



# Hyaluronic acid derivatives and their healing effect on burns, epithelial surgical wounds, and chronic wounds: A systematic review and meta-analysis of randomized controlled trials

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## ABSTRACT

Hyaluronic acid (HA) is a polysaccharide common to most species and is found in many sites in the human body, including the skin and soft tissue. A systematic review of the literature and meta-analysis was performed to identify randomized controlled trials, evaluating the use of HA derivatives in healing burns, epithelial surgical, and chronic wounds. Nine studies were identified, which met the search criteria and clinical endpoints of complete healing and percent wound size reduction when using HA vs. either an active or passive comparator. It was found in the vast majority of randomized controlled trials (eight of nine) that HA derivatives significantly improved the healing of wounds vs. traditional therapies or placebo (either via complete healing or a significant reduction in wound size) occurring from burns, venous insufficiency, diabetes, neuropathic insufficiency, and surgical removal of the epithelial layer (for tattoo removal). In the other remaining trial, one formulation of HA was compared with another, with the higher concentration showing improved application characteristics. Further, it was found in a meta-analysis in subsets of patients with diabetic foot ulcers (neuropathic) that HA derivatives healed these types of wounds significantly faster than standard of care. These studies in aggregate show that HA derivatives accelerate the healing process in burns, epithelial surgical wounds, and chronic wounds.

## BACKGROUND

### History of hyaluronic acid (HA) derivatives

Early in the 1990s, a way was discovered of binding HA with benzyl alcohol (a process of esterification), which rendered HA manageable in other forms (such as pads/film for use in the human body) without HA losing its identity or function. Since then, HA and its derivatives have been used to treat dermal and subcutaneous wounds of various etiologies. All of these forms were evaluated in this review on their effect in wound healing.

### Uses of HA in medicine

Because HA is hydrophilic, it can be used as a lubricating agent—with one of its indications for intra-articular injections (knee, ankle) for osteoarthritis, postarthroscopy, and for joint lesions shown to provide sustained pain relief and improved patient function when compared in randomized controlled trials (RCTs) with other anti-inflammatory medicines<sup>1,2</sup> (e.g., corticosteroids, nonsteroidal anti-inflammatory drugs) and placebo (e.g., saline injections).<sup>3-7</sup> Further, because HA contributes to tissue hydrodynamics (including the movement of cells), HA membranes have also been shown to reduce the

incidence, extent, and severity of adhesions in abdominal surgery.<sup>8</sup>

### HA and its role in wound healing

HA are polysaccharides that occur naturally in the human body throughout connective, epithelial, and neural tissues. HA also provides two very important functions in wound healing as part of cell proliferation and migration. First, HA provides a temporary structure in the early stages of the wound.<sup>9</sup> This structure helps facilitate the diffusion of nutritional supplies and helps rid the wound of waste products from cell metabolism. Second, and most importantly, HA is closely involved in keratinocyte (cell type of the epidermis or outermost layer of the skin) proliferation and migration.<sup>10</sup> Ultimately, this temporary structure is replaced, as the wound matures, by the addition of protein molecules—proteoglycans (whose function is to provide hydration and swelling pressure to the tissue enabling it to withstand compressional forces) and collagen.<sup>10</sup> Further, because HA is a hygroscopic macromolecule, it is highly osmotic, allowing for control of hydration during periods of wound repair and the inflammatory process associated with it (when HA levels are elevated). The presence of elevated HA levels during this process is also of particular relevance to cell proliferation and migration. Due in

part to HA's presence, cell anchorage to the extracellular matrix is weakened, permitting detachment and facilitating cell migration and division.<sup>11</sup>

As granulation tissue matures, the HA is degraded, and as the levels fall, more protein molecules are produced. The proteins bind to the HA to become proteoglycans and continue the healing process to build up tissue resilience.<sup>12</sup> HA molecules are able to absorb up to 3,000 times their own weight in water. HA therefore also has an important role as a hydrating agent for tissue as mentioned earlier.<sup>13</sup>

### Objective

The objective of this review is to determine whether HA and its derivatives, used as a therapy, provide a clinically beneficial healing effect in burns, epithelial surgical, and chronic wounds vs. other therapies or placebo.

## METHODS—USE OF SYSTEMATIC REVIEW AND META-ANALYSIS

Systematic reviews attempt to collate all empirical evidence that fits prespecified eligibility criteria in order to answer a specific research question. These reviews also use explicit, systematic methods that are utilized with a view to minimizing bias, thus providing more reliable findings from which conclusions and decisions can be made.<sup>14</sup> Systematic reviews may also contain meta-analyses. Meta-analysis is the use of statistical methods to summarize the results of independent studies with similar outcomes. In selecting studies for incorporation in a meta-analysis, the following criteria are used (and were used later)<sup>14</sup>:

- The quality of the study—with RCTs being of the highest quality
- Well-specified research question—e.g., does HA have an effect on wound healing?
- Decisions on which type of data to use—e.g., published or unpublished data (with the goal of using unpublished data to reduce publication bias. Publication bias occurs when the published literature is not representative of the entire population of completed RCTs. This may result in a reader drawing the wrong conclusion from what the entire body of research shows).
- Decisions on which dependent variables (outcomes) are allowed and whether they should be discrete (e.g., wound healed—yes or no) or continuous (e.g., percent of wound that is healed).

By combining information from relevant studies identified, meta-analyses can provide more accurate estimates of the effects of health interventions than those obtained from the individual studies included within a systematic review.<sup>14</sup> Meta-analyses can also facilitate investigations of the concurrence of evidence across studies and can also be used in examining the differences across studies.<sup>14</sup> Outputs of this specific methodology are as follows:

- An assessment of how compelling the findings are based on a thorough analysis of the biases present in each study included<sup>14</sup>; and
- A systematic presentation and synthesis (e.g., meta-analyses where possible) of the characteristics and findings of the included studies.<sup>14</sup>

### Search methods for identifying studies

Criteria for considering studies for inclusion in analysis are discussed in the next section.

### Types of studies

Prospective and RCTs evaluating the effect of skin substitute products composed of HA vs. an active or passive (e.g., placebo) comparator.

### Types of participants

Included in the analysis were patients exhibiting the following conditions: diabetic foot ulcers down to and including bone (Wagner class 4), diabetic and neuropathic lower extremity ulcers, venous leg ulcers, partial or full skin thickness burns, and surgical removal of the epithelial layer of skin.

### Types of interventions

Interventions which included the following HA product formulations were included in the analysis: HA-impregnated inert pads, HA gel, or cream; pad or matrix composed entirely of HA (e.g., hyalofill or hyalomatrix); HA pad used as a substrate for later autologous tissue grafts.

