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Review

Trends in alginate-based films and membranes for wound healing

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Abstract Wound healing management is one of the most expensive and most common procedures in modern hospitals worldwide. The modern trend in wound healing is using bioactive compounds, either by themselves or in a blended form that enhances their advantages in order to ensure a fast and possibly scar-free healing. One of these biomaterials is alginate, a heteroglycan with anti-inflammatory, anti-microbial, anti-oxidant and hemostasis-induction properties, being capable to entrap drugs, proteins or growth factors that help wound healing. After a rigorous search of the findings made after 2013, we included the *in vitro* and *in vivo* studies that used alginate films and membranes, as well as the physicochemical differences between them, providing new perspectives on using different compositions when creating a material that could enhance the wound healing process.

Keywords Alginate, biomaterial, dressing, *in vitro* studies, *in vivo* studies, wound healing.

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Introduction

From the first wound healing dressing used in ancient Egypt and Sumer, like honey, herbs or animal grease, medicine has come a long way into developing new and multi-efficient biomaterials (SHAH [1]). A biomaterial is considered to be "a natural or synthetic substance that is not a drug, which can be introduced into body tissue as part of an implanted medical device or used to replace, treat, or augment a tissue, an organ or a bodily function" (ARAMWIT [2]).

The ideal wound dressing should be biocompatible, biodegradable, bio-adherent to the wound surface but non-adherent to the wound bed, elastic but resistant, semi-permeable, non-antigenic and cost effective. Moreover, this dressing should also modulate the moisture level of the wound, be able to carry drugs or other molecules, give protection against bacterial, infectious, mechanical, and thermal agents, while helping the wound heal (DAS & al [3]; ERDOGAN & al [4])

The biopolymers most used as biomaterials for wound healing are: alginate, cellulose, chitosan and collagen (ARAMWIT [2]; DAS & al [3]; LIU & al [5]; MIHAI [6]).

Of these, alginate is used in hydrogels, fibers, membranes, polyelectrolyte complexes or composite multi-layered films for exuding wounds like various types of ulcers (i.e. diabetic foot, pressure, venous leg), or after a traumatic event (i.e. burn, cavity, pilonidal cyst removal, surgery, tumor necrosis) (CLARK [7]; DUMVILLE & al [8]).

Recently many *in vitro* and *in vivo* studies targeted creating new or better alginate-based bioproducts with wound healing properties, the most common ones being under film or membrane form.

Alginates

Alginates (ALG) are **natural anionic polysaccharides** obtained from brown algae (*Ascophyllum nodosum, Cystoseira barbata, Laminaria digitata, Laminaria hyperborea, Laminaria japonica, and Macrocystis pyrifera, Padina* sp., *Phaeophyceae* sp., and several *Sargassum* sp.) cell walls and from some bacteria strains (*Azotobacter, Pseudomonas*) with a linear structure composed of 1,4-linked β-D-mannuronic acid (M) and 1,4 α-L-guluronic acid (G) residues (RHEIN-KNUDSEN & al [9]; IVANCIUC [10], BADEA & al [11]). Alginate usually means alginic acid, its salts and its derivatives (ARAMWIT [2]; LEE & al [12], SZEKALSKA & al [13]).

ALG esters and monovalent salts are **water soluble** and make viscous solutions, but alginic acid is not soluble either in water or in organic solvents. Alginates have a high **swelling capacity**, being able to absorb up to 20 times their own weight, transforming themselves into a hydrophilic weak gel that keeps the wound environment moist and helps grow new epidermis (ARAMWIT [2]; HONG & al [14]). The solubility is influenced by structure, the protonated/deprotonated state of the carboxylic groups, concentration, ionic strength, temperature, "gelling ions" amount and the solvents pH (pKa under 3.28-3.65 is able to make the polymer precipitate). If aromatic groups or long alkyl chains are attached to the ALG backbone its solubility will change. When the alginate has protonated carboxylic groups it will not dissolve neither in water nor in any other solvent, while sodium alginate will dissolve completely in water but not in organic solvents. Environmental pH also influences ALG mucoadhesive capacity where the polymers carboxyl groups bind with mucin, and if it is higher the carboxylic groups become deprotonated (CLARK [7]; LEE & al [12]; SZEKALSKA & al; [13] PAWAR [15]).

