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Tiago Daniel Moreira Fernandes

Hyaluronic Acid compounds in the treatment of skin lesions. A review

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Hyaluronic Acid compounds in the treatment of skin lesions. A review

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Faculdade de Medicina da Universidade do Porto, 25/3/2022

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MINI-REVIEW

Tiago Fernandes et al

Hyaluronic Acid compounds in the treatment of skin lesions. A review

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Abstract: The skin is the main barrier of defense against infections. After the skin is damaged by an external agent, a complex process with a goal of repairing the damaged area is started. To achieve a faster and more favorable healing of skin lesions, different biomaterials have been created, among these, are sponges, hydrogels and electro spun membranes. Different polymers have been used for the preparation of these biomaterials, among which, is Hyaluronic Acid, an extracellular matrix compound with anti-aging effects and hydrating properties, making it an extremely efficient molecule for biomedical usage. Our goal is to provide is a short overview of the different HA-based wound dressings that are being tested in vivo.

Keywords: Hyaluronic Acid, Nanoparticles, Dressing, Wound, Skin

Introduction

The skin is the main barrier of defense against infections, made up of three layers, the epidermis, the dermis, and the hypodermis. As it interacts with the environment, the skin acts as a protective

barrier against diseases, dehydration, UV damage, physical lesions while also helping the homeostasis of temperature. (1) After the skin is damaged by an external agent, a complex process is started, with a goal of repairing the damaged area. Dehydration of the site, excessive inflammation, and infections are often associated with the formation of chronic wounds and ulcers by delaying or even inhibiting the healing process.

To achieve a faster and more favorable healing of skin lesions, different biomaterials have been created, among these are sponges, hydrogels and electro spun membranes. The goal is a perfect dressing, capable of maintaining a correct hydration of the lesion site, promoting angiogenesis and connective tissue regeneration, impeding bacterial growth with an anti-bacterial effect, while being permeable to gas and nutrient exchanges, being biodegradable and having no cytotoxicity. To achieve this goal, different polymers have been used for the preparation of dressings, among which is Hyaluronic Acid (HA). (2)

HA is a negatively charged, linear hydrophilic polysaccharide. HA is also a macromolecule with anti-aging effects and hydrating properties. It can be used in a variety of pathologies, like osteoarthritis, embryo implantation, and skin lesions. It can also be used in cosmetic and dermatologic compounds, to increase the skin elasticity and hydration status, but its particle size limits its penetration through the stratum corneum, limiting its effects to more shallow ones.

As an extracellular matrix compound, it can function as a sponge and helping fixate water, offering elasticity, but because of its particle size limits its penetration through the stratum corneum, limiting its effects to more shallow ones. HA benefits are limited as it cannot penetrate the skin as easily. (3)

On the other hand, HA can be hydrolyzed to be of a lower weight, increasing its permeability capabilities. Some studies have suggested that hyaluronic acid nanoparticles (HANP), using HA as an anionic component have increased the permeability of hyaluronic acid through passive diffusion. HANP are expected to facilitate a deeper penetration through the skin, while offering the benefits of hyaluronic acid like increased elasticity and hydration to the skin. Because of this, applications of biopolymers of Hyaluronic Acid have been gathering more interest.

The main difficulty in the delivery of the drugs through the skin is the stratum corneum, the main obstacle to the transdermic penetration of most compounds. Usually, active drugs and compounds of higher molecular weight cannot pass through this barrier through passive diffusion, while lipophilic molecules with a weight lower than 500 Da can penetrate the stratum corneum. Many studies have been made to help understand how to increase transdermic penetration of drugs and composites with low percutaneous permeability.

The two main types of skin permeability enhancers are chemical or mechanical. The chemical methods are applied to low molecular weight compounds. Some examples are ethanol and ℓ-menthol, considered enhancers of transdermic absorption. Other ways of increasing this ability are techniques using ionic liquids, adhesive plasters, and gauze plasters. These approaches alter the structure of the stratum corneum, reducing its function as a protective barrier and ultimately increasing the absorption of the associated compounds. The main physical methods can be applied to higher molecular weight molecules and typically include micro needles, iontophoresis, and electroporation. The downside of these methods is damage to the skin and the need of specialized equipment and personnel to apply these methods. (4)

Our goal is to better understand in what ways nanoparticles, nanofibers, and other composites of HA are being used to treat skin lesions, and our focus is on the dressings that are being tested in vivo.

Material and methods

The research was performed on PubMed database by combining the following terms: “Hyaluronic Acid” AND “nanoparticles” AND (“wound” OR “ulcer” OR “skin lesion”) AND “dressing”. We limited our literature review to articles written in English, published from January 2017 to February 2022. We excluded all studies that were not done in vivo or focused on skin lesions, and wound treatment with hyaluronic acid based composites and studies that were categorized as reviews. From the original 23 articles found using the query cited above, 12 were excluded after abstract analysis. From the remaining 11 articles, 2 were removed after full text analysis.

