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MINI-REVIEW

Biology and biotechnology of hyaluronan

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Abstract The hyaluronan (HA) polymer is a critical component of extracellular matrix with a remarkable structure: is a linear and unbranched polymer without sulphate or phosphate groups. It is ubiquitous in mammals showing several biological functions, ranging from cell proliferation and migration to angiogenesis and inflammation. For its critical biological functions the amount of HA in tissues is carefully controlled by different mechanisms including covalent modification of the synthetic enzymes and epigenetic control of their gene expression. The concentration of HA is also critical in several pathologies including cancer, diabetes and inflammation. Beside these biological roles, the structural properties of HA allow it to take advantage of its capacity to form gels even at concentration of 1 % producing scaffolds with very promising applications in regenerative medicine as biocompatible material for advanced therapeutic uses. In this review we highlight the biological aspects of HA addressing the mechanisms controlling the HA content in tissues as well as its role in important human pathologies. In the second part of the review we highlight the different use of HA polymers in the modern biotechnology.

Keywords Proteoglycans · Glycosaminoglycans · Extracellular matrix · UDP-sugars · O-GlcNAcylation · AMPK · Hydrogel

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Abbreviations

HA	Hyaluronan
UDP-GlcUA	UDP-D-glucuronic acid
UDP-GlcNAc	UDP- *N*-acetyl-D-glucosamine
HAS1 2 and 3	Hyaluronan synthase 1, 2 and 3
OGT	O-GlcNAc transferase
HMWHA	High molecular weight hyaluronan
LMWHA	Low molecular weight hyaluronan
ECM	Extra cellular matrix
GAG	Glycosaminoglycan

Introduction

Hyaluronan (HA) is an unbranched and unsulphated glycosaminoglycan (GAG), the only one produced outside of the Golgi. The enzymes involved in HA synthesis are located on the cellular membrane and are able to extrude the polymer outside of the cell in the extracellular matrix (ECM). HA is a polymer constituted by disaccharide units of D-glucuronic acid (GlcUA) linked to *N*-acetyl-D-glucosamine (GlcNAc) with a beta 1–3 linkage between GlcUA and GlcNAc and a bond beta 1–4 between GlcNAc and GlcUA, as reported in Fig. 1. The disaccharide unit is repeated 25,000 times to reach a molecular mass of over a million Dalton, generating a linear polymer with a molecular mass ranging from 5×10^5 to 5×10^6 [1]. HA is widely distributed in chordates and in few bacteria, interestingly it appears late in the evolution in comparison to the other structural carbohydrate polymers as glycosaminoglycans, chitin and cellulose [2].

In mammals there are three enzymes involved in HA synthesis, HAS1, 2 and 3, whose genes are located on different chromosomes, probably products of an ancestral gene duplication [3]. All these enzymes on the cell membrane produce and extrude the HA by using the UDP sugars present in the

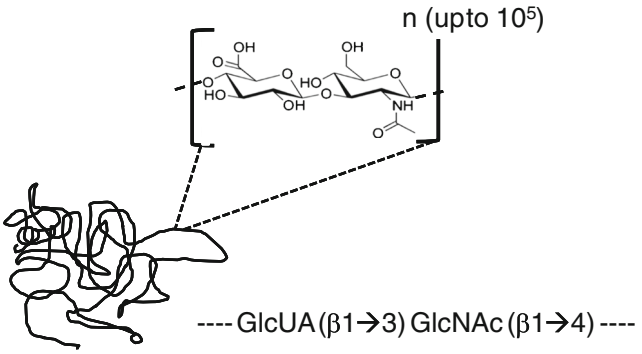

Fig. 1 Structural aspects of Hyaluronan polymer. In the brackets is highlighted the structure of disaccharide unit which is repeated up to 10^5 times. In the lower part of the figure are reported the specific linkage between disaccharide moieties

cytoplasm: UDP-GlcUA and UDP-GlcNAc. The peculiar aspect of these enzymes is the presence in their structure of a double catalytic domain able to interact with the two different substrates and to generate disaccharide units necessary to produce the polymer. The kinetic properties of the HASes are extensively studied even in the absence of crystallographic information all kinetic explanations are still hypothetical [4]. It is not completely clear why three different enzymes have been developed in nature to produce HA. It has been proposed that the different enzymes are able to produce polymers with different length, as HAS1 and 2 produce long length polymers and HAS3 shorter HA chains [5]. An increasing body of literature supports the concept that besides the length of chains produced, the HASes differ to each other also in the regulation of their catalytic properties. In fact, only HAS2 is regulated by covalent modifications such as phosphorylation [5–7], O-GlcNAcylation [8] and ubiquitination [9], whereas the activity of HAS3 is regulated by travelling to the cell membrane linked to the specific protein Rab10 [10]. Information on the regulation of HAS1 is very scanty due to the low expression level of this enzyme in tissues.

