Cross-linked hyaluronic acid in pressure ulcer prevention

Objective: Long-term bedridden patients are at high risk of acquring pressure ulcers (PUs). In this group of patients, prevention is necessary to cut the health costs, improve quality of life and reduce the mortality. Here, we evaluated the effectiveness of a cross-linked hyaluronic acid (HA) as plastic bulking-agent filling and remodelling the deep dermis and subcutaneous space of the skin areas exposed to the risk of necrosis. Our work hypothesis has been to inflate a sub-dermal elastic cushion, filled with a natural ECM component, with the aim to induce a stronger tissue background resistant to the ulcerative process.
Method: All the patients had an increased risk of PUs, at the sacral, ileum or heel skin. Patients were being nursed accordingly to the standard orthopaedic ward management with a pressure relieveing air mattress. The standard protocol consisted in body mobilisation every 3 hours, 24 hours a day and accurate cleaning of the skin with liquid soap and water without any towel friction and without adding any cream or lotion for the skin protection. Our filling protocol enclosed: accurate disinfection of the skin to be injected with povidone-iodine solution, followed by a local anaesthesia with 28G 13 mm needle, injecting 1.5 ml of 1% xylocaine. Then slow, deep, subcutaneous injection of cross-linked HA was performed with a 18G long needle, in order to deliver a homogeneous, soft gel layer underneath and around the whitish erythematous skin edges at risk of ulceration. Patients' tolerability of the compound and adverse events were also recorded.
Results: There were 15 patients (78–94 years old) who participated in the study. All tolerated the procedure very well and no serious side effects were declared. No skin pressure ulceration was detected in the four weeks follow-up
Conclusion: We have demonstrated the safety and tolerability of a cross-linked HA subdermal injection in PUs prevention. The compound stratifies in a soft, elastic, interstitial bulk into the deep dermis, thus reducing the exogenous pressure stress: thus, the induction of a thick hydrophilic substrate supports adequate mesenchymal and blood cells traffic to immediately restore any early or impending damage to the skin.
Declaration of interest: The authors certify that there is no conflict of interest with any financial organisation regarding the material discussed in the manuscript.

healing • hyaluronic acid • prevention • tolerability • ulcers

Pressure ulcers (PUs) can be a life threatening risk for bedridden sick patients which require highly expensive multimodal treatment protocols and long-term follow-up.[1] Prevention is the best approach, and several external appliances, such as sheep skins, silicon pillows, hydrocolloid and polyurethane laminae, as well as air and water beds, have been used to minimise friction and counteract the permanent damage to microcirculation.[2]

In our university, we have extensive experience with cross-linked hyaluronic acid (HA) as bulking substance for either uplifting depressed scars or remodelling anatomic hypoplastic regions, such as in the breasts or buttocks.[3–7] High volumes of cross-linked HA (macrolane) have been safely injected in several body sites. This HA remodels depressed areas,[8] fills gaps[9] and enhances the breast or buttocks volume[10] for rejuvenation procedures, offering a turgid, soft elastic appearance with the lowest invasiveness.[11,12] Face aging is due to multiple factors, such as atrophy, soft tissue dehydration and loss of elastic fibres and laminae, along with superficial textural changes of skin wrinkling improving dramatically with cross-linked injections.[12]

HA is a polysaccharide synthesised as a large, negatively charged, un-branched polymer, consisting in sequence of disaccharides of glucuronic acid (GlcUA) and N-acetylglucosamine (GlcNAc): [-β(1,4)-GlcUA-β(1,3)-GlcNAc-]n. Usually HA encloses 2,000–25,000 disaccharides, with relative molecular masses of 106–107KDa and polymer lengths of 2–25µm.[13] HA is a natural compound widespread in the tissues, especially the skin, but also in liver and parenchyma where it is structural component of the extracellular matrix (ECM). The ECM represents a supportive frame for the cells (tissues and organs) and creates the internal environment for nutrient transport, product metabolism and signal conduction (for proper functioning and protection against external factors).[14] The high viscosity and hydrophilic ability of HA contributes to the parenchyma architecture and porosity.[15] HA also functions as a cell signalling molecule activating cell surface receptors to stimulate cell proliferation, migration, differentiation and gene expression.[16,17] It has different molecular weights, and it is usually degraded by endogenous hyaluronidases