Results

Silver nanoparticles

Silver nanoparticles have antioxidant activity and act as scavengers of superoxide radicals, decreasing the amount of free radicals created by the inflammatory stage of wound healing, contributing to a faster wound healing, with less scarring and maximum function recovery. They also have antibacterial and antifungal properties, making them a good candidate for application on wound dressings. Because of this, several compounds involving silver nanoparticles are being studied in vivo. In Table 1, there is a short review of the compounds being tested in vivo involving silver and hyaluronic acid nanoparticles.

These articles studied the created compounds for their cytotoxicity, their antibacterial effects, and healing capabilities in vivo. M.R. El-Aassar, et al. was the only one who did not test the cytotoxicity of the materials. All the articles added histological analysis of the wounds to confirm the degree of skin regeneration.

In the cytotoxicity studies, all the compounds tested had good cytotoxicity results, but a common theme was shown, in W. Li et al. and Xushan Chen et al. that showed some degree of cytotoxicity with the increase in concentration of the silver nanoparticles, while the blank compounds, without any silver, showed good cytocompatibility.

In the antibacterial studies, all the compounds tested had good results in vitro against both gram-positive and gram-negative bacteria, except in Júnior, D.M. et al., where the all the compounds created were ineffective at creating a halo of inhibition of bacterial growth. On the other hand, both ODex/HA-ADH/HACC from Xushan Chen et al. and DES-DASH from W. Li et al. had better antibacterial effects than the control samples, which is thought to be caused by the cationic group of quaternized chitosan (HACC) in the ODex/HA-ADH/HACC hydrogel causing damage to the bacterial membrane and leading to bacterial lysis and death, while the eutectic solvent (DES) in DES-DASH hydrogel had quaternary ammonium salt component of choline chloride. These effects were then amplified by the addition of Silver (Ag) which gave all the compounds a good antibacterial effect, making all of them a potentially viable wound dressing.

The results of cytotoxicity and antibacterial effects showed that while the silver particles can be cytotoxic in high concentrations, they add antibacterial effects to the dressings, and some balance needs to be maintained to achieve a concentration of silver low enough to keep the compounds low on cytotoxicity while reaching levels high enough to reach the desired antibacterial effects.

B. Lu et al. tested the compounds in vivo on rabbits while the others tested their compounds on mice. All the in vivo testing was done in the same way, by surgically creating a full-thickness wound in the back of the animals, applying the wound dressing as a treatment, and comparing with control groups of either negative controls, or positive controls. Images and tissue excisions for histological purposes were taken on fixed days and analyzed. The rate of healing was then calculated by the percentage of the wound that was closed on those days.

In vivo testing of wound repair was done by all the articles, with M.R. El-Aassar et al. testing the (Ag-PGA/HA)-PVA and (PGA/HA)-PVA particles against two controls, a negative control of saline and a positive control of Garamycin, an antibiotic for dermatologic uses. The (Ag-PGA/HA)-PVA group showed 96% average wound contraction on day 10 and 99% on day 14, while the group with (PGA/HA)-PVA showed 88% and 97% contraction. On the other hand, the positive control group, with Garamycin showed 83% and 92% contraction, while the saline group showed 68% and 83% wound contraption on the same days. Histologically, the results were similar with the nanofiber groups showing no ulcers and almost normal skin on day 10 and 14, with well-formed epidermis and dermis and basic structures like blood vessels and hair follicles. While the positive control group showed inflammatory cells on day 10, with the ulcer disappearing only on day 14, with the formation of normal looking skin on the same day. On the other side of the results, the saline group showed a greater ulcer width along with marked inflammation, granulation tissues and absence of both sebaceous glands and intact hair follicles at both day 10 and 14. (5)

B. Lu et al. selected the CH-L-GA/HA (without silver), CH-L-GA/HA/Ag-1 (3mg $AgNO_3$), and CH-L-GA/HA/Ag-2 (6mg $AgNO_3$) to be tested in the wound healing experiments against a control group that was not treated. The control group became infected on day 3 and had edema and ulceration with little to none wound contraction (3%). On the other hand, the wounds treated with the composites showed signs of wound healing on day 3 and contraction percentages of 47-69% with

better results on the highest concentration of silver and lowest results on the group with no silver. On day 11, the wounds treated with the composites containing silver had a crust, while the one without silver and the control group did not. Wound healing was about 8 days faster with Ag-1 and Ag-2 when compared to the gauze alone, with 22 average healing days in the gauze group, 16,3 days in the CG group, 14,7 days in the CG/Ag-1 and 14,2 in the CG/Ag-2 group. Histologically, on day 3 all groups had acute necrosis and edema. On day 7, the length of neoepithelium on the CG/Ag-1 and Ag-2 groups was larger than on the CG and gauze group, with the latter showing large numbers of inflammatory cells and granulation tissue. On day 11, all the groups with the composite showed almost complete reparation while the control group showed calluses and limited reparation with little to no wound area covered with epithelium. (6)