The amount of the HA in human body is remarkable. In fact the total HA in all tissues is about 15 g and is mainly located in the skin, with about 30 % of this polymer is replaced every day [11]. The rapid turnover in the human tissues is obtained through the activity of hyaluronidases, hydrolytic enzymes which degrade HA in small fragments. The HA fragments have a mass of few thousands Da and are internalized by cells and destroyed in the lysosomes [11]. The amount of HA in the tissues is strictly regulated by a finely tuned balance between synthetic and catabolic activities. Part of the polymer is also removed from tissues by lymphatic system, which drives HA to liver for the final degradation. Beside the hyaluronidase activity, other conditions can affect the HA integrity, such as free radicals produced by inflammation and UV radiations, which are able to generate HA fragments. An increasing body of literature showed that the HA fragments are able to be recognized by Toll Like Receptors (TLR) triggering specific inflammatory pathways [12–14].

HA has a simple chemical structure without typical modifications present in other glycosaminoglycans as branches or sulphation residues. This simple, invariant linear structure implies that the HA size contains most of its biological information [15]. It is described that HA can react with inter-alpha trypsin inhibitor (I-alpha-I) complex. I-alpha-I carries two heavy chains (HC) that are covalently bound by an ester bond to chondroitin sulfate (CS), which itself is attached to bikunin. By trans-esterification reaction the HC can be transfer onto HA polymer. This process, catalized by TSG6 is important in several conditions for HA stabilization in ECM [120].

The role in inflammation represents the main feature distinguishing HA and other biopolymers. In addition, viscoelastic and mechanical properties that are strongly dependent on polymer size and concentration, HA has important biological roles in cell behavior, including cell motility, proliferation and tissues hydration. Tissue hydration is effectively controlled by the presence of HA, as 1 g of HA can bind up to six liters of water [16].

Moreover, in the last decade it has been proven that HA is not only a passive molecule with remarkable mechanical properties, ranging from space filler to molecular sieving, but it emerged that this polymer has numerous biologically active properties that enlist this glycosaminoglycan among the most biological active molecules in the body. It is not surprising that HA plays a role in several physiological processes including development [17], wound healing [18], cell migration [19] and proliferation [20], but it is also involved in several pathologies, such as cancer [21], vascular diseases [22, 23] and diabetes [24].

Hyaluronan biology

Hyaluronan metabolism

The HA is normally present in most mammal tissues, but its amount markedly differs in organs. In fact the HA concentration is high in synovial fluid where the viscoelastic properties are important for lubricant functions, whereas in other tissues, as brain or cartilage, HA content is lower. For these reasons the balance between HA production and degradation is very well regulated. Despite its simple linear structure HA appears late in the evolution starting in chordates [2]. The appearance of HA in biology was particularly related to cell migration and organ development. How some pathological bacteria learned to produce HA from other cells is still unclear, nevertheless *Streptococcus equisimilis* and other related bacteria are able to produce this polymer in order to block host immune system. Noteworthy, the bacteria producing HA need three genes, which encode for UDP-Glucose pyrophosphorylase (*hasC*),

UDP-Glucose dehydrogenase (*hasB*) and HA synthase (*hasA*), all located in an operon [2, 4, 25].

