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REVIEW ARTICLE

Hyaluronan in Medical Practice

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Abstract: Hyaluronan is the major extracellular matrix glycosaminoglycan polymer present in vertebrate tissues, with a molar mass that can reach several megaDaltons. It is particularly prominent in the matrix of tissues undergoing rapid turnover, in fetal tissues, and wherever regeneration and repair are occurring. Hyaluronan has highly varied biological functions often dependent on molar mass, however they are highly dependent on source of hyaluronan, its purity and nature of contaminants. Hyaluronan of high-molar-mass is known for its anti-angiogenic, anti-inflammatory and immunosuppressive properties, unlike hyaluronan of low-molar-mass that has the opposite effects. Hyaluronan also has a broad range of clinical applications, such as intra-articular injection, in ophthalmology, otolaryngology, wound healing, and commercially in the cosmetic industry, as well as in drug delivery systems. Currently, polymers of hyaluronan are modified in order to improve their properties, including bioavailability and resistance to degradation. Because of greatly increased interest currently in hyaluronan, the multiple functions of the polymer are presented here, including medicine and industry, as well as recent progress in the formulation of hyaluronan-based materials.

Keywords: Hyaluronic acid, hyaluronan production, tissue engineering, viscoelasticity, viscosupplemenation, viscoprotection.

INTRODUCTION

Hyaluronan (hyaluronic acid, HA, Fig. **1a, 1c**), a linear polymer composed of two disaccharides-*D*-glucuronic acid and *N*-acetyl-D-glucosamine, is linked *via* β-1,4 and β-1,3 glycosidic bonds. Unlike other GAGs (glycosaminoglycans), HA lacks a sulfate group. The number of disaccharide units in HA can vary from 25 to 25,000, with a linear uncoiled polymer length from 10 nm to 25 μm [1]. Molar mass of HA reaches from 100 kDa in serum to 8 MDa in the vitreous of the eye [2] (Table **1**).

In addition, various aspects of the metabolism of HA are indicated in Table **1** for purposes of orientation. The biological significance of the tetrasaccharide and the oligomeric HA with ~ 25 disaccharide units within the metabolic cycle are pointed out. A remarkable property of HA is its capacity to surround itself with a large volume of water that can reach up to several thousand-fold its original weight [3-6]. The very large

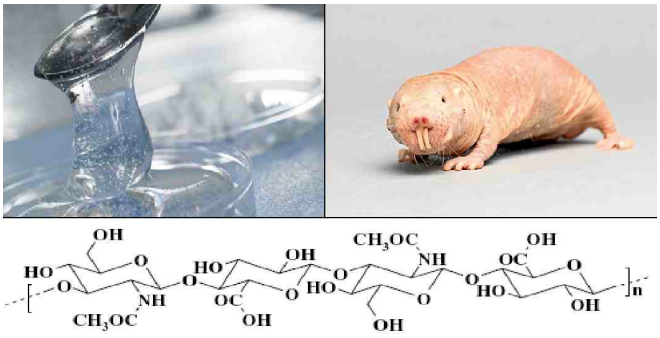

Fig. (1). A highly viscous solution of HA; an adult naked mole rat [36]; structure of HA demonstrating a tetrasaccharide or two repeating units of *N*-acetyl-D-glucosamine and D-glucuronic acid.

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Table 1. The size, uncoiled length, radius of gyration, hydrodynamic radius, and molar mass of HA molecules [7-10].

Physiological moiety	Number of disaccharide units	Uncoiled length	Radius of gyration, R_g[a](nm)	Hydrodynamic radius, Rh[b](nm)	Molar mass (Da)
HYAL 1 cleavage product[c]	1	10 Å	-	-	400
	2	20 Å	-	-	800
	10	10 nm	~ 3.8	~ 2.4	4.0×10^3
HYAL 2 cleavage product[d]	25	25 nm	~ 6.7	~ 4.2	1×10^4
Serum HA	75	75 nm	~ 12.9	~ 8.1	3×10^4
High-molar-mass HAs	250	250 nm	~ 26.5	~ 16.6	1×10^5
	2,500	2.5 μm	~ 106	~ 66.3	1×10^6
	10,000	10[e] μm	~ 242	~ 151	4×10^6
HA in the vitreous of the eye	25,000	25.0 μm	~ 420	~ 263[f]	1×10^7

[a]R_g in 0.2M aqueous NaCl.

[b]Since R_g/Rh = 1.5-1.8 for linear flexible chain of hyaluronan, an approximate estimation of the hydrodynamic radius $Rh = R_g/1.6$ (R. Mendichi, personal communication).

[c]The tetrasaccharide is the predominant cleavage product of hyaluronidase-1 (HYAL 1), the acid-active lysosomal enzyme. The tetrasaccharide corresponds to the size that fits precisely into the active site of the enzyme [7].