Abdel-Mohsen AM et al. tested both HA/Ag-NPs-1mg HA/Ag-NPs-2mg against a group treated with only Hyaluronic Acid and a control group of untreated mice, on both nondiabetic and diabetic mice. On the 5th day after surgery, the wounds of the control groups showed 33% closure for nondiabetic and 22% closure on diabetic mice, while the groups treated with plain HA was 55% and 45% respectively. The groups treated with HA/Ag-NPs-1mg showed a wound closure percentage of 67% on nondiabetic mice and 57% on diabetic mice. After 15 days of surgery, the differences were even greater, with the treated groups reaching between 95% and 85% wound closure while the plain HA group had between 85 and 75% and the control groups only achieved 65% on nondiabetic mice and 50% wound closure on diabetic mice. The histology of the wounds was analyzed and at day 5, the results showed small differences between the diabetic and nondiabetic groups. Meanwhile, on day 15, the diabetic groups showed some necrotic tissue, indicating a slower healing process, while in the HA/Ag-NPs-2 mg groups, a complete wound regeneration was achieved, with some hair follicles and sebaceous glands beginning to form. (7)

X. Chen et al. created an infected burn wound model on rats. The compounds were then tested as ODex/HA-ADH, ODex/HA-ADH/HACC, and Ag@ODex/HA-ADH/HACC and compared against Aquacel® Ag, a commercial silver ion antibacterial dressing. On the 3rd day after surgery, almost all the wounds were enlarged compared to the surgery day, due to the ulceration of wound caused by the infection. This enlargement was not seen in the Ag@ODex/HAADH/HACC group, possibly

caused by the antibacterial effect of the silver particles. On the 7th day, the groups treated with both Ag@ODex/HA-ADH/HACC and ODex/HA-ADH/HACC had wound closures of about 60% while the other groups had more than 60% wound still visible. On the 14th day of treatment, the groups treated with Ag@ODex/HA-ADH/HACC and ODex/HA-ADH/HACC were almost completely healed, while the other groups still had more than 20% of the original wound remaining. After 21 days of treatment, all the groups were fully healed, with the Ag@ODex/HA-ADH/HACC group achieving the smallest scars when compared to the original wounds. Histological analysis also showed that the Ag@ODex/HA-ADH/HACC group was the fastest in healing and was beginning to form hair follicles and dermal papilla. On the 21st day the results were similar with the Ag@ODex/HA-ADH/HACC group showing a skin comparable to the skin tissue of healthy mice. (8) W.Li et al. testing was done by creating 4 groups, the control group, the DES-SH (Sodium Hyaluronate) group, the DES-DASH (Dopamine conjugated Sodium Hyaluronate) group and the DES-DASH@Ag (Dopamine Conjugated-Sodium Hyaluronate loaded with silver nanoparticles) group. On the 3rd day, the all the treated groups had started to close, with only 13% of the wound left on the DES-DASH@Ag group, while the DES-DASH and DES-SH@Ag showed 24% and 34% wound left, and the control group around 40% wound left. On day 12, the wounds of DES-DASH@Ag group were almost completely cured with the wound area approaching 0% while the other groups still had more than 10% of the wound remaining. The regenerated dermis and epidermis were analyzed in histology and the results showed a thicker wound tissue in the DES-DASH@Ag group, when compared to the other groups, confirming the faster healing process related to this treatment. (9)

S. Pérez-Rafael et al. Used the 1.5%_0.2 HA-SH/Ag@Lig NPs because of its greater performance in the antibacterial testing, when compared to the other 8 hydrogels created. The hydrogel was then tested against a positive control group of Dermazin cream, an antibacterial cream for topical use made of silver sulphadiazine. Dermazin was applied to nondiabetic rats (group 1) while the HA-SH/Ag@Lig NPs hydrogel was applied to both nondiabetic (group 2) and diabetic rats (group 3). The groups treated with HA-SH/Ag@Lig NPs hydrogel showed rapid healing of the wound with a fast contraction at the end of the experiment, showing a complete retraction of the wound after

15 days, with no scar formation visible to the naked eye. The histological analysis showed that complete regeneration was achieved in both group 1 and group 2, with group 3 showing less regeneration of skin structures like hair follicles and sebaceous glands. The results showed that HA-SH/Ag@Lig NPs hydrogel led to a rapid and efficient wound healing, even when compared to commercially available products. (10)

All the studies showed good results of their compounds, with all of them achieving a satisfactory cytocompatibility while maintaining good antibacterial effects created by the silver particles, against both gram-positive and gram-negative bacteria and reducing the wound area when compared to the control groups. These results show great potential in new dressings to treat skin lesions.