Calcium alginate is used as a nonwoven dressing in infected surgery wounds or exuding injuries because of its ability to exchange ions with the wound fluid, the Ca^{2+} exchange the Na ones in the wound bed, making the dressing non-adherent to the wound bed and thus the removal will be pain-free. Also, alginate dressings are able to self-adhere to the peri-wound area. Fibers made of alginate can be washed away without pain or destroying the new tissue if they are applied on exuding wounds (CLARK [7]). The question of bio-incompatibility arises only when the alginate is impure (CLARK [7]; LEE & al [12]). The viscosity and other physicochemical properties are influenced by the amount of ALG, by increasing the ALG amount or its concentration the viscosity and the bead size will also increase (LOTFIPOUR & al [16]). **Sodium alginate** has better gel-forming characteristics and longer release time in the case of spray-dried particles of sodium alginate at pH 1.2 and it can be used as a carrier in oral drug distribution of hydrophilic matrix controlled-release form because it forms viscous solutions and it gels in contact with aqueous media. The environmental pH, coating technique, drug type and solubility influences the speed and absorption rate (BHARDWAJ & al [17]). The used concentration of ALG varies from 0.001% w/v to 95% depending on the dressing type (PEREIRA & al [18]; SINGH & al [19]; SMITH & al [20]).

Alginates were proved to exhibit angiogenetic, anti-anaphylactic, anti-microbial, anti-inflammatory, antioxidant, immuno-modulating, hemostatic, and regenerative properties, either by themselves or in various blends. Researchers have proven that the anti-infectious properties of an alginate-based wound dressing is influenced by the formulation type, environmental pH, M/G-block ratio, molecular weight, and structure modification (SZEKALSKA & al [13]; WIEGAND & al [21]; RAPOSO & al [22]; RAGUVARAN & al [23]; PAWAR & al [24]; LIAKOS & al [25]). Alginates demonstrated their **antibacterial activity** against *Bacillus cereus, E. coli, Pseudomonas aeruginosa,* and various species of *Acinetobacter*, *Proteus* and *Staphylococcus* (RAPOSO & al [22]; RAGUVARAN & al [23]) and the **fungal inhibition** of *Candida albicans* (BADEA & al [11]; SPADARI & al [26]) Furthermore, essential oils like chamomile blue, cinnamon, elicriso italic, eucalyptus, lavender, lemon oils, lemongrass, peppermint, sage, and tea tree were encapsulated in Na ALG, with their **bacterial** and **fungal growth inhibition** varying according to the oils concentration and type (LIAKOS & al [25]). These activities are highly important because infection is one of the leading causes for a wound to become chronic (WIEGAND & al [21]). Also, fractions containing alginic acid showed their **antiviral activity** against several virus families such as *Flaviviridae*, *Herperviridae*, *Rhabdoviridae*, and *Togaviridae*, either because of the electrical charge interaction between the sulfated alginate and the host cell (both being positive) that prevents the virus from entering the cell, or because the ALG creates a barrier surrounding the cell that the virus cannot penetrate (SZEKALSKA & al [13]; WIEGAND & al [21]; RAPOSO & al [22]; RAGUVARAN & al [23]; PAWAR & al [24]; LIAKOS & al [25]). Thus, some biomaterials for various tissue regeneration (i.e. blood vessels, bone, cartilage, and muscles) are often derived from ALG as a physical support for cells or tissue, or as a hurdle between two media. Alginate wound dressings may be found in **various forms** because of their high absorbency and strong hydrophilic gel formation ability, such as: flexible fibers, films, foams, freeze-dried porous sheet, nanofibers, electrospun scaffolds, composite hydrogels, beads, microparticles, and microspheres, each having its specific use because they limit the wound exudates and decrease contamination, for example the flexible fibers are used to fill cavity wounds (LEE & al [12]; BOATENG & al [27]; CHAUDARI & al [28]), as we will present in a few articles.

Alginate-based films for wound healing

The alginate-based **films** can be obtained by different techniques such as the solvent/casting-evaporation process, freeze-drying or microwaving (PEREIRA & al [29]), but most authors refer to the solvent casting method because of the results regarding smoothness, high malleability, appropriate thickness, transparency, film light transmission values, and many more, summarized in Table 1.

When describing the mechanical properties of a wound dressing material researchers usually invoke the tensile strength, the elongation at tensile strength and Young's modulus. Pașcalău et al. (PAȘCALAU & al [30]) describe how they analyzed these parameters and how the cross-linking level and the ALG – k-carrageenan (k-CG) ratio (AC_{1ab}: 1.5 g ALG, 1.5 g k-CG, 6,8 glycerol, 1.62 g $CaCl_2$ AC_{3ab}: 2 g ALG, 1 g k-CG, 7.5 g glycerol, 1.62 g $CaCl_2$, AC_{4ab}: 1.5 g ALG, 4.5 g k-CG, 8.6 g glycerol, 1.74 g $CaCl_2$) affects the mechanical properties and swelling ability of ALG-based composite films, while using calcium as the cross-linker and glycerol as a plasticizer. Fully cross-linking leads to more rigid, water resistant, clear and translucent films with an appropriate swelling behavior, higher Young's modulus and ultimate stress values but with lower values for the elongation at tensile strength. The results showed that AC_{1ab} and AC_{3ab} have higher Young's modulus, ultimate stress values, the highest Ca^{2+} content, with better flexibility, and AC_{4ab} has good swelling behavior and water resistance (PAȘCALAU & al [30]).