### Types of outcome measures

Studies which evaluated the following primary and secondary outcomes were included in the analysis: Primary – complete wound healing (defined as complete epithelialization of the wound without any septic drainage; Secondary – wound area reduction.

### Search methods for identification of studies

#### Electronic searches

- PubMed using following MeSH terms: Hyaluronic acid, or hyaluronate, or hyaluronan, and wound healing, and randomized controlled trial. Searched conducted on March 25, 2011 and on November 25, 2011.
- Cochrane Central Register of Controlled Trials (CENTRAL) using the search terms hyaluronic acid, or hyaluronate, or hyaluronan, and wound healing. Searched conducted on March 25, 2011 and on November 25, 2011.
- Journal Web sites including (and using the search terms: hyaluronic acid, or hyaluronan, or hyaluronate, and randomized controlled trial) *Journal Wound Care*, *Advances in Skin and Wound Care Journal*, *International Wound Journal*, *Wound Repair and Regeneration*, *Ostomy & Wound Management*, *Journal American Podiatric Medical Association*, *Journal Foot and Ankle Surgery*, *Diabetes Care*, *Diabetic Medicine*, *Diabetes Research & Clinical Practice*, *American Journal Clinical Dermatology*, *Annals Plastic Surgery*, *Journal Plastic and Reconstructive Surgery*, *Journal of Plastic Reconstructive & Aesthetic Surgery*, *Archives Surgery*, *New England Journal of Medicine*, *Journal of the American Medical*

Association, *Lancet*, *International Journal Lower Extremity Wounds*. Searched conducted on March 25, 2011 and on November 25, 2011.

- Technology assessment Web sites including (using the following search terms: wound care or wound healing) Agency Health Research and Quality, Canadian Agency for Drugs and Technology in Health, Health Technology Assessment as part of the National Institute for Health and Clinical Excellence (NICE), California Technology Assessment Forum, and Blue Cross Blue Shield (BCBS) Tech Assessment. Searched conducted on March 25, 2011 and on November 25, 2011.
- Clinical guideline Web sites (using the search term[s] hyaluronic acid, or hyaluronan, or hyaluronate): Institute for Clinical Systems Improvement, National Guideline Clearinghouse, NICE, Scottish Intercollegiate Guidance Network, Wound healing society. Searched conducted on March 25, 2011 and on November 25, 2011.
- Google using search terms hyaluronic acid, hyaluronan, hyaluronate, wound healing, randomized controlled trial (first eight pages of hits). Searched conducted on March 25, 2011 and on November 25, 2011.
- HA manufacturer Web sites were searched. Manufacturer Web sites included: Anika Therapeutics, Institut Biochimique SA (IBSA), and LAM Pharmaceuticals. Searches conducted on March 25, 2011 and on November 25, 2011.

### Searching other resources

The reference section of the RCTs identified through the above electronic searches were reviewed to identify other RCTs. Additionally, manufacturers of HA wound-healing products (Anika Therapeutics/Fidia Advanced Biopolymers, Abano Terme, Italy; IBSA, Budapest, Hungary; LAM Pharmaceuticals, North York, Ontario, Canada), were contacted regarding published unpublished trials. Further, RCT studies that were mentioned as being undertaken as a result of published pilot study results were followed up on.

### Data collection and analysis

Two review authors (JV, VD) screened the titles and abstracts of all studies identified (and independently of each other) in the search strategy. Full text versions were obtained of all studies identified as being potentially relevant, and they were assessed by two review authors for inclusion, using an eligibility pro forma screening document—which was based on prespecified inclusion/exclusion criteria. Any disagreement between the two review authors was resolved by discussion.

A data extraction form was developed to aid in the collection of details from the included studies. One review author independently extracted the data and a second review author validated the extracted data. This data extraction form was developed by the Cochrane Wounds group (University of York, United Kingdom) and used with very minor modifications for the purpose of extracting data for this analysis.

If more than one publication arose from the same study, all versions were considered to maximize data extraction and the primary publication was identified along with the secondary references.

Two review authors independently assessed each included study using the Cochrane Collaboration tool for assessing risk of bias.<sup>14</sup> This tool addresses six specific domains, namely sequence generation, allocation concealment, blinding, incomplete outcome data, selective outcome reporting, and other issues (e.g., extreme baseline imbalance). Blinding and completeness of outcome data were assessed for each outcome separately. A risk of bias table was completed for each eligible study. Any disagreement among review authors was discussed to achieve a consensus. If consensus could not be reached, a third independent party was to be used (note that during the assessment process, third-party adjudication was not necessary).

An assessment of risk of bias using a “risk of bias summary figure,” which presents all of the judgments in a cross-tabulation of study by entry, was evaluated. This display of internal validity indicates the weight the reader may give the results of each study.

We incorporated the results of the risk of bias assessment into the review through systematic narrative description and commentary about each of the domains, leading to an overall assessment of the risk of bias of included studies and a judgment about the internal validity of the results.

Each study is reported separately. The results of binary outcomes (e.g., complete healing—yes/no)—are presented as risk ratios (RRs) with corresponding 95% confidence intervals (CI). For continuous data (e.g., wound area reduction), we used the mean difference if outcomes were measured in the same way between trials. Further, if pooling of data was not possible, we used the statistics utilized in the study for analyzing treatment effect.

In cases of missing data, we attempted to contact authors where data were missing and requested it. We also addressed the impact of missing data in the discussion section. In the case of abstracts, we attempted to contact authors to see if a study has been published in a peer-reviewed journal. If an article had been generated from an abstract but was unpublished, we attempted to obtain it from the author.

If trials could be combined, assessment of statistical heterogeneity was made using the  $I^2$  statistic in order to determine appropriateness for meta-analysis. If the  $I^2$  statistic was at or below 60%, the heterogeneity was considered moderate and meta-analysis was appropriate. If the value was greater than 60%, sensitivity analyses was undertaken in an attempt to identify which studies were most likely causing the problem. If there were only few such studies, and they could be identified, the reasons for their difference were explored and the appropriateness of removing these studies was determined. When appropriate, the meta-analysis was performed excluding any such studies. As well, in examining small-sized studies and heterogeneity, a comparison of fixed and random effects models were employed. If the estimates were similar, it was concluded that any small-study effects would have little effect on the intervention effect estimate.<sup>14</sup> Lastly, weighting of the participant studies in the meta-analysis was based on the sample sizes of the individual studies included in each meta-analysis.

We used a funnel plot to assess reporting bias. Each primary outcome was reported separately. Furthermore, an assessment was made of publication bias (including a review of unpublished studies), location bias (types of journals), and language bias.