The regulation of HA synthesis is critical and still includes poorly understood different mechanisms. One of these is the substrate availability. It has been described that the amount of cytosolic UDP-sugars, the substrates for HA synthesis, is able to influence the HA synthesis. In fact modulating in primary cell cultures the UDP-sugars availability it was evident an increased HA production as well as an increased expression of HAS2 and 3 [17]. This aspect is confirmed by using the 4-methyllumbelliferone, a drug able to bind the UDP-GlcUA and therefore to reduce the availability of this precursor [19, 26]. Another way to regulate HA synthesis involves the activity of adenosine monophosphate activated protein kinase (AMPK), the sensor of cellular energy level [27]. It was demonstrated that the activation of AMPK induces the phosphorylation of a specific threonine (Thr 110) in HAS2, blocking the enzyme activity [7]. It is remarkable that this regulation is specific for HAS2 and does not influence the other two HA synthases. Moreover, AMPK activation affects only HA production leaving unaltered the synthesis of other GAGs [5]. This observation correlates the energy level with the capacity of cells to specifically produce HA. Another way to regulate HAS2 activity is related to the presence of high levels of UDP-GlcNAc. UDP-GlcNAc influences the HA synthesis not only as precursor, but also because the HAS2 itself is target of O-GlcNAcylation, a recently described protein covalent modification [27]. High levels of cytosolic UDP-GlcNAc due to an increased hexosamine pathway, induce the activity of the enzyme O-GlcNAc transferase (OGT) which catalyzes the β-*O*-linkage of one residue of GlcNAc to serine 221 of HAS2 [8]. Again, this modification is specific for HAS2 and does not affect the HAS1 and 3 activities and the synthesis of other GAGs. The O-GlcNAcylation is a protein covalent modification described by Torres and Hart in 1984 [28] and it is now widely described in several physiologic and pathologic conditions, including cancer and chronic diseases [29, 30]. The protein O-GlcNAcylation is strongly related to general metabolic conditions as UDP-GlcNAc is a general sensor of energy level in mammal cells [31]. The mechanism involved throughout O-GlcNAcylation of HAS2 affects the half life of the enzyme. In fact, without the O-GlcNAcylation the HAS2 remains on the cell membrane for 17 min, whereas after O-GlcNAcylation the enzyme can remain active on cell membrane for more than 5 h, increasing the HA content in extracellular matrix [8]. The relationship between enzyme stabilization and HA production is also evident considering the ubiquitination, which occurs in lysine 190 residue in HAS2. Even in this case, the modification of the enzyme alters the HA content that depends on the enzyme stabilization on membrane, indicating that ubiquitination plays an indirect role in HA production [9]. Similarly, the proteasome inhibition is able to reduce HAS2 turn over, increasing HA production [8]. It is noteworthy that all covalent modifications, summarized in Fig. 2, have been described only in HAS2. Nevertheless, the regulation of HAS 1 and 3 is critical in several processes. For instance, HAS1 seems to be important in hyperglycemic conditions, cancers and in early phase of development [32] and the hyperglycemic conditions can influence the expression of all HASes with a mechanism still unclear [33]. HAS3 is involved in the formation of particular microvilli structures in cancer cells with a still undefined function [34]. Furthermore, the kinase ERK is able to increase the activity of all the three HASes indicating that protein phosphorylation in different residues from those identified in AMPK activity, can lead to HA accumulation [20]. The regulation of HA content in tissues depends also by the expression of synthetic enzymes at transcriptional level [35]. Usually in the inflammatory conditions induced by cytokines or ER stress, an increase of HA synthesis as well as an up regulation of HAS transcription have been reported [36]. Recently, HAS2 and 3 have been demonstrated to be up regulated by oxidized LDL and 22-oxysterol in an *in vitro* atherosclerotic model [37].

Beside direct covalent modification of enzymes, it has been described an epigenetic control of transcription of HAS2 gene, involving a long non-coding RNA. It was recently described a HAS2 antisense (HAS2-A1), which is able to increase the HAS2 transcription acting as gene activator in *cis* [38]. The presence of the antisense transcript and the machinery of its activity were demonstrated in different cell models and *in vivo*, as described in murine and human atherosclerotic models, acting as a general gene expression regulation [38]. It is noteworthy that HAS2-A1 activity involves NF-kB signaling cascade throughout the transcription factor P65. The interaction of this nucleoprotein with antisense promoter may explain the correlation between inflammatory signals and HAS2 expression.

Even if several aspects of HASes activity are still elusive, it is evident that the complexity of the regulation of their activity, as it depends on the general metabolic conditions of the cells. The machinery of HA synthesis is regulated by different systems including covalent modifications at protein level as well as epigenetic modifications of gene expression [22].