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Fig 1. HA syringes employed in the current study

(HYAL). HA in the ECM can be partially degraded by HYAL activity or oxygen free radicals and diffuse away through the lymphatic system.[18] Alternatively, HA can be taken up by adjacent cells and be subject to lysosomal degradation in the tissue of origin.[19]

There are six HYAL in the human genome; HYAL1 and HYAL2 are ubiquitous.[20] HYAL3 is often enzymatically inactive and appears to have only a supportive role in HYAL1 expression.[21] HYAL4 expression is very limited, and it might be a chondroitinase rather than HYAL enzyme.[20,22] HYAL5 and HYAL6 have been found in rodent sperm, which are released by the acrosomal reaction of the sperm after reaching the oocyte–cumulus complex for penetration.[23]

The linear exogenous glucosamine derivatives degrade very quickly, in a few hours, but they are cross-linking, a process that creates steady hydroxyl bonds with water molecules; enzymatic degradation can take weeks or months. Crosslinking agents such as polyethylene glycol (PEG), 1,4-butanediol diglycidylether (BDDE) and glutaraldehyde and are commonly used for extending the soft tissue around the skin and for cosmetic use as anti-wrinkling compounds. In addition, we have used these two agents as crosslinking agents, since they induce macro aggregation (more than 1,000,000Da molecular weight of HA).

Our aim was to investigate if the experimental subcutaneous embedding of body areas at risk of PUs. We hypothesise that expanding the sub-dermal elastic cushion underneath would reduce the possibility of pressure ulceration. The tolerability and possible side effects of the procedure were also evaluated.

Fig 2. Accurate disinfection of the ilium area with povidone iodine solution

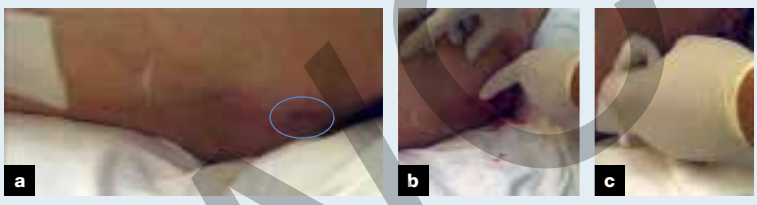
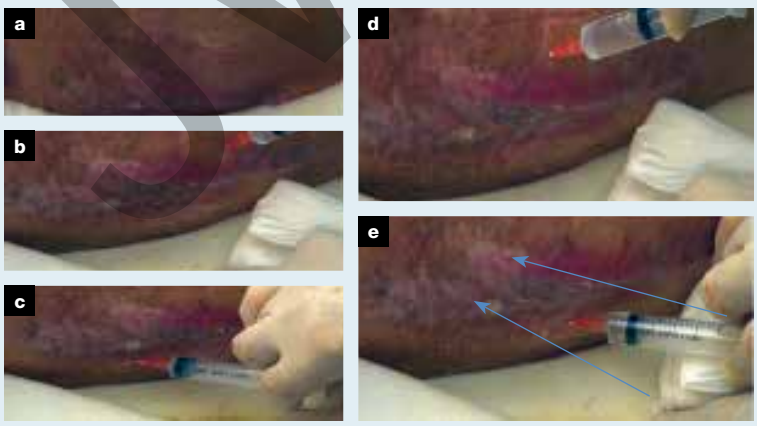


Fig 3. Subcutaneous infiltration of 50cc of cross-linked HA in both sides of the dorsal scarred area; 77-year-old patient, who had a previous healed pressure ulcer (PU) due to spine severe scoliosis, was admitted to hospital for peritonitis. PU prevention was successful: note the erythematous turning to blenching, skin, predictive of high PU risk



Materials and methods

The study was approved by our clinical department university committee, being compliant with the ethics rules. Written informed consent was obtained from all patients.