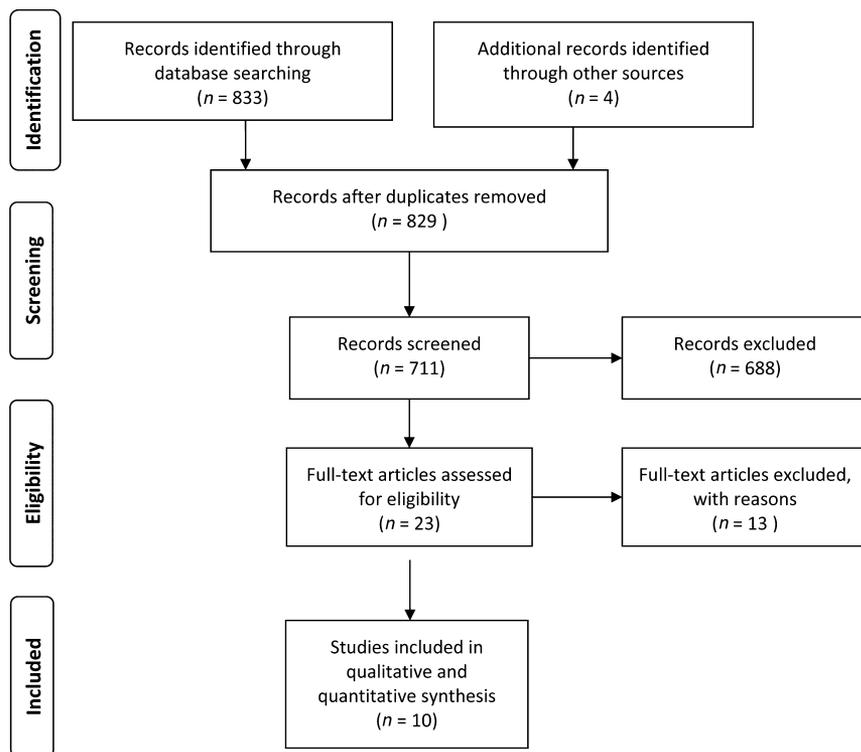


Figure 1. PRISMA diagram—search summary.

**RESULTS**

**Results of search**

See Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) figure (Figure 1) for search summary (specific search methodology and findings available upon request).

**Risk of bias**

Figure 2 shows the overall risk of bias assessment for all included studies. It shows that biases existed in the nonblinding of patients and clinicians performing the procedures, allocation concealment (when patients were allocated to a particular treatment group and when treatment started), and in other types of biases (e.g., study support from manufacturers).

English and Italian language only articles were identified in the search.

No unpublished studies were identified in the search.

Funnel plot analysis of combined trials showed symmetry indicating minimal reporting bias (figure not shown).

**Included studies**

Descriptions of included RCTs (see Table 1 later for specific details on each study) are explained in the next section.

*Studies examining the effect on healing of HA vs. traditional/accepted therapy in venous leg ulcers*

Two studies examined the effect of HA vs. the accepted standard of care for treating venous leg ulcers. In one trial

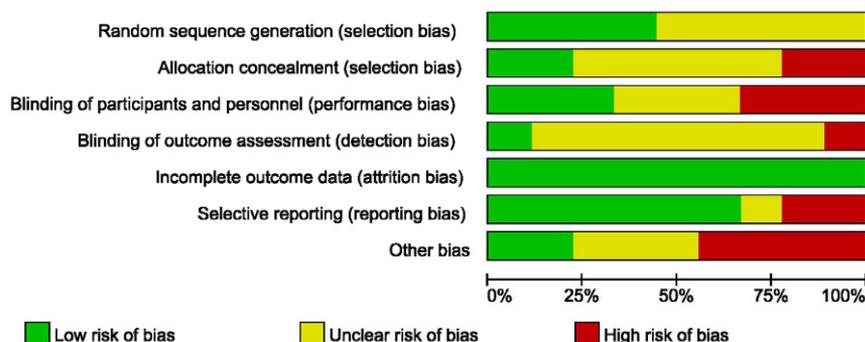


Figure 2. Overall risk of bias assessment.

**Table 1.** Randomized controlled trials using hyaluronic acid (HA) derivatives (either alone or in combination with other therapies) for treating lower extremity wounds (chronic and acute) of various etiology and type

Study	Participants	Interventions	Outcomes	Wound category
Ortonne JP. A controlled study of the activity of hyaluronic acid in the treatment of venous leg ulcers. <i>J Dermatol Treatment</i> 1996; 7: 75-81. <sup>15</sup>	Hyaluronic group (HA): average age = 66.2 ± 3.1; Males/female (M/F) = 16/10; surface area of wound at baseline: 20.8 ± 4.4 cm <sup>2</sup> ; wound present for at least 3 months. Dextranomer group: average age = 69.7 ± 3.6; M/F = 17/7; surface area of wound at baseline: 23.18 ± 4.4 cm <sup>2</sup> ; wound present for at least 3 months. No difference between groups at baseline. Study performed in France.	After initial ulcer debridement: HA impregnated pad applied daily for 21 days (n = 26); wounds cleaned daily prior to HA application. Dextranomer paste (standard of care; SOC) applied daily (n = 24); wounds cleaned daily prior to dextranomer application.	Surface area (cm <sup>2</sup> ) showed a statistically significant difference in reduction in favor of HA at the end of the 21-day treatment period (p < 0.05; Mann-Whitney test). Treatment with HA caused a significant reduction in the incidence and severity of edema (p < 0.001) vs. no significant reduction in the SOC group. A significant decrease in the incidence and severity of oozing was seen in the HA group by day 14 (p < 0.001). A significant decrease in the incidence and severity of oozing was not seen in the SOC until day 21 (p < 0.001).	Venous leg ulcers
Bettinger DA, Mast B, Gore D. Hyaluronic acid impedes reepithelialization of skin graft donor sites. <i>J Burn Care Rehabil</i> 1996; 17: 302-4. <sup>17</sup>	In 11 patients (age range: 21-58 years) two separate partial thickness wounds (1" x 1" x 0.16") were created with a dermatome.	0.5 mL of 1.5% HA placed in wound site and covered with Tegaderm—reapplication of HA and occlusive dressing occurred daily. 100% glycerin placed in wound site and covered with Tegaderm—reapplication of glycerin and occlusive dressing occurred daily.	Glycerin group healed significantly faster than HA group; 9.1 ± 1.6 vs. 10.3 ± 2 days (p < 0.05). On subsequent examination of the wounds, there was no apparent difference in the cosmetic appearance of the resultant scars at 6 weeks and 3 months.	Burns