Hyaluronan functions and metabolism

The functions of HA in tissues depend on its structural features. HA is a very hydrated polymer and controls the water content in all tissues. A well hydrated ECM is a perfect environment for cell migration and proliferation [39]. For example, the presence of a well hydrated and soft ECM is critical for embryo development, as described in the microenvironment of oocytes which is mainly constituted by HA [40]. The surface of organs in the body takes advantage from the rheological properties of natural HA present, as in cartilage and in muscle bundles, playing a role as osmotic buffer regulating the

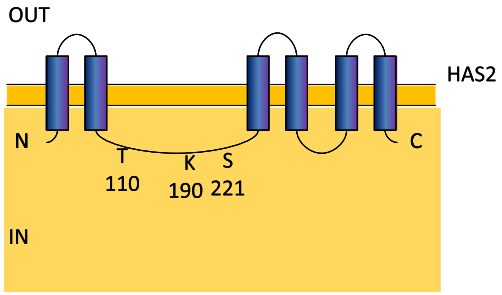

Fig. 2 Schematic model of HAS2 covalent modifications. The residues involved in the modification are reported: T110 for phosphorylations, S221 for O-GlcNAcylation of serine, K 190 for leucine 190 ubiquitination. All residues are in the cytoplasmic loop of the protein. *N* N terminus, *C* C terminus. The *bars* represent the intra-membrane domains of the enzyme

water content in the tissues. Due to its viscoelastic properties HA has critical roles in the biomechanical functions of various tissues, from corpus vitreous to derma [41]. It is important to consider that HA can form gel when its concentration is above 1 % and this concentration is often reached in mammal tissues. For instance, between keratinocytes in the epidermis, in synovial fluid or in Wharton jelly in umbilical cord the HA concentration is enough to form gels and in this form the polymer can act as space filler and shock absorbing macromolecule. The important biological functions of HA are also due to the interactions with other proteins called "hyaloadherins". Among those ECM molecules can be included several proteoglycans, *e.g.*, aggrecan, neurocan and versican; the network between HA and hyaloadherins becomes the tissue architectural scaffold [16].

It is noteworthy to observe that HA activities strongly depend on polymer size. High molecular weight HA (HMWHA) shows anti-angiogenic, immune suppressive and anti-inflammatory activities and induces tissues reparative processes as described in wound healing [18, 41, 42]. It was recently described that in naked mole rat, an African rodent, an HA chain was characterized by its incredible large size (more than 12 millions Da). These animals have an unusual long life, about ten times longer than other rodents, and an incredible resistance to tumor development and spreading, when cancer cells are injected in dermis [43]. In an opposite fashion the fragments of HA, called oligosaccharides with a size of less than 200 kDa, show the capacity to induce inflammatory process as well as angiogenesis throughout the interactions with specific receptors [12, 15]. In view of the HA-oligosaccharides properties, the HA fragments can be considered part of the defense system of the organism and could be considered part of the "alarmin protein family" [44].

The biological activities of HMWHA and oligosaccharides are mediated by interactions of receptors present on cell membrane. The almost ubiquitous HA receptor is CD44, which is a proteoglycan widely distributed on cell membrane and particularly abundant on inflammatory and cancer cells [21]. The interaction of HA with this receptor triggers the signal cascade which eventually activates ERK 1/2 [45]. The main function of CD44/HA interaction has been reported in inflammatory cells. In fact, the HA helps to maintain immune cells at inflammation sites [46–48]. The signalling cascade triggered by CD44 also includes PI3K/PDK1/Akt activity as well as Ras phosphorylation pathway, which involves RAF1, MEK and eventually ERK1/2, as reported in Fig. 3 [49]. The complex regarding the CD44 signal also involves other proteins, as ezrin, merlin and ERB-B1,2 [21].

The binding of HA to CD44 is critical for cell adhesiveness and this interaction triggers the phosphorylation of cytoplasmic CD44 domain which is also required for cell migration and infiltration [50]. CD44 signalling is also important in wound healing. In fact, CD44 on fibroblasts drives their migration in wounded area from perilesional stroma [51]. In this context it should be noted that CD44 *per se* does not induce cell migration but only the interaction of CD44 with HMWHA can promote cell migration and therefore the progression of the wound healing process. Moreover, the directionality of cell migration is strongly dependent on CD44 expression and on HA gradient within the ECM [52].