Other compounds

As hydrogels receive more and more attention as a wound dressing due to their potential to offer an ideal environment for the wound healing process, and their intrinsic features. Their capacity to act as a drug delivery system have made them the focus for a transportation system for various kinds of composites and drugs. In Table 2, there is a short review of the compounds being tested in vivo.

Cefepime loaded hydrogel

Cefepime is a fourth-generation cephalosporin, an antibiotic effective against gram-positive as well as gram-negative bacteria. It is water-insoluble and thus there is a need to develop a controlled delivery of cefepime that does not involve puncturing the skin, reducing its dosing frequency, and safely releasing the drug in a sustained manner. Nanomaterials are a good candidate for the delivery of topic cefepime, increasing stability and increasing shelf life. Incidentally, reducing the need for a higher dosing frequency and increasing the bioavailability of the drug by incorporating cefepime into a nanoparticle matrix, the drug can be released in a more controlled manner. Maryam Shafique et al. created chitosan nanoparticles loaded with cefepime, that would then be loaded into a hydrogel membrane created from HA, pullulan, PVA and glycerol. Different concentrations of the first 3 components were used to create 9 different formulations of hydrogel membranes (F1-F9).

The hydrogel membranes were then characterized and tested by their thickness, water vapor transmission rate, percentage of gel fraction, oxygen permeability, swelling behavior, drug release, antibacterial effect, and degradation.

F1-F3 demonstrated that increasing the concentration of HA resulted in an increase in the thickness of the membrane. Likewise, the increasing the concentration of the Pullulan polymer in F4-F6 formulations also caused an increase in the thickness of the produced membranes. In the same way, increasing PVA quantity in F7-F9 also increased the thickness.

A higher water transmission rate causes faster drying of the wound and if the rate is lower, moisture accumulates in the wound bed and leads to the delay of the healing process. The presence of higher hyaluronic acid increased the water transmission rate, while the increase in pullulan decreased the transmission rate. The presence of PVA also increased the transmission rate. The drug release rate was also increased by the concentration HA. The same result was shown in the increase of pullulan concentrations.

The antibacterial effect of the cefepime loaded hydrogel was strong, as expected following the drug release rates and the antibacterial properties of the antibiotic. No cytotoxicity was shown in the testing of the hydrogel membrane samples.

The in vivo testing of the wound repair capabilities showed that the drug-loaded hydrogel achieved a wound closure of 80% on the 7th day, compared to the 70% in the blank hydrogel membrane, 60% in the positive control group containing cefepime cream and 40% in the negative control treated with saline. On the 14th day, the loaded membrane showed a full recovery of the wound, while the blank hydrogel achieved 90% wound closure and the 80% and 60% of the positive and negative control, respectively.

Based on the results of this study, we can conclude that the nanoparticle loaded hydrogel was capable of an efficient, sustained-release profile of the antibiotic, as well as increasing the rate of healing in the in vivo studies. (11)

Cellulose based nanocrystalline composites

Hyaluronic acid biopolymers for wound dressings also have downsides, like the poor mechanical properties, which can limit their clinical applications. To combat this problem, HA wound dressings can be combined with other polymers to reinforce and improve the mechanical properties of the HA dressing.

The unique characteristics of Nanocrystalline cellulose, such as its mechanical properties, as well as being a safe hydrophilic polymer, have created more and more attention surrounding this compound. Nanocrystalline cellulose can be added to natural polymer based biocomposites in an effort to increase their mechanical strength. One of these composites is Hyaluronic acid dressings. Because of this, the main goal of Karimi Dehkordi, N., et al. was to prepare an effective HA wound dressing. The resulting composite was a CNC-HA composite loaded with granulocyte-macrophage colony stimulating factor (GM-CSF) containing chitosan nanoparticles, to increase the wound healing rate of a simple HA composite.

The composites were created with different concentrations of both the CNC/HA and GM-CSF-Chi-NP dispersion. GM-CSF containing chitosan nanoparticles were added to the CNC-HA composite creating CNC-HA/ GM-CSF-Chi-NPs composite to achieve a faster wound recovery rate with higher re-epithelialization and granulation tissue formation than empty CNC-HA composite. Different concentrations of each component were created to increase the chances of a better final composite.

The composites were then tested for swelling and mechanical characterization, where it was perceived that increasing the amount of CNCs in the structure of the composites increases the swelling ratio and decrease the flexibility of the composite. At the same time, while varying the amount of HA did not have an effect on the tensile strength of the composites, its increase can increase the flexibility of the compounds. The optimal composite was chosen, with the highest swelling ratio and tensile strength. The optimal composite was then tested on an in vitro release study, where it showed a release profile of 22.8±2.45 % in the first 4h, followed by a sustained release achieving around 100% released GM-CSF at 48h. The wound healing properties of CNC-HA/ GM-CSF-Chi-NPs composite were evaluated on full-thickness 1,5cm excisional wounds in rats.