**Table 1.** Continued.

Study	Participants	Interventions	Outcomes	Wound category
Liguori V, Guillemin C, Pesce GF, Mirmanoff RO, Bernier J. Double-blind, randomized clinical study comparing hyaluronic acid cream to placebo in patients treated with radiotherapy. <i>Radiother Oncol</i> 1997; 42: 155-61. <sup>18</sup>	All patients treated with radiotherapy for head and neck, breast, or pelvic carcinomas. Patients were treated prophylactically for acute radioepithelitis. HA (Ialugen cream, IA): average age = 59.9 ± 12.7; M/F = 34/36; Placebo: average age = 55.7 ± 11.8; M/F = 40/24; no difference between groups at baseline.	IA (n = 70) applied twice daily to the irradiated skin—1 to 2 hours postirradiation and in the evening for a period of 6 weeks with 4-week follow-up. Placebo (n = 64) applied twice daily to the irradiated skin—1 to 2 hours postirradiation and in the evening for a period of 6 weeks with 4-week follow-up.	Irradiated skin evaluated on skin reaction scores to HA cream vs. placebo on a scale of 0-5 (0 = normal skin; 5 = ulcer). Pearson chi-square test with Yates correction of skin reaction scores (0-1 vs. 2-5) between two treatment groups showed a significant difference in favor of the IA group (appearance of more "normal skin" in the IA group) starting at week 3 and though out the radiotherapy treatment ( $p < 0.01$ from week 3-7).	Burns
Edmonds M, Foster A. Hyalofill: a new product for chronic wound management. <i>Diabetic Foot</i> 2000; 3: 29-30. <sup>23</sup>	Hyalofill group: average age = 58 ± 12; mean duration of ulceration = 45 ± 55 weeks; of the 15 patients included, there were 13 ulcers with sinuses and 13 with bone exposed. Control group: average age = 55 ± 12; mean duration of ulceration = 48 ± 64 weeks; of the 15 patients included, there were nine ulcers with sinuses and nine with bone exposed.	Hyalofill plus standard treatment (sharp debridement, pressure relief and infection control) (n = 15). Hyalofill applied at weekly intervals for a period of 12 weeks or until ulcer healed. Control received standard treatment only (n = 15).	Statistically significant difference seen favoring HA group vs. control in healing rate at 12 weeks: 10/15 ulcers healed in HA group vs. three/15 in control ( $p < 0.05$ ).	Diabetic foot ulcers

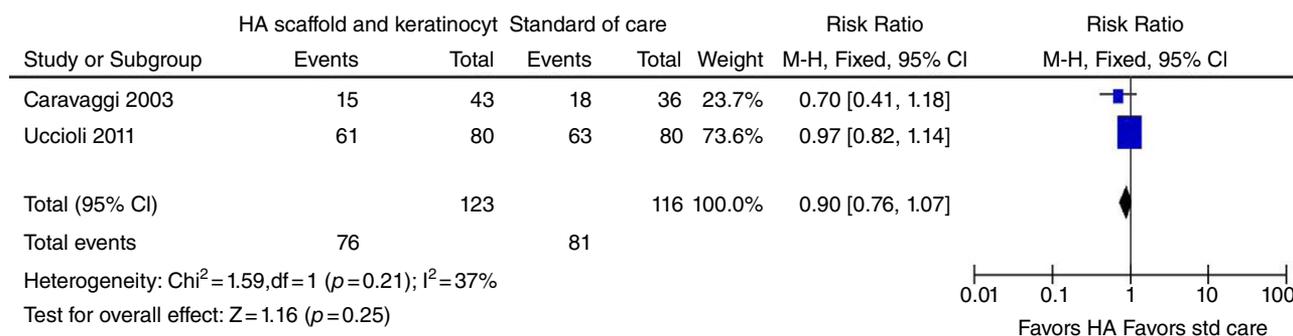
<p>Mekkes JR, Nahuys M. Induction of granulation tissue formation in chronic wounds by hyaluronic acid. <i>Wounds</i> 2001; 13: 159-64.<sup>16</sup></p>	<p>Ten consecutive patients who nonhealing ulcer caused by venous insufficiency (<math>n = 8</math>) or vasculitis (<math>n = 2</math>) had one side of their wound treated with HA vs. IntraSite Gel in a randomized fashion.</p>	<p>HA sheet (<math>n = 10</math>) covered with polyurethane film—applied once per day until 80–100% of wound surface covered with granulation tissue. Wounds then grafted.                  Intrasite gel (Smith &amp; Nephew) (<math>n = 10</math>) covered with polyurethane film—applied once per day until 80–100% of wound surface covered with granulation tissue. Wounds then grafted.</p>	<p>Time to grafting was reduced by 29% with HA (<math>p = 0.004</math>). Total time to healing was reduced by 31% with HA (<math>p = 0.0003</math>).</p>	<p>Venous and vascular leg ulcers</p>
<p>Caravaggi C, De Giglio R, Pritelli C, Sommaria M, Dalla Noce S, Faglia E, Mantero M, Clerici G, Fratino P, Dalla Paola L, Mariani G, Mingardi R, Morabito A. HYAFF-11 based autologous dermal and epidermal grafts in the treatment of noninfected diabetic planar and dorsal foot ulcers. <i>Diabetes Care</i> 2003; 26: 2853-9.<sup>20</sup></p>	<p>Autologous tissue-engineered grafts placed on HYAFF-11 substrate group: ulcer area = <math>5.3 \pm 6.76 \text{ cm}^2</math>; ulcer duration = <math>4.0 \pm 10</math> months; type 1 or 2 DM = 9/34; ABI = <math>0.73 \pm 0.3</math>; HbA1c = <math>7.9 \pm 2.13</math>; Wagner score 1-2.</p>	<p>Prior to treatment, each wound debrided and cleaned. Autologous tissue-engineered grafts placed on HYAFF-11 substrate and implanted onto wound site (<math>n = 43</math>). Patients evaluated for complete healing outcomes at 11 weeks. Paraffin gauze and secondary dressing of sterile cotton pads and gauze (<math>n = 36</math>).</p>	<p>No statistical difference was found in complete healing end point (for both planar and dorsal foot ulcers) at 11 weeks between groups (<math>p = 0.191</math>). However, when examining dorsal foot ulcer subgroup, a statistically significant difference in complete healing was shown, favoring the HYAFF-11 group (<math>p = 0.049</math>).</p>	<p>Diabetic planar and dorsal foot ulcers</p>
<p>At the end of the study, exudates was absent in 86% vs. 69.4% of the HYAFF-11 group vs. control, with a significant difference seen in dorsal ulcers.</p>				

