sterilization results in lower values for the content of free amino acid groups.[60] This would mean that fewer backbone groups are available for additional interactions with other molecules, which might result in lower protein binding capacities. However, no significant loss of the binding affinity for the tested cytokine and proteases or radical scavenging ability of a bovine collagen type I containing wound dressing was detected. Therefore, it has been concluded that β- and γ-irradiation up to a maximum dose of 20 kGy as well as ethylene oxide treatment are possible methods to sterilize this wound dressing.[55] Concisely, collagen possesses a high binding capacity for different inflammatory mediators, like proteases and cytokines, and antioxidant potential *in vitro*. [42,55] Additionally, previous studies have shown that a wound dressing composed of bovine collagen type I is able to bind significant amounts of PDGF-BB, protect it from proteolytic degradation and maintain its biological activity.[61] Hence, collagen containing wound dressings should have a beneficial effect on wound healing. Furthermore, collagen also provides haemostatic properties.[62,63]

Alginate

Alginates are a family of linear polysaccharides found in marine algae and some bacteria. Most alginate is obtained commercially from three genera of the marine brown algae, Phaeophyceae (*Macrocystis pyrifera*, *Laminaria digitata*, and *Laminaria saccharina*). They constitute copolymers of (D)-mannuronic acid (M) and (L)-guluronic acid (G) units. These homopolymeric regions, called M-blocks and G-blocks, are interspersed with regions of mixed sequence, referred to as MG-blocks. Within the algae the function of alginates is thought to be primarily skeletal, with the gel providing the strength and flexibility required to withstand water movements.[64] The chemical composition and sequence of M- and G-units depend on the biological source, growth conditions, and environmental effects.[65,66] The gelling characteristics of alginates are distinctly influenced by monomer composition and sequence.[66] The higher the content of guluronic acid in the alginate, the greater the interaction and the more stable the resultant gel. In high-M alginates the calcium ions are less firmly attached to the molecule and can be more easily replaced by sodium ions, resulting in increased fluid uptake, fiber swelling, and faster gel formation.[64] Alginate dressings are widely used in the treatment of exuding wounds.[67–69] The alginate takes up wound exudate, the ion exchange occurring between the calcium ions of the dressing and the sodium ions in the exudate results in the formation of a gel on the surface of the wound. This gel absorbs moisture and maintains an appropriate moist environment.[2] Alginates also have hemostatic properties.[67] Furthermore, it has been shown that patients treated with alginate dressings felt a reduction of pain associated with the wound and the dressing

changes.[69] Moreover, it could be shown that alginates have the potential to actively influence the pathophysiological mechanisms in the chronic wound as they are able to bind considerable amounts of PMN elastase, TNF-α, IL-8 and to inhibit free radical formation *in vitro*. [70] Additionally, alginates inhibit the growth of gram-positive and gram-negative bacteria by immobilizing bacteria within their fibrous matrix. The alginate fibers absorb water and swell, the spaces between the fibers are closed and any bacteria that are carried in the wound exudate are trapped in the wound dressing.[71] While alginate exhibits a strong bacteria static effect, it shows only a slight inhibition of *Candida albicans* growth. The yeast cells are bigger than the bacteria cells and therefore might not be trapped as efficiently in the forming gel.[70]