Following hospital admission, all the included patients lay on a water bed for 3–5 days before treatment. They were nursed for the following 30–45 days, according to the standard orthopaedic ward management, with water PU-prevention mattresses (Tecnomed, Pescara, Italy). The water mattresses distribute the body pressure on a more extended surface. In this way, the body pressure was inferior to arterial pressure, avoiding exposure body areas to PUs.

As soon as any blenching skin erythema was detected during the daily inspection in the sacral,[15] ilium,[3] and heel areas,[2] we immediately started with our preventive strategy injecting a high volume of HA accordingly to the following procedure (Fig 1).

- Accurate disinfection of the perineal area with a povidone-iodine solution, covering anus-perianal space with povidone-embedded sponge
- Local anaesthesia with eutectic mixture of local anaesthetics (EMLA) for 20 minutes (Fig 2)
- Injection of 0.5 ml 1% lidocaine with an insulin needle in the areas requiring larger needle access
- For the sacral and ilium areas 30–50 cc of cross-linked HA was injected using a 16G 15 cm needle 3–5cm at the lateral side of the erythematous skin
- The subcutaneous injection had to be slow and deep, with careful progressive back-and-forth movement, to create an homogeneous soft gel layer underneath and around the erythematous edges, at least 2–3cm thick (Fig 3 and 4)
- Skin thickness was checked at the end of the procedure with a simple pinch test
- The needle was withdrawn, the hole disinfected, and moderately squeezed to rule out and remove any blood oozing and then sealed with transparent tegaderm dressing.
- For the heel areas, we adopted the same procedure, injecting 2 cm underneath the medial malleolus with an overall volume not exceeding 5–7 cc.

The skin was inspected every day to evaluate any local reaction to HA injections. Monitoring was carried out by the nurse for the following 4 weeks, during the morning while cleaning the perineal area (soaked with liquid soap, rinsed with water and dried with a hair-drier) and by the doctor every week, to evaluate the effective PU prevention, the skin quality and any possible complications.

We assessed the patients' declaration of procedure tolerability as follow: (a) optimal (b) good (c) satisfactory (d) not tolerable.

Cross-linked HA injection-related pain was reported as: (a) absence (b) moderate (c) medium (d) strong.

Adverse events were reported in terms of: (a) granuloma (b) leaking of gel (c) bleeding (d) bulging (e) itching (f) blots (g) haematoma (h) infection; (i) inflammation.

Results

The study was undertaken on 15 subjects aged between 78–94 years old (eight male and seven female) who had become suddenly immobile for orthopaedic or surgical reasons (fracture treatment). The patients enrolled had a body mass index (BMI) between 22–31.

The risk factors identified were:

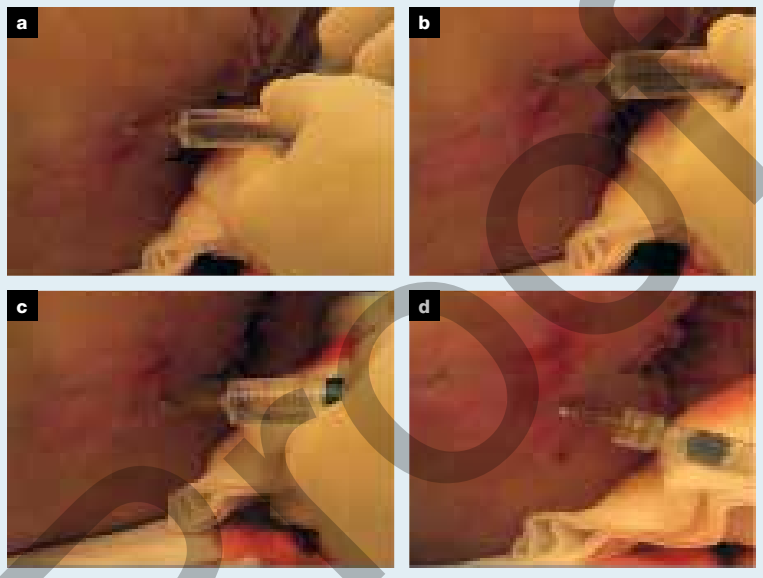
- Overweight or obesity (7/15)
- Severe scoliosis with exceeding pressure of the spine against the skin (3/15)
- Previously healed PUs (3/15)
- Severe weight loss/impending under-nutrition (3/15) faecal incontinence: (5/15)
- Urinary catheter 12/15. (Table 1).