The composites tested were CNC-HA/ Chi-NP, a CNC-HA/ GM-CSF-Chi-NP, and a control group with saline. The groups treated with the composites CNC-HA/ GM-CSF-Chi-NPs composite and CNC-HA/ Chi-NPs showed significantly greater wound closure compared to the control group. The CNC-HA/ GM-CSF-Chi-NPs composite was also significantly better than the CNC-HA/ Chi-NPs composite. At day 13, the wound closure of the full composite showed almost 100% wound closure, while the CNC-HA/ Chi-NPs composite achieved closer to 94% closure. The faster wound closure is thought to be related to the effects of GM-CSF.

Histological and immunohistochemical studies were also done to the wounds, where a significantly lower level of inflammation was shown in the treated groups compared to the control groups. There were also differences between the groups with CNC-HA/GM-CSF-Chi-NPs showing lesser inflammation than the CNC-HA/Chi-NPs group. This shows that the CNC-HA/GM-CSF-Chi-NPs composite has the potential to be used as an effective wound dressing, being easy to create, apply and having good mechanical properties, while also having a good, controlled release of the GM-CSF.

The results of the study done by Karimi Dehkordi, N., et al. showed that the created composites had good swelling properties that can help moisturize the wound environment to help wound repair. To add to that, CNC-HA/ GM-CSF-Chi-NPs showed greater wound repair capabilities than the non-loaded compound and normal saline. With all this in mind, this compound (CNC-HA/ GM-CSF-Chi-NPs) is capable of being thoroughly tested for use in the wound dressing field. (12)

Graphene/copper oxide

Graphene oxide nanosheets have been drawing attention for their acceptable cytotoxicity and antibacterial properties. Combined with copper, a cost-effective agent with antibacterial effects due to its metallic surface, and beneficial effects on wound healing by promoting VEGF, vascular endothelial growth factor. GO and Cu have the ability to be used as treatment of infected wounds. With this in mind, Yang, Y., et al. aimed to create a new wound dressing by combining graphene oxide/copper nanocomposites (GO/Cu) with chitosan/hyaluronic acid, that can provide a new alternative for therapeutic intervention of infected wounds.

The composites were created by preparing nano composites of graphene oxide/copper nanocomposites that were then incorporated on a dressing of chitosan/hyaluronic acid. The dressing was then characterized by their morphology and chemical characteristics using electron microscopy, spectrophotometer, and a thermogravimetric analyzer.

The nanocomposites were tested for antibacterial activity in vitro, against S.Aureus and S.epidermidis, on which they tested a control group with only Mueller-Hinton Broth, and 3 groups containing Chitosan/Hyaluronic Acid, Chitosan/Hyaluronic Acid/Graphene Oxide and Chitosan/Hyaluronic Acid/Graphene Oxide/Copper dressings. The results of the antibacterial tests showed considerably lower numbers in the Chitosan/Hyaluronic Acid/Graphene Oxide/Copper group than those of the other groups, which confirmed the theory that the C/H/GO/Cu dressing would be a good candidate for the treatment of infected wounds.

The composites also went through a cytotoxicity in vitro test, on which the previous composites were tested on mouse fibroblasts, and although the number of apoptotic cells was higher than the control group, the C/H/GO/Cu group showed no obvious cytotoxicity to the tested cell lines. When considering the results of the antibacterial tests, its cytocompatibility was deemed appropriate by the investigators. Having said that, the dressings could also be improved by reducing the concentration of its cytotoxic components to the optimal balance between antibacterial effect and cytotoxic behavior

The dressings were then applied on a full thickness cutaneous defect infection model to evaluate the wound healing capabilities of the C/H/GO/Cu dressings. In these tests, the wounds treated with C/H/GO/Cu displayed the best results with lower wound size and abscesses at day 10 and 14. Meanwhile, the C/H/GO also showed better results than the group with C/H and the control group. On the sacrifice day, minimal wounds with no complications were found in the C/H/GO/Cu group while all the other groups had nonhealing areas and mild abscesses. The C/H/GO/Cu group showed a 60% decrease in wound size at day 10, while the C/H/GO group showed 30% decrease and the C/H and control group showed no difference to the original wound size. On day 14, C/H/GO/Cu showed a 90% wound decrease and C/H/GO showed 60% wound decrease. This

difference was attributed to the GO/Cu antibacterial effect, creating a better controlled infection on the wound site, and allowing its regeneration.

After day 14, the mice were sacrificed and the wounds were analyzed on Histopathology and Immunofluorescence, as well as the internal organs of the animals to test systemic toxicity.