**Table 1.** Continued.

Study	Participants	Interventions	Outcomes	Wound category
Costagliola M, Agrosi M. Second-degree burns: a comparative, multicenter, randomized trial of hyaluronic acid plus silver sulfadiazine vs. silver sulfadiazine alone. <i>Curr Med Res Opin</i> 2005; 21: 1235–40. <sup>19</sup>	HA plus silver sulfadiazine (SSD) group with IIa (superficial) and IIb (deep) dermal burns: average age = 38.2 ± 12.4; M/F = 33/23; burn area = 97.3 ± 100.7 cm <sup>2</sup> . SSD group with IIa (superficial) and IIb (deep) dermal burns: average age = 38.5 ± 15.1; M/F = 36/18; burn area = 91.4 ± 55.9 cm <sup>2</sup> . No difference in baseline characteristics. Multicenter study performed in France, Croatia, Slovenia, and Germany.	HA and SSD topical cream (n = 56) applied daily for 4 weeks. (Connectivina Plus cream, Fidia Farmaceutici SpA, Abano Terme [PD], Padua, Italy) SSD topical cream (n = 54) applied daily for 4 weeks.	Statistically significant shorter time to healing with HA and SSD (9.5 days) vs. SSD (14 days) (p = 0.0073). This shows the improved wound healing activity of HA.	Superficial and deep dermal burns
Price RD, Das-Gupta V, Leigh IM, Navasria HA. A comparison of tissue-engineered hyaluronic acid dermal matrices in a human wound model. <i>Tissue Eng</i> 2006; 12: 2985–95. <sup>25</sup>	Twenty patients with tattoos that were surgically removed. HYAFF p80 group; mean age = 36 ± 7; M/F = 2/8 HYAFF p100 group; mean age = 34 ± 6; M/F = 5/5. Study performed in the UK.	HYAFF p80 dermal matrix applied—changed weekly during 2-week period (n = 10); grafting of cultured autologous keratinocytes at 2 weeks. HYAFF p100 dermal matrix applied once during 2 week period (n = 10); grafting of cultured autologous keratinocytes at 2 weeks.	No statistical difference seen at 2 and 4 weeks as it relates to wound epithelialization between two groups (p = 1.0 and p = 0.79), respectively. Advantage of a p100 matrix was that it only had to be applied once during 2-week period vs. twice for the p80 matrix. Scars were assessed using the Vancouver Scar Scale. At 4 weeks, there were no significant differences in the component scores or in the totals.	Dermal wounds

Abbruzzese L, Rizzo L, Fanelli G, Tedeschi A, Scatena A, Goretti C, Macchiarini S, Piaggese A. Effectiveness and safety of a novel gel dressing in the management of neuropathic leg ulcers in diabetic patients: a prospective double-blind randomized trial. <i>Int J Low Extrem Wounds</i> 2009; 8: 134–40. <sup>24</sup>	HA (Vulnamin gel); average age = 61.8 ± 8.9; type 1 or 2 diabetes = 2/13; duration diabetes = 21.9 ± 6.7 years; HbA1c = 8.8 ± 1%; ABI = 1.1 ± 0.2; ulcer area = 25.8 ± 8.8 cm <sup>2</sup> ; ulcer duration (weeks) = 30.8 ± 16.7. Inert gel; average age = 62.4 ± 7.4; type 1 or 2 diabetes = 3/12; duration diabetes = 19.8 ± 4.2 years; HbA1c = 8.6 ± 1.2%; ABI = 1.0 ± 0.1; ulcer area = 27.3 ± 10.4 cm <sup>2</sup> ; ulcer duration (weeks) = 22.9 ± 18.6. No difference in baseline characteristics except for ulcer duration. Study performed in Italy.	HA plus elastocompressive bandage (n = 15); patients treated for 3 months or until ulcer healed. Inert gel plus elastocompressive bandage (control) (n = 15); patients treated for 3 months or until ulcer healed.	Ulcer area significantly reduced in the HA group over a 4-week period vs. control (p < 0.05; -58.7% vs. -23.4%, respectively). Percentage of lesional area covered by granulation at 4 weeks was significantly higher in HA group than control (62.8 ± 14.7% vs. 28.3 ± 10.2%, p < 0.01).	Neuropathic leg ulcers
Uccioli L, Giurato L, Ruotolo V, Civaarella A, Grimaldi MS, Piaggese A, Teobaldi I, Ricci L, Scionti L, Vermigli C, Seguro R, Mancini L, Ghirlanda G. Two-step autologous grafting using HYAFF scaffolds in treating difficult diabetic foot ulcers: results of a multicenter, randomized controlled trial with long-term follow-up. <i>Int J Low Extrem Wounds</i> 2011; 10: 80–5. <sup>21</sup>	Autologous tissue-engineered grafts placed on HYAFF-11 substrate group: ulcer area = 8.8 ± 9.4 cm <sup>2</sup> ; ulcer duration = 7.4 ± 6.6 months; type 1 or 2 DM = 11/68; ABI = 0.9 ± 0.2. Paraffin gauze group: ulcer area = 6.7 ± 7.7 cm <sup>2</sup> ; ulcer duration = 7.3 ± 7.8 months; type 1 or 2 DM = 6/74; ABI = 0.9 ± 0.7. Note that ulcer area larger in treatment group (p = 0.016). Study performed in Italy.	Autologous tissue-engineered grafts placed on HYAFF-11 substrate and implanted onto wound site (n = 80). Patients evaluated for healing rate, complete healing, outcomes at 12, and 20 weeks, and at 18 months. Paraffin gauze and secondary dressing of sterile cotton pads and gauze (n = 80).	A 50% reduction in ulcer area was achieved significantly faster in the treatment group (mean 40 vs. 50 days; p = 0.018); dorsal ulcers healed significantly faster in the treatment group (p = 0.047).	Diabetic foot ulcers

M, male; F, female; DM, diabetes mellitus; ABI, ankle brachial index; HbA1c, hemoglobin A1c.



**Figure 3.** Meta-analysis diabetic foot ulcers (plantar and dorsal)—number of nonhealed ulcers (defined as events above) in each group at 12 weeks.

(Ortonne 1996;  $n = 50$  patients),<sup>15</sup> the percent wound area reduction was significantly increased with HA (HA impregnated pad) vs. standard of care over a period of 3–8 weeks. In the other trial (Mekkes 2001;  $n = 10$  ulcers),<sup>16</sup> wounds were randomized to where one side of the wound received HA (HA sheet) and the other side received IntraSite Gel (Smith & Nephew, London, UK). Time to skin grafting was reduced significantly as well as time to wound healing with HA. The HA was provided in a pad form for both trials.