RHAMM is another important HA receptor described in cancer cells and in endothelial cells [53, 54]. RHAMM is acronym of Receptor for Hyaluronan Mediated Motility and is also known as CD168. The signalling pathway involving RHAMM is still not completely characterised, but it is known that it includes ras-oncogene activity [55, 56]. It has been also described that RHAMM shares ERK1/2 activation pathway with CD44 [57]. In several models RHAMM is critical for cell migration and in inflammation development, as well as in tissue healing [58, 59].

Another receptor present on cell membrane is stabilin-2, also known as HARE, acronym for Hyaluronan Receptor for Endocytosis. This receptor was initially described in endothelial cells, in lymph nodes, in spleen and in liver [60], playing a critical role in these organs development [61]. More recently HARE was also found in eye, brain, kidney and heart. Interestingly HARE is able to interact also with other GAGs as chondroitin sulphates [62]. Usually the HARE function is related to the HA endocytosis and for this reason it is close to clathrin molecules in cell membrane. Moreover, even if its role in endocytosis is demonstrated, its involvement in lysosome degrading pathway is still not clear and its activity at molecular level is unknown [63].

Lymphatic Vessel Endothelial hyaluronan receptor 1 (LYVE 1) is the HA receptor specifically expressed on endothelial cell in lymphatics but it was also described in lymph nodes and in sinusoidal endothelial cells in liver [64–66]. Considering its presence on lymphatic cells, LYVE 1 is generally considered a molecular marker of those cells [67]. The LYVE 1 is able to interact with HA and can be internalized and digested in lysosomes [68]. LYVE 1 function is to absorb

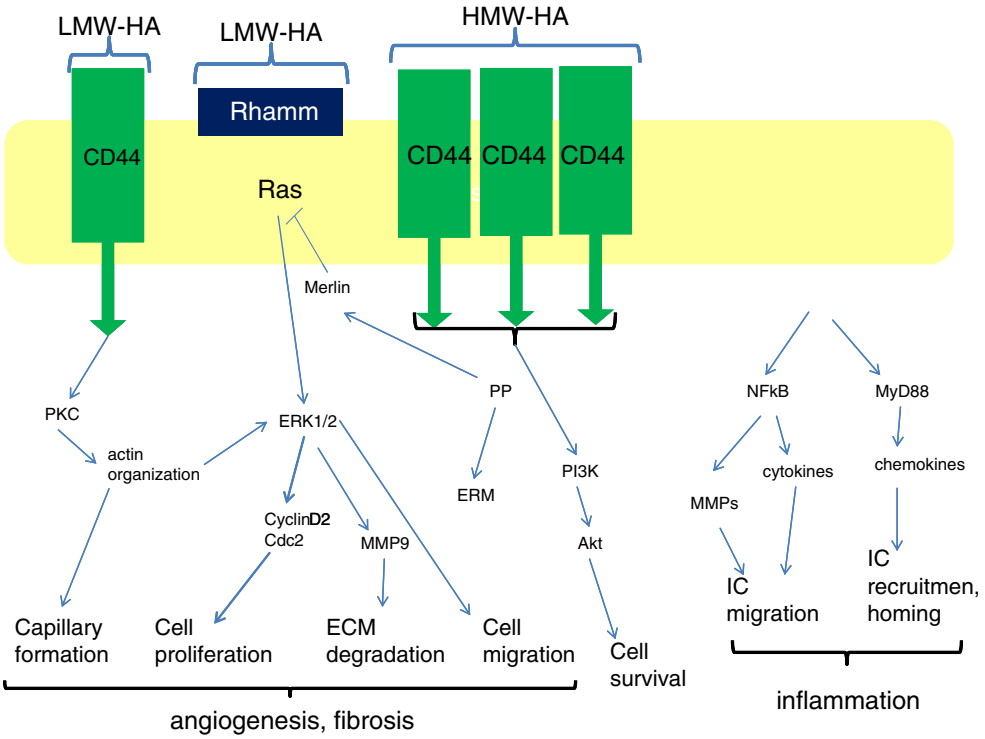

Fig. 3 Schematic model of signaling pathways triggered by the Hyaluronan receptors. For each receptor are reported the signaling pathways triggered by interaction with polymers. In the lower part of the figure is reported the specific biological effect of the activation of these pathways. *HMWHA* high molecular weight hyaluronan, *LMWHA* low molecular weight hyaluronan. In the figure is represented the effect of HMWHA on receptor "clusterization"

HA from tissue driving it to the lymph and playing as regulator of tissue hydration. Interestingly, the abrogation of LYVE 1 in the lymphatics generated animals with normal behaviour, and these findings indicate that probably other unknown receptors are able to act in similar way in mammals [69].