The same results were found on the histological examination of the wounds, with the better results of re-epithelialization and organized granulation tissues being found in wounds treated with C/H/GO/Cu dressings at day 14. On the other hand, signs of infection around the wound like development of inflammatory cell infiltration, local abscesses, fibrosis, and hyperplasia were observed mainly in the CTRL, C/H groups, while the C/H/GO group had some signs of inflammation but not as marked as the other ones and appeared closer to the C/H/GO/Cu group.

The organ toxicity was evaluated by analysis of the heart, liver, and kidneys. The slices of these organs showed no evident changes that would lead the investigators to believe there were any systemic toxicity. (13).

Discussion

Hyaluronic Acid is a main component of the extracellular matrix of the skin, having a central role in inflammation and regeneration of the skin after a lesion. Furthermore, it promotes the proliferation of fibroblasts/keratinocytes as well as the differentiation of fibroblasts into myofibroblasts. Aside from these biological effects, HA is distinguished by its hydrophilicity, biocompatibility, and capacity to be chemically changed, allowing it to be used in a variety of applications. This can widen its possibilities of being used in many applications, having shown to be helpful in many pharmacological activities, of which we decided to focus on the creation of wound dressings. In this approach, several HA-based wound dressings, such as sponges, hydrogels, nanofibers, and other biopolymers have been developed, with various tactics used to overcome HA's low water stability and mechanical qualities, as well as to improve its biological performance.

Overall, the inclusion of HA in these dressings improved their oxygen and nutrient exchanging capabilities by increasing their porosity and swelling properties, facilitating exudate absorption, and enhancing cell migration and proliferation. These properties helped the granulation and re-

epithelization processes, while also favoring angiogenesis, which might explain the overwhelmingly positive results in wound healing rates in all the compounds tested. (14)

Silver nanoparticles

In the Silver nanoparticle compounds, the antibacterial effect of the silver nanoparticles associated with hyaluronic acid in not well understood, but it is thought to be caused by the electrostatic interaction between the negatively charged microorganism cell membrane and positively charged nanoparticles.(15) Silver's antibacterial effect works by affecting the protein synthesis within the cell, as well as the metabolic pathway and nucleic acid production.(16) This combination of effects is possibly synergetic, with the damage to the membrane caused by the electrostatic interactions helping the silver nanoparticles enter the cells and affect all the different processes vital to a cell.

While almost all composites created had at least acceptable cytotoxicity results, Bitao Lu et al.(6) found that increasing the concentration of AgNO3 present in the composite created increasing cytotoxicity effects. This cytotoxicity is probably due to the same mechanisms that are associated with the antibacterial effects of the silver particles.

The wound healing capabilities were tested by all the studies, with all of them achieving a good result when compared to either positive control groups with commercially available products or saline negative control groups. These overwhelmingly positive results in the wound healing rates can be associated with multiple factors, including the antibacterial effect of the silver nanoparticles, the facilitated transportation of oxygen and nutrients provided by the hyaluronic acid hydrogel, the good cytocompatibility profile that all the particles tested in vivo showed, and the effects of hyaluronic acid on the skin, inflammation and cell proliferation and differentiation. All these factors help reduce inflammation caused by the infection of the tissue, a common cause of slower wound healing rates and chronicity of wounds. (17)

B. Other compounds

Cefepime loaded hydrogel

Being a clinically used antibiotic, cefepime was expected to create a zone of inhibition showing antibacterial effects on the in vitro testing, as long as the loaded hydrogel was capable of incorporating and releasing the drug in high enough concentrations to be capable of having its expected effect. With the confirmed results of both the in vitro drug release testing and in vitro antibacterial testing showing good results, as well as the cytotoxicity testing showing good biocompatibility, the wound healing effects of the in vivo testing were also expected to be positive, in essence, applying an antibiotic topically to a skin lesion with the added benefits of the Hyaluronic Acid hydrogel. In fact, the cefepime loaded hydrogel exceeded the results of the commercially available cefepime cream, which had lower results compared to the blank membrane, with no demonstration of antibacterial effects on the in vitro testing. This result shows that, in a clean wound, the unique features of hyaluronic acid and chitosan's antibacterial action, low toxicity and ability to provide a moist environment to the injury site (18) can be more favorable to the wound repair than a stronger antibiotic like cefepime.