**Studies examining the effect on healing of HA vs. placebo or HA plus silver sulfadiazine (SSD) vs. SSD in burn patients**

There were three trials examining either complete healing or rate of healing in burns patients. In each of these trials, HA was delivered in a cream formulation. Two of the trials compared HA vs. placebo. These two trials (Bettinger 1996<sup>17</sup>;  $n = 11$  patients; Liguori 1997<sup>18</sup>;  $n = 114$  patients) examined complete healing (Bettinger) and rate of healing (Liguori). It was found in the Bettinger trial that complete healing occurred significantly faster with placebo (9.1 vs. 10.3 days). In the Liguori trial, in which patient’s skin was treated for radiotherapy burns (radiotherapy used in the treatment for cancer), significantly faster healing occurred throughout the trial at all points with HA vs. placebo. In the third trial (Costagliola 2005<sup>19</sup>;  $n = 110$  patients), the use of HA plus SSD resulted in significantly faster complete healing of burns vs. SSD alone (9.5 days vs. 14.5 days)—showing the clinical efficacy of HA.

**Studies examining the effect on healing of HA vs. standard of care in patients with diabetic plantar and dorsal foot ulcers (Wagner class 1,2, or 4)**

Two trials were identified examining the healing effect of HA on diabetic foot ulcers. In the Caravaggi 2003<sup>20</sup> trial ( $n = 74$  patients), the use of an HA pad seeded with keratinocytes plus autologous graft in patients with Wagner class 1–2 diabetic foot ulcers (graft placed approximately 7–10 days after HA application) was found to heal dorsal foot ulcers significantly faster than the standard of care. In a follow-up to the Caravaggi 2003 trial, Uccioli 2011<sup>21</sup> published a multicenter RCT examining a similar patient popu-

lation but with larger numbers of patients in both groups and with longer term follow-up—20 weeks ( $n = 160$ ). At 20 weeks, it was found, as mentioned, that in the dorsal ulcer subgroup, an HA pad seeded with keratinocytes plus autologous graft treatment vs. standard of care (paraffin gauze) had a significant effect on wound healing. Further, a 50% ulcer area reduction was achieved significantly faster in the HA group (mean 40 vs. 50 days).

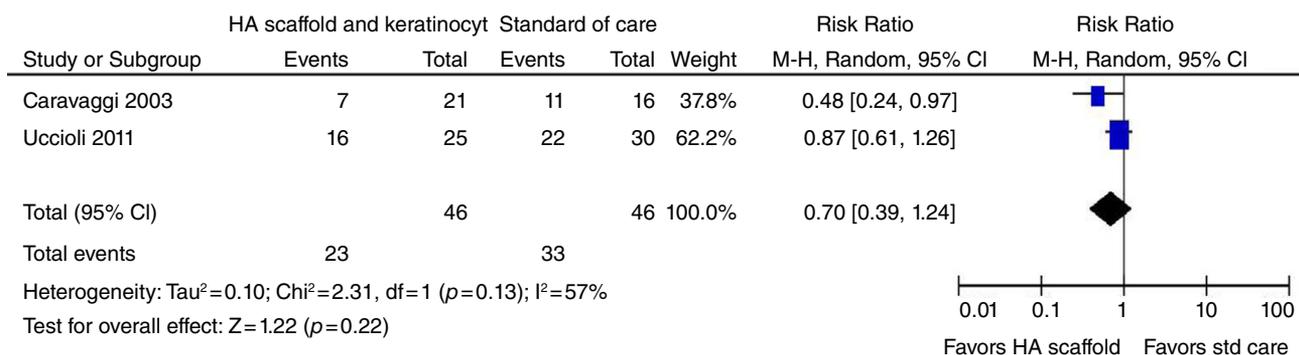
In a meta-analysis examining all diabetic foot ulcers evaluated (plantar and dorsal), it was found that there was no healing effect of HA scaffolding plus keratinocytes vs. standard of care at 12 weeks, although there was a trending toward improved healing:  $\text{RR} = 0.90$ ; 95% CI (0.76–1.04);  $p$ -value 0.25;  $I^2 = 37\%$  (Mantel-Haenszel [M-H] fixed effects model, Figure 3).<sup>1</sup>

A subset of these patients was pooled from the Caravaggi and Uccioli trial and analyzed for HA’s effect on dorsal ulcers. It was found in this meta-analysis that again, there was no healing effect of HA scaffolding plus keratinocytes vs. standard of care at 12 weeks, although there was a trending toward improved healing:  $\text{RR} = 0.70$ ; 95% CI (0.39–1.24);  $p$ -value 0.22;  $I^2 = 57\%$  (M-H random effects model, Figure 4).<sup>†</sup>

Lastly, a non-RCT pilot study was undertaken on the use of hyaluronan therapy in neuropathic foot wounds.<sup>22</sup> In this pilot, it was mentioned that a multicenter RCT on diabetic foot ulcers was being undertaken using the findings gained from the pilot study. In an e-mail follow-up with the author of the pilot study, it was mentioned that the results of this multicenter RCT were negative (in other words, the use of HA did not show a statistically significant difference [improvement] in healing vs. the control) and thus were not published. It was further mentioned that this lack of an effect with HA may have been due to patients not being offloaded effectively and that the lack of effective offloading may have had a confounding effect on the results in this RCT.<sup>‡</sup>

<sup>†</sup>September 5, 2011, email correspondence between Luigi Uccioli and lead author in order to obtain complete healing data from study cited in reference #22.

<sup>‡</sup>March 28, 2011, November 24–26, 2011, email correspondence between David Armstrong and lead author in order to obtain data on follow up RCT mentioned in reference #24.



**Figure 4.** Meta-analysis diabetic dorsal foot ulcers—number of nonhealed ulcers (defined as events above) in each group at 12 weeks.

*Studies examining the effect on healing of HA vs. standard of care in patients with neuropathic foot ulcers*

Two trials were identified on the use of HA derivatives and their healing effect on neuropathic foot ulcers. In the Edmonds 2000<sup>23</sup> trial (n = 30 patients) in which patients with Wagner class 4 diabetic foot ulcers (exposed bone) were treated with an HA matrix vs. standard of care, it was found that complete healing at the end of the study period of 12 weeks was significantly better with HA vs. the standard of care. One trial examined the effect of HA (gel formulation) plus standard of care vs. placebo plus standard of care (Abbruzzese 2009<sup>24</sup>; n = 30 patients) showed a statistically significant effect on reduction in ulcer area size over a 4-week period using HA when compared with placebo/standard of care.