A large body of evidence supports the role of TLR in HA signalling. TLRs are receptors of innate immunity, present on the membrane of all mammal cells [70, 71]. The TLRs involved in HA signalling are TLR2 and 4 [14]. The TLR2 is able to interact with mycobacteria and Gram positive bacteria whereas TLR4 is involved in LPS recognition. When HA interacts with TLR 2 or 4, the signalling cascade in macrophages starts the expression of chemokine genes, a process strongly dependent on the adapter protein MyD88 presence [14]. In such interaction, the signalling triggered by HA interaction is strongly dependent on HA size. In fact, since the TLR clustering on cell membrane is critical for the signalling cascades, the HMWHA promotes cell survival whereas HA oligosaccharides are able to induce inflammatory stress and cell death [14]. HA oligosaccharides induce dendritic cell maturation throughout the phosphorylation cascade of MAPK and nuclear translocation of NF-kB and eventually TNF-alpha synthesis. The role of HA oligosaccharides are also related to the transplant rejection indicating that these fragments are critical for alloimmunity [72]. The inflammation induced by oligosaccharides throughout TLRs can be attenuated by HMWHA interaction with CD44 and TLR4. In fact, this binding increases the expression of IL1-R associated kinase-M that blocks the cell activation [73]. Also IL-8 and MMP2 syntheses are stimulated by HA interaction with TLR4 [74]. In osteoclast differentiation, the HA interaction with TLR4 blocks the signalling triggered by macrophage colony-stimulating factor (M-CSF) [75]. Recent literature highlights the possibility that HA interactions with TLRs are not only critical for innate immunity response, but also for tissue metabolism as described in gut [76]. The role of HA oligosaccharide in tissue metabolism seems to involve the synthesis of defensins, a family of small proteins that beside the antibiotic properties stimulate tissue regeneration in several in vivo and in vivo models [76, 77].

The amount of HA in tissue is also regulated by its degradation that usually is obtained by the activity of the enzymes hyaluronidases. In mammals six hyaluronidases have been described (Hyal −1, −2, −3, −4, P1 and PH20), which catalyze the hydrolysis of linkage bonds beta1-4 between hexosamine and glucuronic acid residues. Interestingly, in bacteria there are several lyases that act as hyaluronidases [78]. In mammals the activity of Hyal 1 and 2 are synergistic. In fact, Hyal 2 degrades the HA in fragments of 20 kDa and then the Hyal 1 degrades these fragments in smaller ones of about 800 Da [15, 78]. The degradation of HA polymer could be also due to the action of free radicals that break HA polymer in fragments without a specific size [79]. The HA fragments are internalized by the cells throughout endocytosis (mainly controlled by CD44 and HARE), but in the presence of free radicals or in excess of oligomers, those oligomers may remain in the ECM interacting with HA receptors. The minimal size able to trigger a cell response has been extensively addressed. It was

reported that oligomers characterized by 4–6 disaccharide units (4-6mers) are responsible for NF-kB signalling and metalloprotease synthesis [74], whereas the oligosaccharides with a range of 4 to 16 mers are able to activate the dendritic cells by TLR receptors [80, 81].

Hyaluronan in disease

The HA is necessary for mammal survival. Indeed, the abrogation of the expression of HAS2, the principal enzyme for the HA synthesis, is lethal [82]. These observations are confirmed by the findings described in naked mole rat, where great amount of large size HA guarantees to these animals an incredible survival and tumour resistance [43]. The HA presence is critical during embryonic development and the expression of three HA synthases is tissue and time specific [32]. The depletion of HA is a common phenomenon in aging, which can cause specific symptoms as those described in articular degeneration where a reduced concentration of HA affects the lubricant properties of the synovial fluid. The reduction of HA in synovial fluid is a phenomenon in arthritis and the articular viscosupplementation restores the correct HA concentration in synovial fluid by injecting HA solution in the articular space [83]. The diseases related to HA are caused by an altered balance between production and degradation, which in turn drives to an altered HA concentration and size. It is demonstrated that skin exposed to UV light progressively looses HA, that cannot be continuously replaced, generating a chronic inflammatory condition. As a consequence, the HA in skin diminishes both in dermis and epidermis causing the wrinkles formation. The wrinkle formation is a phenomenon basically due to tissue de-hydration and the HA replacement in aged skin by using gel-filler tries to restore the correct HA content.