Cellulose based nanocrystalline composites

Nanocrystalline cellulose is a safe hydrophilic polymer with good mechanical properties, making it a good choice of polymer to provide an increase in mechanical strength of a wound dressing of hyaluronic acid, which are still rapidly biodegraded and have low mechanical stability. Providing HA based hydrogels with better mechanical properties and a better biodegradation profile is still a challenge, without a reproductible process and with the necessity of using toxic agents in their production. Karimi Dehkordi N et al. created a composite that has the potential of being in the future of wound healing, with a controlled release of GM-CSF, that showed superior wound healing effects, such as rapid wound closure, inflammation status and granulation tissue creation, when compared to either CNC-HA/ Chi-NPs, without the GM-CSF, and saline. (12)

Graphene/copper oxide

Yang Y, et al created a new wound dressing with the goal of providing an alternative for therapeutic intervention of infected wounds. This was done by loading a chitosan/hyaluronic acid dressing with Graphene Oxide, a derivative of graphene, with good cytotoxicity and antibacterial properties, and copper which has good antibacterial activity as well as wound reparation promoting effects. The results of the study done on the composite are very promising, with good results of antibacterial testing, no obvious cytotoxicity to the tested cells and with in vivo results of wound healing rates surpassing the other groups, confirming the theory that the C/H/GO/Cu dressing would be a good candidate for the treatment of infected wounds and can be a promising dressing to be applied in the future in clinical practice.

Conclusion

Hyaluronic acid based dressings show promising results in healing rates of infected or non-infected wounds, but the manufacturing process of these composites is still an expensive and non-reproductible method. The results of the in vivo testing of the various types of wound dressings based on hyaluronic acid show that the attention to these dressings is merited, but in the next years, the problem of in vivo degradation of HA based dressings and the usage of cytotoxic materials like Silver in complex reactions with no possibility of systematic reproducibility need to be addressed in order to create the ultimate HA based dressing, with no cytotoxicity, and easier and cheaper development.

Disclosure

The author reports no conflicts of interest in this work.

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Table 1 Silver nanoparticle infused hyaluronic acid composites being tested in vivo.

Article	*Compound*	*Preparation process*	*Main Findings*
M.R. El-Aassar(5)	(Ag-PGA/HA)-PVA nanofibers	-Dissolution of PGA in distilled water followed by the addition of AgNO3 under magnetic stirring. -Addition of HA solution to the Ag-PGA that was then freeze dried into a powder. -A solution was prepared by dissolving PVA 8%, and then 1% of Ag-PGA/HA dry powder. -The solution was then electrospun to create dry nanofibers.	Particles with an average diameter of 326nm. AgNP demonstrated antibacterial activity against both gram-positive and gram-negative bacteria. Maximum wound reparation compared to a positive control.
Bitao Lu (6)	CH-L-GA/HA/Ag sponges	-Dissolution of 2g L-GA in 100 mL acetic acid, followed by 3g chitosan and stirred for 30 min, followed by 10-mL volume of HA (2mg/mL) stirred for 60min, and 10mL of AgNO3 solution containing either 3, 6 or 10 mg, forming CG/Ag-1, CG/Ag-2, and CG/Ag-3, respectively. -The mixture was then freeze dried for 24h to obtain CG/Ag composites.	Increasing concentrations of AgNO3 achieve stiffer hydrogels, with larger pore size and irregular pore structures. Bonds between Chitosan and Hyaluronic acid create a more stable final structure. AgNP demonstrated antibacterial activity against both gram-positive and gram-negative bacteria. Increasing concentrations of AgNP increase the cytotoxicity of the hydrogel.

			Maximum wound reparation was achieved with and CG/Ag-1, CG/Ag-2, when compared to the other groups.
A.M. Abdel-Mohsen (7)	HA/Ag-NPs fibers	-Dissolution of 40mg of sodium hyaluronate in distilled water. Silver nitrate solutions were then added independently dropwise into the solution. -After confirming the formation of AgNP, the solution was then put in a coagulation bath to obtain HA nanofibers that were then washed and dried. -The fibers were then used to create a dressing sheet.	Achieved formation of uniform Ag nanoparticles of 20-25 nm. The addition of Ag-NPs to the plain HA granted better mechanical properties. Antibacterial effect against gram-negative bacteria and good cytocompatibility. Maximum wound reparation was achieved with HA/Ag-NPs fabrics when compared to the other groups.
Xushan Chen (8)	Ag@ODex/HA-ADH/HACC hydogel	-Formation of ODex by oxidizing dextran. -Dissolution of Hyaluronic acid in deionized water. -Adipic dihydrazid (ADH) was added to the Hyaluronic acid solution. -The ODex/HA-ADH hydrogel was formed using the ODex solution and HA-ADH solution. -Quaternized chitosan (HACC) was then added to the solution before the hydrogel formed to create ODex/HA-ADH/HACC hydrogel. Finally, the hydrogel was soaked in a silver nitrate solution to reduce the silver ions into Ag-NPs and creating Ag@ODex/HA-ADH/HACC Hydrogel.	Good rates of degradation, and swelling properties, essential characteristics for its use in a clinical setting. Antibacterial activity against both gram-positive and gram-negative bacteria. Ag@ODex/HA-ADH/HACC hydrogel treated wound were repaired faster than the other compounds tested.