In a meta-analysis performed on the aforementioned trials (Edmonds 2000, Abbruzzese 2009) examining the effect of HA on neuropathic diabetic foot ulcers, it was found that at 12 weeks postinitiation of therapy, HA derivatives showed a significantly improved healing rate vs. standard of care—with a lower number of nonhealed ulcers in the HA group: RR = 0.24; 95% CI (0.24–0.49); p-value <0.0001; I<sup>2</sup> = 0% (M-H random effects model, Figure 5).

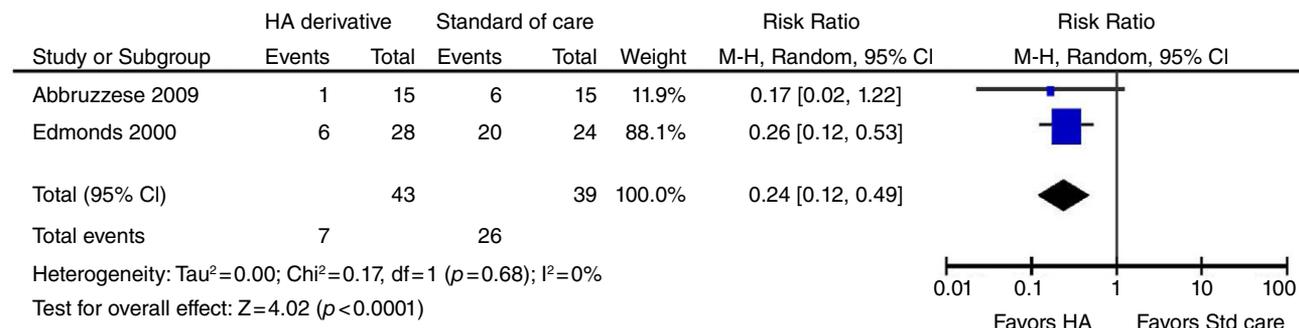
*Studies examining the effect on healing of one formulation of HA vs. another in tattoo removal (removal of epithelial layer of skin) patients*

One trial examined the effect on healing rates of one formulation of HA matrix vs. another (Price 2006<sup>25</sup>; n = 20 patients). There was found to be no difference in the healing rates (epithelialization) over a 2-week period. However, it was found that the p100 (higher concentration of HA) formulation had the advantage of less wound applications over this time period.

**Excluded reviews and studies (n = 13)**

Six RCTs were excluded for the following reasons:

- One RCT evaluated the use of topical HA in the management of oral lichen planus vs. a placebo in 124 patients.<sup>26</sup> The patients treated with HA cream showed a significant reduction in the size of the ulcerated area after 28 days (p < 0.05). The reason for the exclusion was due to the fact that the HA was not used in the earlier indications for inclusion—but in the oral cavity.
- One RCT evaluated the use of an HA/carboxymethylcellulose packing after endoscopic



**Figure 5.** Meta-analysis diabetic neuropathic ulcers—number of nonhealed ulcers (defined as events above) in each group at 12 weeks.

surgery vs. an unpacked side in order to reduce post-operative scarring.<sup>27</sup> It was found in this trial that after 8 weeks, while there was no difference in scarring, there was a significant reduction at all time points measured (2, 4, and 8 weeks) in nasal congestion favoring the HA packing. Again, the reason for the exclusion was due to the fact that the HA was not used in the earlier indications for inclusion—but in the sinus cavity.

- Two RCTs evaluated the use of zinc hyaluronate in the treatment of diabetic foot ulcers.<sup>28,29</sup> It was found in the Tankova 2001<sup>28</sup> trial that the combination of zinc plus HA applied as a cream to diabetic foot ulcers along with standard of care (i.e., debridement, local antiseptics, immobilization of the foot, and antibiotics) vs. standard of care alone resulted in a faster healing rate ( $p = 0.008$ ). This study was excluded due to the potential confounding effect of zinc in healing. In the Cuevas 2004<sup>29</sup> trial, it was also found that the zinc hyaluronate cream when applied to diabetic foot ulcers vs. conventional treatment (not defined in trial) resulted in a faster healing rate ( $p = 0.01$ ). Again, this study was excluded due to the potential confounding effect of zinc in healing. Topical zinc oxide used in other RCTs as a primary therapy for wound healing has shown a positive healing effect.<sup>30,31</sup> Thus, in this particular trial, it could not be determined whether zinc or HA or a combination of the two accelerated the healing of the diabetic foot ulcers.
- Two RCTs evaluated the use of a water-in-oil formulation containing HA, shea butter, glycyrrhetic acid (GrA), *Vitis vinifera*, and telmesteine (Xclair™, Sinclair Pharmaceuticals, Godalming, UK) for treating radiation-induced dermatitis postirradiation therapy for breast cancer.<sup>32,33</sup> The combination of these compounds is believed to contribute synergistically and independently to the minimization of radiation-induced skin reactions. While the use of Xclair™ showed a positive healing in these double blind studies, it was not possible to determine which of these compounds within Xclair™ actually contributed to the healing. Therefore, both studies were excluded.

Other reviews and studies excluded in the PRISMA chart earlier ( $n = 7$ ):

- Three of the excluded studies were Cochrane reviews on “dressings” for burns,<sup>34</sup> venous leg ulcers,<sup>35</sup> and arterial leg ulcers<sup>36</sup> and included only one HA study identified in this review (which ultimately was excluded—see reason later). In the Cochrane review on burns,<sup>34</sup> two of the trials identified earlier and included (Bettinger 1996, Liguori 1997) were not included in this Cochrane review. Perhaps these studies were not found based on the search methodology employed. In the Cochrane review on venous ulcers,<sup>35</sup> only the Taddeucci 2004<sup>37</sup> trial was evaluated as part of their systematic review. However, the Taddeucci 2004 trial was not an RCT as ulcers were not assigned in a randomized fashion (i.e., ulcers were assigned sequentially to treatment groups). In the Cochrane review on arterial ulcers,<sup>36</sup> there were no studies identified using HA as one of the treatment groups. This is consistent with the findings earlier—as no studies using HA with arterial ulcers were identified.
- Another study identified in the search was excluded due to the fact that it was not a truly randomized trial but

used a selection of patients via an “every other” selection.<sup>38</sup> As with the Taddeucci 2004 earlier, because this type of assignment (sequential/every other) can be predicted in advance, it is therefore not truly random. It thus can be open to manipulation and affect outcomes being studied.<sup>39</sup> Lastly, Galasso 1978<sup>40</sup> and Passarini 1982<sup>41</sup> were excluded due to the fact that they were not randomized trials.