Chitosan

Chitosan is a cationic polysaccharide composed of D-glucosamine and *N*-acetyl-D-glucosamine, and can be obtained by the partial deacetylation of chitin. Chitin is a linear polymer of repeating N-acetylglucosamine residues and constitutes the second most abundant polymer found in nature after cellulose and is a structural component of shellfish, insects, and the cell walls of bacteria and fungi.[72,73] Chitin and chitosan have been widely used in various fields such as wastewater treatment, paper making, agriculture, cosmetics, food processing, and as a biomaterial for pharmaceutical and biotechnological purposes.[74] Chitosans are biocompatible, biodegradable, and exhibit low toxicity.[72,73,75] Chitosan and chitosan oligomers have gained considerable interest due to their biological activities such as antimicrobial properties, antitumor effects[76], haemostatic assets[77], and the beneficial influence on wound healing.[78] Hence, chitosan scaffolds present a promising matrix for skin tissue engineering[79], for orthopedic applications[80], and as basic material for wound dressings.[73,81] In experimental animal models, chitin and chitosan were shown to affect all stages of wound repair.[82,83] Chitosan can also interact with neutrophils and macrophages modulating their migration behavior and modifying subsequent repair processes such as fibroplasia and re-epithelialisation.[82,84] The term chitosan is actually used to describe a series of chitosan polymers with different molecular weight, crystallinity index, and degree of deacetylation (40-98%).[85] These parameters influence solubility, viscosity, water retention capacity, and charge density of the polymer.[86,87] For example, chitosans with a relative low degree of deacetylation (40%) have been found to be soluble up to a pH of 9, whereas chitosans with a high degree of deacetylation (85%) are only soluble up to pH 6.5.[73] Furthermore, the increasing degree of deacetylation also increases the viscositiy of a chitosan solution.[87] It could be shown that chitosans exhibit a concentration dependent antifungal activity against *Candida albicans*, *Candida krusei*, and *Candida glabrata* which is influenced by the molecular weight.[88] The polycationic character is crucial for the antifungal acitivity because it is lost when the functional groups are masked, e.g. by carboxymethylation of chitosan.[88]

Antimicrobial Activity by Introduction of Silver or Other Antimicrobials

Excessive exudation is mainly caused by infection which is an important factor in compromised wound healing.[4,89] Increased presence of bacteria in a wound amplifies and/or perpetuates a pro-inflammatory environment and hinders re-epithelization. Several studies of bacterial profiles on chronic wounds have been published; chronic wounds are colonized with *Staphylococcus aureus*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, coagulase-negative staphylococci, *Proteus* species and anaerobic bacteria.[25,90] As a specific example, *S. aureus* is considered to be the most problematic bacterium in traumatic, surgical, and burn wound infections, primarily based on the knowledge that its incidence is high in these, and other types of wounds.[25,26] In order to improve the

opportunity for wound healing, it is necessary to reduce the level of bacterial burden. Silver (Ag^+) is effective against a broad range of micro-organisms such as yeast, mold and bacteria, including MRSA and VRE, when it is provided in appropriate concentrations.[27,29,91] Silver ions are highly reactive; they react with inorganic compounds, organic acids, proteins, DNA, and RNA. The micro-organisms are killed by Ag^+ through various mechanisms of action, such as inhibition of cellular respiration, interference with DNA replication, and alteration of cellular membrane permeability.[27] The commonly used forms are silver coated dressings that have been demonstrated to be effective at killing a broader range of bacteria than cream-based silver applications. They are less irritating than silver nitrate solution and better tolerated. Hence, silver has been incorporated into a wide variety of wound dressings consisting of collagen[92,93], bacterial cellulose[94], or alginate.[70,95] By incorporating silver into alginate fibers, a highly absorbent wound dressing with antimicrobial properties is obtained.[70] In order to attach silver ions, calcium alginate fibers can be treated with aqueous solutions of silver nitrate. The silver ions in the solution exchange partly with the calcium ions in the fiber, resulting in the formation of calcium alginate fiber containing silver ions. Silver can also be introduced in form of unique Ag^+/Ag^0 complexes by the use of nanotechnology (SILCRYST™ nanocrystals, NUCRYST Pharmaceuticals). From a materials perspective, a nanocrystalline material has a crystal size less than 20 nm. This is a functional definition related to the changes that occur in the physical and chemical properties as the crystal or grain size drops below 20 nm. This technique is reported to give the highest sustained release of silver ions to a wound without the risk of mammalian cell toxicity.[96] Silver containing alginate dressings exhibit significant antibacterial and antifungal activity.[70] However, detrimental effects on cutaneous cells due to silver have been observed.[70,97,98] Hence, the use of antimicrobially active substances like silver or the employment of materials with inherent antimicrobial properties such as chitosan may have damaging effects on healthy cutaneous cells and impair their proliferation (own observations). Therefore, their utilization has to be carefully evaluated so that they provide a favorable influence on wound prognosis. Other antimicrobial agents such as polihexanide (PHMB) have been shown to be effective against a broad spectrum of micro-organisms while possessing low cytotoxicity and improving wound healing.[99–103] Hence, they potentially provide a more biocompatible alternative as additives in antimicrobial dressings. A wound dressing consisting of bacterial synthesized cellulose containing polihexanide (Suprasorb® X + PHMB, Lohmann & Rauscher GmbH & Co.KG) is commercially available for the treatment of colonized or infected chronic wounds. A co-culture system of HaCaT keratinocytes and *Staphylococcus aureus* used as a model for infected cells in a non-healing wound enables the assessment of antiseptic solutions and wound dressings containing antimicrobials for their potential to positively influence wound healing by reducing the bacterial burden. It could be shown that the extract of the wound dressing was able to reduce the bacterial contamination and establish normal cell growth.[104]