Wen Li (9)	DES-DASH@Ag hydrogel	-Synthesis of dopamine-conjugated Sodium hyaluronate by adding SH in a MES buffer, followed by EDC and NHS. DASH created with the addition of dopamine -Lyophilization of DASH in DES to create a hydrogel. -Silver nanoparticles were then added by submerging the hydrogel in a silver nitrate solution, creating DES-DASH@Ag. -DES-SH and DES-DASH were created by the same process.	Good physical properties. No cytotoxicity while achieving good antibacterial effect against both gram-positive and gram-negative bacteria. Maximum wound healing was achieved by the DES-DASH@Ag hydrogel when compared to the other groups.
Sílvia Pérez-Rafael (10)	HA-SH/Ag@Lig NPs hydrogel	-Preparation of HA-ADH and HA-SH, the HA and ADH were dissolved in MilliQ water, then the solution was pH adjusted with HCl to form a solution of 4.8 pH which was then thiolated, with the HA-SH being the byproduct of the thiolation of AH-ADH. The preparation of Ag@Lig NPs was done using lignin and silver ions centrifugated and resuspended on an ultrasound bath. -The hydrogels were then prepared by mixing HA-SH and Ag@Lig NPs.	Lignin was essential for the structure of the biopolymer while having a functional role with antibacterial and antioxidant effects. The created hydrogels were capable of inhibiting MPO, MPPs and ROS production, factors of wound chronicity. Good antibacterial effects and biocompatibility. Complete wound recovery of both diabetic and non-diabetic mice in 15 days of treatment, with faster recovery than commercially available products.

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479 **Table 2** Other Hyaluronic Acid based composites being tested in vivo

Article	***Compound***	***Preparation process***	***Main Findings***
Maryam Shafique(11)	Cefepime-loaded hydrogel	-Dilution of Chitosan in acetic acid. -Incorporation of Cefepime in the chitosan solution. -Dissolution of HA in distilled water. -Addition of Pullulan in the HA solution -Addition of PVA in the HA-pullulan solution -Addition of glycerol to the PVA-HA-pullulan solution. -Lyophilization of the chitosan nanoparticles. -Incorporation of the lyophilized chitosan to the hyaluronic acid formulation during the hydrogel formation.	Good stability with good biodegradation and swelling capabilities. Safe and sustained release of cefepime from the hydrogel Antibacterial activity against gram-positive and gram-negative bacteria Maximum wound reparation when compared to a non-loaded hydrogel, and both positive and negative controls
Nakisa Karimi Dehkordi(12)	CNC-HA/ GM-CSF-Chi-NPs composite	-Dissolution of Chitosan in acetic acid. -Addition dropwise of TPP into the Chitosan solution. -GM-CSF was added to the TPP-Chitosan solution, creating GM-CSF-Chi-NPs. -Addition of MCC to a sulfuric acid solution and the solution hydrolyzed. Collection of the supernatant (CNC), which was then dispersed in deionized water. The suspension was then Freeze-dried. -Addition of CNC to deionized water.	The size of the GM-CSF-Chi-NPs was 366.9±9.15 nm with a zeta potential of +43.52±2.39 mV CNC had a size of 93.2±21.1 and a zeta potential of -43.16±5.31 Increasing the amount of CNCs in the structure of the composites, increases the swelling ratio and decrease the flexibility of the composite

		-Addition of HA to the suspension, creating CNC.HA blend. -Addition of the GM-CSF-Chi-NPs to the CNC-HA blend creating CNC-HA/ GM-CSF-Chi-NPs.	The release rate of GM-CSF was 22.8±2.45 % in the first 4h with about 100% over the first 48h. CNC-HA/ GM-CSF-Chi-NPs have better healing results in vivo than the control and the CNC-HA/Chi-NPs.
Ying Yang(13)	C/H/GO/Cu dressing	-Preparation of GO by the Hummer method, then created a GO suspension with an ultrasonic bath. -Addition of cupric sulfate pentahydrate and ethylene diamine tetraacetic acid disodium salt dihydrate to the GO suspension. Addition of a solution containing sodium hydroxide and sodium borohydride to the mixture. Centrifuging of the mixture and drying to obtain GO/Cu nanocomposite. -Preparation of Chitosan and HA solutions. -Preparation of a GO/Cu solution dispersed in deionized water. -Crosslinking of the solutions using STMP. -Pouring of the solutions into a PTFE mold, creating C/H/GO/Cu.	Greatly improved antibacterial activity of the C/H/GO/Cu. Significantly improved antimicrobial performance and angiogenesis capacity Good biocompatibility Better wound healing capabilities. When compared to the C/H/GO, C/H, and control treatments.

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Table 1 [Table titles are in sentence case and do not end with a full-stop.]

Notes:

Abbreviations: AUC, area under the curve; LS, least squares; NE, not estimable. [These are examples of format.]

Figure 1 [Title of figure is in sentence case and ends in a full stop. Please see https://www.dovepress.com/author_guidelines.php?content_id=3511 for our Figure requirements.]