## DISCUSSION

There appears to be an overall positive effect of HA in the healing of chronic wound ulcers of various etiologies, burns, and epithelial surgical wounds no matter the form in which HA is delivered topically (i.e., pad, cream, substrate), with eight of the studies identified in the comprehensive search performed showing a significant improvement in the healing rates (with either complete healing or a reduction in wound size). In two trials, Bettinger 1996<sup>17</sup> and Price 2006,<sup>25</sup> healing rates were not superior with HA versus the control. In the Bettinger 1996 trial, the placebo was significantly better than HA (albeit a very small sample size of 11 patients). In the Price 2006 trial, which examined the effect of one HA formulation versus another on skin regeneration in tattoo removal, the higher concentration of HA was found to have improved user characteristics (ie, the need for less applications), but the healing rates were found to be similar.

There also appears to be specific evidence, based on this comprehensive search, supporting the positive healing effect of HA in patients presenting with venous leg ulcers, burns, and diabetic foot ulcers (neuropathic) (when used either alone or as adjunctive therapy for autologous grafts). While there was no statistical difference in the healing effect of HA on dorsal foot ulcers at 12 weeks, there appeared to be a trend of a positive effect. Both of the trials<sup>20,21</sup> evaluated in the meta-analysis on HA and its use with dorsal ulcers were small in size, and the results were likely affected by the small sample sizes.

What appears to be most interesting in these findings is that healing in the most difficult to treat ulcers among chronic wounds (i.e., diabetic foot ulcers) is accelerated with HA vs. using HA in other types of ulcers that were studied—a surprising finding considering the pathology of diabetes. Diabetes is a chronic inflammatory disease. Initial granulation tissue formation is a high inflammatory process with a high rate of tissue turnover. HA assists in this initial granulation process and is found in great abundance in early granulation tissue—in other words HA assists in this inflammatory process early on. Contradictory to its early inflammatory function, HA may also act as a moderator to inflammation in the healing process.<sup>42</sup> As it relates to diabetes, perhaps HA derivatives, when used, have a “modulating” effect on the chronic inflammatory process commonly seen in diseases such as diabetes—thus, accelerating the healing rate. In other diseases such as osteoarthritis, HA has shown an anti-inflammatory and analgesic effect.<sup>43</sup> Further, this anti-inflammatory effect has also been shown in cellular research.<sup>44,45</sup> As it relates to the diabetic plantar foot ulcerations and a lack of difference shown on the outcome of complete healing in both the Caravaggi and Uccioli studies between treatment and control, plantar ulcerations may be more sensitive to off-loading.<sup>20,21,46,47</sup> The Caravaggi trial stated as such—namely that what is fundamental to the

healing of plantar ulcers is off-loading and not the type of wound care product applied. This suggests that differently designed trials may need to be developed in order to show what type of treatment(s) is efficacious. Lastly, a follow-up to Vazquez 2003<sup>22</sup> did not show a superior healing effect of HA vs. control. However, as mentioned in this trial, the lack of effective off-loading may have had a confounding effect—as none of the patients with diabetic foot ulcers in this trial were effectively off-loaded. This issue of off-loading was evaluated in an RCT<sup>48</sup> comparing total contact casts (TCCs), removable cast walkers (RCWs), and half shoes to heal neuropathic foot ulcers in patients with diabetes. It was found that the therapy that provided the most effective reduction in pressure (off-loading) (i.e., TCC) resulted in a significantly higher proportion of patients healed at 12 weeks vs. the other modalities (OR 5.4, 95% CI 1.1–26.1;  $p = 0.026$ ).

One of the other issues with the previous findings, especially as it relates to wound healing in diabetic foot ulcers, is the ulcer area at the time of initiation of treatment, with a  $>5 \text{ cm}^2$  area being predictive of healing in a  $>4$ -month time frame.<sup>49,50</sup> In both the Caravaggi and Uccioli studies,<sup>20,21</sup> the ulcer area exceeded this amount. This may also have affected the 12-week results for plantar and dorsal ulcers combined as reported on earlier—which did not show a statistical difference in wound healing between the groups. Both studies therefore may have benefited from a longer follow-up period for wound healing evaluation based on a larger wound area.

A question that may arise in reviewing the included and excluded studies appearing in the results section is why HA plus keratinocytes was included for analysis and why HA plus other compounds (i.e., zinc, shea butter, GrA, and EXCLAIR) was excluded. The reason for inclusion of HA plus keratinocytes was that prior to grafting of this combination, the keratinocytes were seeded onto an HA biodegradable scaffold and continued to grow for a period of 8 days prior to their being grafted onto the wound site—indicating a potential positive effect of HA on keratinocyte proliferation.<sup>20</sup> With the excluded studies, HA plus the other compounds was placed directly on to the wound.

We were unable to pool similar studies on the outcome of wound area reduction based on different lesions and durations of treatment—e.g., there was no common outcome identified.

Unfortunately, many of the studies identified were of short duration, lasting less than 12 weeks. Again, important differences in healing rates may have arisen with longer follow-up.

### Limitations in the analysis

There were biases identified in the risk of bias assessment that may have affected the outcomes—e.g., nonblinding of clinicians performing the procedures and evaluating the outcomes. Further, one cannot rule out that there are other non-English, non-Italian language articles that have been published and studies that have not been published. We did not identify any unpublished studies. This is not to say they did not exist. The majority of the published articles appeared in chronic disease (e.g., diabetes) and wound journals, appropriate journals for publishing on this type of therapy. This may have minimized the issue of location bias. These facts need to be taken into consideration when evaluating the results.

### Evaluation of the findings in the excluded studies

In the majority of the excluded studies, it was found that HA alone or in combination with other compounds has a positive wound-healing effect when used in the oral (cream) and sinus cavities (packing material), in diabetic foot ulcers when used with zinc (cream formulation), and when used in radiation-induced dermatitis (water/oil formulation with shea butter and GrA). Lastly, the Romanelli 2007<sup>38</sup> trial evaluated two active agents (Oasis Wound Matrix, Healthpoint Biopharmaceuticals, Fort Worth, TX vs. Hyaloskin, Anika Therapeutics, Bedford, MA). Oasis was found to be superior to Hyaloskin in its wound-healing capabilities. However, this trial had biases, namely an accepted method of randomization was not used and editorial assistance for the development of the article was funded by the manufacturer of the Oasis product. Thus, the finding of superiority may be suspect.

In summary, the data point to a positive effect on wound healing with HA derivatives vs. standard of care. Longer duration trials are needed, especially in larger sized wounds (i.e.,  $>5 \text{ cm}^2$ ) and for greater than 12 weeks duration. RCTs are also needed to examine the effect of HA on arterial ulcers. As well, larger sized trials are likely required to show whether HA derivatives have a robust effect (e.g., complete healing) on other chronic wounds such as venous ulcers, more severe type burns, and epithelial surgical wounds.

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