Comparison of Binding Capacity and Antioxidative Potential

In general, wound dressings are classified as inactive (gauze, fleece material), interactive (hydrocolloids, alginate, hydrogel), and active (protease-modulating matrix, growth factors).[3] Only the last are thought to provide active influence on the chronic wound milieu while the others merely grant a moist wound environment, thermal isolation and protection against invading micro-organisms. However, it is difficult to sort the polymer-based biomaterial containing wound dressings into these three classes. Collagen and collagen/ORC efficiently reduce elastase activity *in vitro* (Figure 5). Moreover, a distinct binding

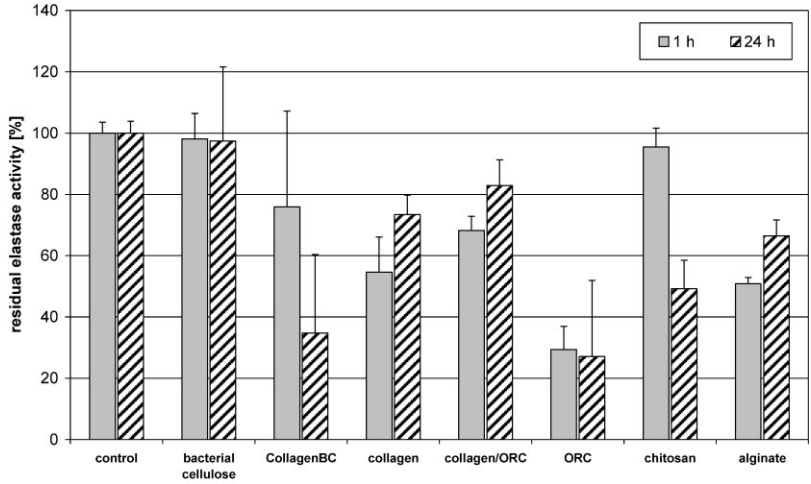

Figure 5.
Measurement of the residual elastase activity after incubation with different biomaterials for 1h and 24h (mean ± SE) according to the protocol previously described in Ref. [42] and [55].