All the patients tolerated the procedure very well, and no skin disruption was observed following the injection of HA. The patients declared the procedure: (a) comfortable and (c) satisfactory in 26.7% of cases (4/15) and (b) good in 46.7% of cases (7/15). This comment stressed the soft and painless feeling of the patients laying for a long time on their back. No patient reported a intolerance or allergy to the injected compound.

Injection–related pain during or soon after administration was absent in 86.7% of cases (13/15) and (b) in 13.3% of cases (2/15), the pain was moderate. No cases of medium and strong pain were observed in the follow-up (0/15).

Minor (c) bleeding from the injection site was observed in 3/15 patients treated with more than 8,000 units of low molecular weight (LWM) heparin. In 5/15 patients, blood oozing arose from the needle track, this stopped after 5 minutes of steady compression. There were also two small (g) haematomas noted in following days, these were gradually reabsorbed and disappeared within 10 days. There was some minor (2–3 cc) (b) gel leaks in three cases from the injection hole: no (h) infection, (i) inflammation, (a) granulomatous reactions, (d) bulging, (e) itching and (f) blots were detected. The skin looked smooth, elastic, and well preserved and the erythema disappeared 2–4 days after injection. In the three-month follow-up, with weekly inspections, no PUs were detected (Fig 5).

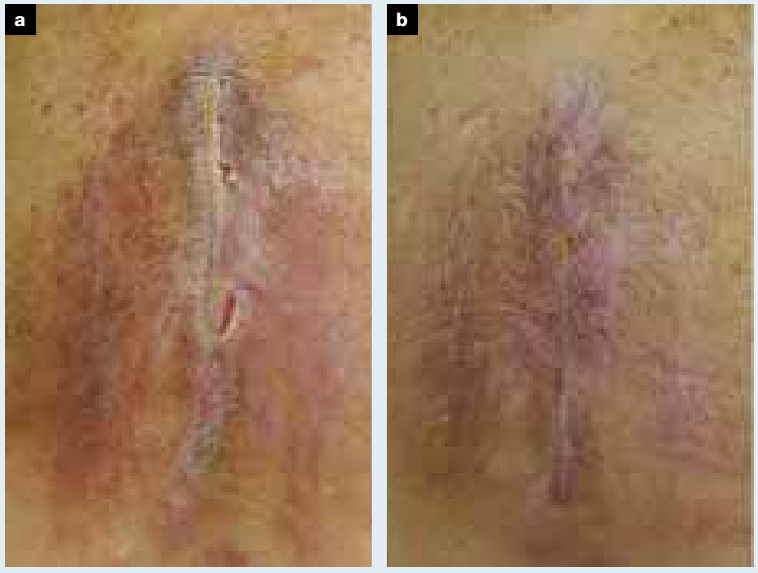
Fig 4. Subcutaneous infiltration of 50 cc of cross-linked HA in the sacral skin area, around a reddened skin spot, heralding an impending bedsore to be prevented



Discussion

In our experience, this is the first article supposing the role of cross-linked HA in PU prevention. We were encouraged to test this new preventive approach by the number of literature reports about the healing properties of HA in chronic wounds.[24] There are a multitude of wound dressings in the market, that aim to preserve hydration within the wound in order to optimise regeneration, protect against infection, and avoid disruption of the wound base. The use of HA ointment, cream, or embedded sponges in the treatment of PUs in spinal cord was adopted in the 1960's.[25] The efficacy of a