The HA overproduction is usually a condition present in inflammation [14], where often HA produced in large amount interacts with inflammatory cells throughout their membrane receptors (CD44, TLR). Usually, the presence of HA in inflammation is followed by tissue repair and new collagen deposition. This phenomenon should be carefully tuned as the excess of inflammation due to high amount of HA oligosaccharides or excess of fibroblasts stimulation by HA oligosaccharides may drive to fibrosis [84]. The relationship between HA production and chronic inflammation is evident in gut pathologies as Crohn disease and ulcerative colitis. In these pathologies an overproduction of HA by smooth muscle cells, which alters the ECM is evident. In this microenvironment the inflammatory cells are able to interact with HA throughout CD44 receptors. In this case, it is also evident the presence of super molecular organization of HA chains that appear organized in structures named cables, usually the target of inflammatory cells [85, 86]. A similar overproduction of HA was also observed in vascular diseases as arteriosclerosis and in diabetes [23]. In these pathologies the amount of HA is remarkable, even then produced by smooth muscle cells. The presence of HA is also coupled with an increase of cell migration, vessel wall tickening and neontima formation, common findings in vascular degeneration [23]. The augmented production of HA is also evident in diabetes *in vivo* and in *in vitro* experiments with cells cultured in hyperglycaemic condition [87, 88] *i.e.*, for kidney cells as well as in kidneys of diabetic mice [89]. Ultimately, even in lung inflammation and several lung diseases, including asthma and pneumonia, has been described an increased production of HA [90, 91]. Interestingly, tumor necrosis factor-stimulated gene-6 (TSG-6) amplifies HA synthesis by airway smooth muscle cells in lung inflammation and stabilizes the HA polymer structure catalyzing the transfer of chondroitin sulphate chains from bikunin to HA chains [91, 92, 120].

The healing processes in all tissues are typically characterized by a complete alteration of ECM and the first step of the healing process is characterized by the synthesis of HA which favours inflammatory cell migration, a correct macrophage, fibroblast migration, new synthesis of collagen and neoangiogenesis [18, 57, 84]. In all of these processes, HA plays a pivotal role and altered HA content affects wound healing altering a normal scar formation [92]. The role of HA content in microenvironment is therefore critical and considering that in most cancers it has been described an inflammatory microenvironment, it is not surprisingly that HA is present in most of the solid tumours [21, 93]. Similarly to the other kind of inflammations described above, the roles of HMWHA and low molecular weight HA are critical in cancer progression [12]. It was proposed that HA may constitute a marker of malignancy in several solid cancers, *e.g.*, breast, prostate, lung and glioma [21, 94]. It is still under discussion if cancer itself is able to produce the HA surrounding tumour or if this polymer is produced by cells in stroma. However, it is demonstrated that most cancer cells have an increased number of HA receptors CD44 which seems to be related to the cancer cells motility [21].

Hyaluronan biotechnology

The last decade was characterized by an increasing use of HA for different purposes, from products with therapeutical use, as in wound healing or in surgery as anti-adherence agent [95], to products related to aesthetic and cosmetic medicine.