affinity for elastase could be observed for alginate, chitosan and ORC. Only bacterial cellulose is not able to decrease the protease amount. In order to explain the varying binding capacities, a closer look at the structure of the biomaterials is needed. ORC contains a large number of functional groups, such as OH, CHO, CO, COOH, which allow various interactions with other molecules via hydrogen bonds and electrostatic interactions. As studies with oxidized, phosphorylated, carboxymethylated and sulphonated cotton-gauze have shown, the chemical modifications lead to an increase in the binding capacity for elastase from chronic wound fluids.[31] The authors suggested that the negatively charged groups of the gauze interact with arginine side chains on the surface of elastase. A similar mode of action by noncovalent interactions has been proposed for collagen[42] and might be applicable for chitosan as well. Bacterial cellulose possesses functional groups but they might not be available for electrostatic interactions with other molecules. Furthermore, all wound dressings exhibit a significant antioxidant potential (Figure 6). The samples were equally effective in inhibiting the formation of reactive oxygen and nitrogen species. While ROS and RNS play an important role in the normal wound healing process by killing invading microorganisms, the overproduction of these species causes indiscriminate cellular damage. The ability of the wound dressings to scavenge free radicals varies to a great extent. The ORC product and the composite of ORC/collagen showed a nearly complete depletion of ROS and RNS. In contrast, pure collagen type I is not as effectively inhibiting ROS and RNS formation. However, the effect of collagen I was clearly more pronounced than the effect of the bacterial cellulose. The effect achieved by the composite material CollagenBC seemed almost additively. Alginate and chitosan also showed a high antioxidant capacity. Hence, the ability to act against superoxide radical species and peroxynitrite seems likely to be due to the numerous functional groups, such as OH and COOH, offering a variety of sites for radical attack leading to electron and hydrogen abstraction from α-carbons. In addition, the ROS scavenging capacity of comparable wound materials such as carboxymethylcellulose, hyaluronan and hyaluronan benzyl esters has been demonstrated.[14] The observed antioxidant capacity of collagen type I can be attributed

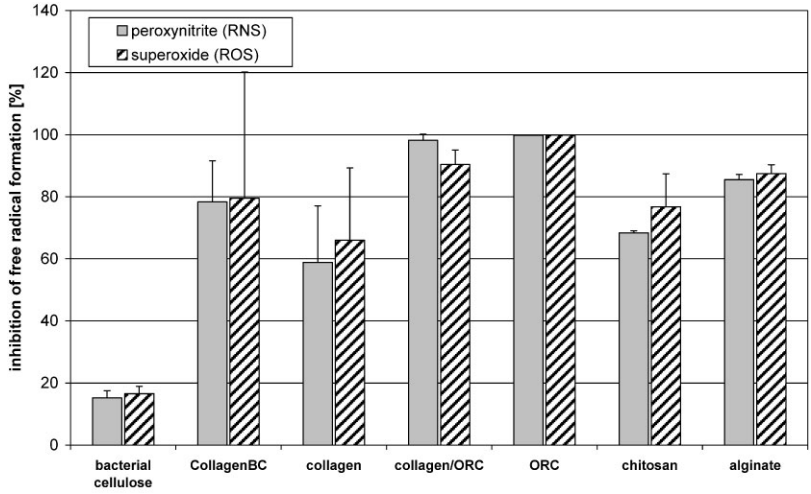

Figure 6.
Inhibition of the formation of reactive peroxynitrite and superoxide radicals by different biomaterials (mean ± SE) measured using the ABEL antioxidant test kits specific for superoxide anion and peroxynitrite anion radicals as described in Ref. [42] and [55].

to the amino acid residues like lysine and proline, which have been reported to be highly susceptible to ROS attack.[105–107] Due to the interaction of these amino acids with free radicals, collagen exhibits radical scavenger properties as well.

Conclusion

The advantage of polymer-based biomaterials, such as cellulose, collagen, alginate and chitosan, for application in wound care products is their ability to form natural bonds with the surrounding tissue and thereby promote the healing process. Furthermore, they exhibit distinct binding capacities for inflammatory mediators like cytokines, proteases and free radicals, which are found in elevated concentrations in chronic-stagnating wounds. Moreover, these polymer-based biomaterials feature intrinsic antimicrobial properties (e.g. alginate and chitosan) or can be easily supplemented with antimicrobial agents (e.g. silver and polihexanide) to improve their performance on infected chronic wounds. Hence, due to their actual composition, wound dressings exhibit different properties. Basic information on advantages and disadvantages of each dressing type is essential and would be helpful in the choice of optimal wound dressings for non-healing wounds. Therefore, *in vitro* methods are increasingly utilized to evaluate the ability of materials to fulfill requirements of biocompatibility, exudate management, antimicrobial activity, and binding capacity for pathophysiological factors in chronic wounds. The further understanding of these properties helps to support the refinement of biopolymers in wound dressings for improved wound healing.

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