Glycerol addition makes the alginate fibers flexible, as the water uptake capacity of such fibers is directly linked to their size and volume (TAMAYOL & al [31]). Glycerol addition might also increase an alginates' film water solubility, swelling degree, and flexibility but it will decrease its mechanical stability. Also, the mechanical properties, solubility and swelling were within the accepted range if 10% glycerol was added to alginate – pectin composite films, while the addition of glycerol to pullulan-alginate films made them less resistant to tensile stress, more soluble in water, and their elongation at break value increased (DA SILVA & al [32]; VENUGOPAL [33]; DA SILVA & al [34]). For example, an ALG-*Aloe vera* film, obtained through the solvent-casting method and $CaCl_2$ cross-linking, was thicker (66.14 µm – 75% ALG vs. 69.00 µm – ALG-glycerol film) and more transparent (2.11 – 75% ALG vs. 1.28 – ALG-glycerol film) when glycerol was present. This film displayed increased tensile strength 42.36–50.91 MPa, and the blended film light transmission varied from 69.32 ± 1.79% – 95% ALG to 73.26 ± 4.76% – 75% ALG. *A. vera* also increased the thermal stability of the film (PEREIRA & al [18]).

Films obtained by blending polyethylene oxide (POLYOX™) with four hydrophilic polymers (carrageenan, chitosan, hydroxypropylmethylcellulose and sodium alginate) and glycerol for plasticizing the gel formulation exhibited high swelling ability, controlled drug release, good flexibility, transparency and bioadhesiveness. The films were loaded with streptomycin and diclofenac and gave optimistic results, enabling drug diffusion over

a long period of time, eliminating the film removal in order to supplement the drug dose, and reducing inflammation and bacterial infection (PAWAR & al [24]). Regarding gelation, when POLYOX™ is mixed with sodium alginate the resulting film is very thin and hard to remove from the cast, in this case a Petri dish (PAWAR & al [24]). If alginate and pectin are formulated as transparent composite films with 10% glycerol, they display limited swelling and low solubility but good mechanical features (VENUGOPAL [33]).

These types of films showed less re-injury when peeling off and better pH stability and drug release, than separate chitosan or alginate dressings when used on wounds, and thus they show a great potential when used on highly exuding wounds (HONG & al [14]; JAYAKUMAR & al [35]). Also, ciprofloxacin HCl was incorporated in a composite bilayer antimicrobial membrane composed of an alginate and a chitosan layer that showed an improved water absorption rate of above 800%, a prolonged drug release time, and bacterial growth control (DONG & al [36]).

Table 1. Alginate-based films with wound healing properties

Composition	Study type/Target	Ref.
4% w/v fibrin – 0.1% w/v chitosan – 0.5, 0.1, 0.15 - 0.25% w/v Na ALG – 1% PEG	Composite film characterization	[38]
9, 20, 33, 43, 50% w Na ALG – PVA in 1% w/v separate solutions	Composite film characterization: tensile strength, differential scanning calorimetry, thermal stability, miscibility	[33]
Ca ALG – *Aloe vera* gel 95:5, 85:15 and 75:25% v/v in hydrogel film	Film characterization	[18]
Ca ALG – k-CG – glycerol – $CaCl_2$	Film characterization	[30]
10% w/v Sago starch – 2% Na ALG – Ag-NPs – 0.018 w/v gentamicin ALG/Ag-NPs ratio 4 : 0.5 – 4 : 3.5	Film characterization, Albino Wister rats wound healing: histological test, healing rate, biochemical assay, healed wound tensile strength	[39] [40]
2 % ALG in 2.5, 5, 10 % Asiaticoside	Film characterization, cytotoxicity test	[41]
1% w/w Polyox® – 25% Na ALG – 7.5%w/v streptomycin 1% w/w Polyox® –25% Na ALG – 2.5%w/v diclofenac	*In vitro* – swelling, scanning, texture, FTIR, drug release	[17] [24]
3% w/v Na ALG – glycerol – Igepal – essential oils ratios: 48:20:16:16 / 30:10:10:50 / 20:8:6:66	Morphology, dispersion degree, bacterial and fungal growth inhibition	[25]
2% chitosan – 3% PVA – 2% w/v Na ALG – 0.5, 1, 1.5 mg/cm^2 ornidazole	Multi-layered film characterization, drug release study, antibacterial effect	[35] [37]
10 mL Na ALG – chitosan – 20% w/w glycerol	Wistar rats with burn wounds	[42]