The biotechnological properties of HA are remarkable and are actually dependent on the particular structure of the polymer. HA structure based on beta linkages allows all of HA bulky groups (the hydroxyls, the carboxylate moieties and the anomeric carbon on the adjacent sugar) to be in sterically favorable equatorial positions, favoring the chemical modification of the polymer. All axial positions, less favorable for

chemical derivatization, are occupied by simple hydrogen atoms [96]. When the polymer is in aqueous solution a network of hydrogen bonds is established and maintains the HA chain stiffness. The axial small hydrogens form a relatively hydrophobic environment whereas the equatorial groups are more hydrophilic and are able to interact with solvent creating a twisting ribbon structure. In solution the HA chain forms an extended random coil structure, which can interact with other chains (Fig. 1). The HA chains in solution can entangle each other even at very low concentration: the chains start to entangle at 1 mg/mL and this spontaneous process explains the particular rheological properties of HA [97]. The helical chain of HA in solution can bind 1000 times its weight in water [98]. At 1 % the HA forms a jelly with soft properties, which allow to manage it using syringe with needles, and it can be defined as "quasi-plastic" material [79]. It was demonstrated that HA chains in solution after and during the entanglement can form double helices [99]. These physical aspects indicate HA gel as perfect product with lubricant properties that can be used for replace synovial fluid or in surgery to prevent post surgical adhesion formation, for example after abdominal surgery procedures .

The HA hydrogel formation allows also the use of HA as innovative bio-compatible product in several applications, in fact they are biodegradable, biocompatible and bioresorbable [100–103]. The viscosity of the HA solution increases with its concentration and the elasticity with the size of the polymer. Elasticity and viscosity are two critical parameters important for commercial HA gel preparation [103]. The viscoelastic properties of HA solution are useful in various scaffold preparations for medical applications, those HA scaffolds induce cell differentiation and growth [45], improve the wound healing without aspecific absorption of proteins [104]. Moreover HA matrices can interact with specific receptors to enhance tissue growth and repair. [105].

The preparation of highly purified HA from bioreactors by using genetically modified bacteria introduced the possibility to obtain large amounts of high pure and large size HA. Using these polymers from bioreactors it is possible to treat arthritic joints restoring the lubrication and recovering the rheological properties of synovial fluid improving osteoarthritis symptoms. In order to ameliorate the chemical properties and stability of scaffolds, in some formulation the HA chains can be chemically modified by use of adipic hydrazide, tyramide, benzyl ester, glycidyl methacrylate, thiopropionyl hydrazide or bromoacetate, either at carboxylic acid of glucuronic acid or at the C-6 hydroxyl group of the *N*-acetylglucosamine [106].

Another important application of HA gels is the drug delivery, as proposed in ophthalmology and otolaryngology [107–109]; the HA gels maintain *in situ* the drug molecules and release them during the HA reabsorbing process by the tissue [110].

Moreover, the re-absorption of HA matrix is the mechanism used to produce new tissue *in situ*, stimulating cells with growth factors as BMP2 in cartilage [105].

In cosmetic application HA gels are described as moisturizing agents restoring elastic properties of the skin even though in scientific literature robust scientific data about HA anti aging properties at the moment do not exist. Noteworthy, sunscreens products containing HA showed important anti free radicals action with protective activity against UV irradiation [111].

A large use of HA gels alone or in stabilized form (cross-linked) are used in plastic surgery as cosmetic filler reducing the facial lines and wrinkles showing greater tolerability compared to collagen products [112–114].

Eventually HA is also present in the nutriceutical market, its use in beverages, food and confectioneries has been approved as health food material worldwide. This particular use in food of HA presents remarkable points of interest as is was demonstrated that specific fragments of HA induce beta 2 defensin synthesis in cells of intestinal mucosa [76] and it suggests the use of HA chains with specific size in several pathologies including colitis and gut inflammation.

Conclusion

The increasing knowledge about HA roles in tissues it is evident in the literature data and this polymer represents a key molecule in ECM from the beginning of life until aging. The aging process of mammals is characterized by a marked decrease of HA content and in chronic pathologies its role emerged as critical aspect in disease outcome. Randomized controlled trials have successfully proved the remarkable properties of this polymer in acute and chronic healing, including burn treatment, surgical anti adhesions and ulcers [115]. In diabetes, in chronic inflammatory diseases, in pulmonary and kidney fibrosis HA has a pivotal role and represents a potential therapeutical target or a therapeutic agent [87, 116–119]. International agencies for drug control are increasingly involved in the control of newly developed medical devices based on HA use [120].

The capacity of HA to interact with other ECM proteins and its viscoelastic properties as well as its synergistic activity with bioactive molecules and drugs open the possibility of several clinical applications. From this point of view the HA hydrogels will be a common engineered tissue in regenerative medicine.

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Disclosures The authors declare that they have no conflict of interest.

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