Fig 5. Sacral area before multiple HA injections (a) and after 4 weeks of follow-up (b)



an HA-matrix was also demonstrated for the treatment of diabetic foot ulcers.[26] The healing was complete in 84% (26/31) of the treatment group ($p<0.05$), after 36.4±17.6 days ($p<0.05$). No adverse events related to HA compounds were observed. Fino et al.[27] successfully reported the local administration of HA in the treatment of leg ulcers. Turley and Torrance[28] found depolimerisation and hydrolysis of HA helps fibroblasts and epithelial cell proliferation and migration.[28] It has also been demonstrated that higher HA concentrations promote fibroblast detachment from dermal matrix and mitosis.[29] Interestingly, HA has a relevant role in angiogenesis and inflammatory events, by promoting fibroblast and the endothelial cell growth.[30,31]

The molecular weight of HA determines how they alter wound-healing mechanisms.[32–36] Studies by Campo et al.[32] found that breakdown products of low molecular weight HA are pro-inflammatory, leading to increased tissue damage. However, high-molecular -weight HA may block the epithelial cells proliferation by inhibiting the formation of capillary networks,[31] and may bind to regenerating wound sites, while medium molecular weight HA accelerates wound repair.[32] In addition, medium-molecular-weight HA promotes wound closure by up-regulating the junction adhesion molecules.[32]

The HA-rich ECM has different cell functions involved in tissue repair, such as cell migration into the regenerative wound matrix, cell proliferation and organisation of the granulation tissue matrix.[37] HA promotes cell migration by specific cell interaction through cell-surface HA receptors such as CD44, ICAM-1 and receptor for hyaluronan mediated motility (RHAMM).[38,39] RHAMM activates several bonds with cell locomotion-linked protein kinases, for example extracellular signal-regulated protein kinase (ERK), p125fak and pp60c–src.[40]

HA also prevents the free-radical damage to cells,[41] an antioxidant activity probably due to the physicochemical characteristics of the large polyionic polymers.[42] In a rat model of free-radical-induced inflammation, HA reduced the damage to the granulation tissue.[43]

HA modulates keratinocyte proliferation, which is essential for normal epidermal function, as well as during re-epithelisation in tissue repair. In healing wounds, HA is expressed at the edges into the connective tissue matrix, inducing CD44 expression in the migrating keratinocytes.[44] Kaya et al.[45] demonstrated that the inhibition of CD44 expression resulted in a defective HA pool in the superficial dermis, followed by specific morphological alterations of keratinocytes and abnormal keratinocyte proliferation. In addition, a reduction of skin elasticity, abnormal inflammatory responses and tissue repair were observed.[45] All these observations support the relevant roles of HA and CD44 in skin physiology and tissue repair.

The frequency of injection is individually variable. Conventional methods required further refilling sessions at 8–10 months.[23] Based on our personal experience we decided to fill with cross-linked HA in skin areas exposed to PU risk to prevent the abrasive, haemorrhagic friction of the skin against the sacral and the ilium bone surfaces or spinal processes during prolonged laying on bed.

Limitations

Our report has limitations in terms of the small number of patients recruited being priamrily a feasibility study. The positive findings achieved support a more extensive investigation comparing this method with other external devices, such as silicon cushions or protective medications; this will be our next challenge to a comparative validation of the procedure.

Conclusions

HA is a very special molecule, with unique physicochemical properties and specific interactions with cells and ECM.

Our aim was been to create a soft elastic background in the deep dermis in order to counterbalance the pressure stress, as well as to guarantee, by the using the best reactive hydrophilic substrate, adequate cellular traffic to maintain microvascular integrity, and facilitate immediate repair of any early or impending damage to the skin by exogenous injuries. **JWC**

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