Furthermore, a three-layered solvent casted drug loaded film with antibacterial properties against *S. aureus* and *E. coli* containing perfectly bonded layers of chitosan, ornidazole-incorporated polyvinyl alcohol (PVA) (1.0 mg/cm^2) and sodium alginate were used and displayed a greater light transmittance, fluid drainage ability and water vapor transmission rate (WVTR) in comparison with a single layer film. The WVTR ranged between 2084 (drug-free) and 2211 and 2295 g/cm/day^{-1} that increased with the drug concentration (PEI & al [37].

Alginate-based membranes for wound healing

Another important class of potential wound healing dressings are the alginate-based membranes which are summarized in Table 2.

The casting-solvent evaporation method can also be applied to obtain biocomposite **membranes** that display good results when applied on wounds. For example, when creating chitosan-alginate- AgSD poly-electrolyte complex (PEC) membranes they were more stable to the pH change and more efficient for drug release than when used as individual dressings, even on exuding wounds (DONG & al [36]; AZUMA & al [43]). Also, these membranes displayed a WVTR of 442 to 618 g/m^2/day, a maximum breaking elongation of 46.28% within the wet membrane, and a maximum breaking strength of 52.16 MPa within the dry membrane. The drug release study pointed out that AgSD had the fastest release rate when the membrane had a 50% alginate content (MENG & al [44]). The WVTR of a biomaterial that is supposed to be used in wound healing is important because the known values for it in a healthy skin is 204 ± 12 g/cm/day, but it increases to 279 ± 26 g/cm/day (1st degree burn) or 5138 ± 202 g/cm/day (granulating wound), hence the midrange that allows a wound to be moist enough whilst not being dehydrated was established at 2000-2500 g/cm/day (PEI & al [37];

RUIZ-CARDONA & al [45]). Furthermore, membranes prepared from Na ALG-silk fibroin fibers display a maximum of an appropriate strength at break (179.94 ± 36.72 N) and tear propagation force (7.63 ± 1.08 N), as well as high flexibility, good swelling capacity, and good thermal resistance (DE MORAES & al [46]).

Table 2. Alginate-based membranes with wound healing properties

Composition	Study type/Target	Ref.
Ca Na ALG – bio-occlusive membrane	Clinical study on healing split-thickness skin graft donor sites	[48]
2% g-PEG-chitosan hydrogel solution – 4% chitosan – 4% ALG scaffold	*In vitro* tissue regeneration after injury, cells-scaffold interaction by applying a 3D bi-layered membrane	[28] [49]
chitosan – 30, 50 and 70 w % Na ALG in PEC membranes	Membrane characterization	[38] [44]
2% w/w chitosan – 0.5% w/v Na ALG	Membrane characterization, antibacterial effect	[35] [47]
ALG – chitosan – ciprofloxacin HCl	Membrane characterization, antibacterial effect	[35] [36]
silk fibroin – 2 w% Na ALG	Membrane characterization, cell viability	[38] [46]
2% w/v Na ALG – chitosan – 10, 20, 30% glycerol	Membrane characterization, cytotoxicity	[50]
2% g-PEG – chitosan hydrogel solution – 4% chitosan – 4% ALG 3D scaffold	Tissue regeneration after injury, cells-scaffold interaction	[28] [49]

Another method used for obtaining alginate-based membranes is coacervation, a process that involves liquid-liquid phase separation under electrostatic influence, one example being the formation of alginate-chitosan membranes used for wound healing. The resulting membranes had different characteristics depending either on their dry versus wet state or depending of the flow and stirring rate. Morphologically, the surface was irregular for the dry ones but smooth with no pores for those that were wet, and the thickness varied between 66 and 80 µm (dry membranes) and 106-633 µm (wet membranes). The mechanical parameters studied were the tensile strength (6.86-31.14 MPa), the elongation at break (3.97-8.42%), and the maximum water uptake (maximum 19 g/g of membrane). Also, these membranes prevented bacterial permeation, and those obtained at 100 rpm at 40mL/h displayed good results regarding their use for highly exuding wounds (RODRIGUES & al [47]).

Conclusions

In the last 30 years since the first sulfated alginate derivative was used, the development of alginate-based biomaterials for wound healing had an accelerated pace. The alginate-based films and membranes wound dressings give patients new hopes regarding a pain-free and possibly scar-free healing. Moreover, the existence of other forms of alginate-based dressings and because new wound dressings are studied around the world, the expected outcome is that a true "ideal dressing" will be developed soon enough.

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