[d]Hyaluronidase-2 (HYAL 2) is the first enzyme encountered by the large HA of the ECM. The enzyme, attached to the cell surface, cuts the polymer into intermediate sized fragments of approximately 25 disaccharide units. These fragments are then internalized and passed to early lysosomes for further degradation by HYAL 1 [1, 8, 9] followed by further cleavage by the lysosomal acid exoglycosidases, β-glucuronidase and *N*-acetyl glucosaminidase. The single sugars are then able to pass out of the lysosomes into the cell cytoplasm, where they participate in other carbohydrate pathways.

[e]HA molecule (10,000 repeats) could be spread to 10 μm if stretched from end to end, a length similar to the diameter of a human erythrocyte [10].

[f]The hydrodynamic diameter, equal to approximately 0.5 μm (Rh ≈ 263 nm), of even a 10 MDa HA molecule is able to flow readily through blood capillaries.

negative charge associated with HA at neutral pH accounts for this extraordinary water domain. In Table **1** the hydrodynamic radius (Rh) and the gyration radius (R_g), related to HA molecules in the dissolved state, are also provided [7-10].

In 1934, Meyer and Palmer became the first to purify a substance that was later identified as HA, isolating it from the bovine vitreous body [11]. It took another 20 years to solve its chemical structure. HA together with cellulose and chitin constitute the three major β-chain sugar polymers on Earth [12]. However, cellulose and chitin, homopolymers of glucose and *N*-acetyl-D-glucosamine respectively, contain exclusively linkage by β-1,4 bonds. It is the interspersed β-1,3 bonds that provide the HA polymer with its enormous flexibility and solubility. HAs can associate physiologically in various manners, either electrostatically or covalently, to form a remarkable variety of forms and structures: single self-associating molecules, as a moiety that binds to proteins (referred to as hyaladherins), or other GAGs, ability to form fibers, cables, fibrous networks, sheets, stacks, as space-filling volume expanders that functions as shock absorbers, or as resistance to compression. An example of the latter is the Wharton's jelly of the umbilical cord. It is 99% HA, and prevents vessel compression during neonatal delivery. The smaller HAs are able to aggregate or self-associate as readily as the higher-molar-mass polymers, following complex size-dependent formulas that are not well understood.

HA occurs as the predominant component in the ECM (extracellular matrix) of almost every tissue of vertebrates, particularly in the umbilical cord, skin, vitreous of the eye, and in heart valves. Additionally, high concentrations of HA are present in lymphatic and synovial fluid (SF) and in brain [13-16]. HA also occurs intracellularly but its function therein remains unclear [17]. The concentrations of HA in several human organs as well as in that of other mammals are provided in Table **2** [18].

HA is involved in cell migration, adhesion, proliferation and differentiation, in other physiological processes including embryological development, wound healing, regeneration and repair, and whenever rapid tissue growth occurs. It functions through a broad range of signal transduction pathways [19, 20]. At a body-wide scale, HA modulates water homeostasis, maintains osmotic pressure, buffers physiological solu-

Table 2. Concentrations of HA (μg/g) in various organs in man and other animal species [18].

Organ or fluid	Human	Sheep	Rabbit	Rat
Umbilical cord	4100			
Synovial fluid	1400-3600	540	3890	
Dermis	200			
Vitreous body	140-338	260	29	
Lung		98-243		34
Kidney			93-113	30
Renal papillae			250	
Renal cortex			4	
Brain	35-115		54-76	74
Muscle			27	
Intestine				44
Thoracic lymph	8.5-18	1-34		5.4
Liver			1.5	4
Aqueous humor	0.3-2.2	1.6-5.4	0.6-2.5	0.2
Urine	0.1-0.3			
Lumbar celebrospinal fluid	0.02-0.32			
Blood plasma/serum	0.01-0.1	0.12-0.31	0.019-0.086	0.048-0.26

tions, lubricates joints, is involved in chondroprotection and functions as a space filling molecule and volume expander [1, 21-24]. HA is also associated with a variety of other processes including inflammation, immune regulation and malignant transformation [25-28]. In fact, the aggressiveness of human cancers often correlates with levels of HA [29].

The sizes of HA macromolecules often determine their biological functions. Under normal conditions, HA is of high molar mass (mean molar mass greater than 5×10^5 Da) and possesses space filling, anti-angiogenic, anti-inflammatory and immuno-suppressive properties. The high-molar-mass HAs usually occur in normal health tissues. Medium-sized-HA-chains (mean molar mass ranging from 2×10^4 to 1×10^5 Da) participate in ovulation, embryogenesis, and wound repair. However, under pathological conditions, HA can be degraded to oligosaccharides composed of 15-50 repeating disaccharide units. Such oligosaccharides are highly inflammatory, immuno-stimulatory and angiogenic, a reflection of tissues under stress. On the contrary, very small HA oligomers (400-4,000 Da) appear to mollify the severity of such reactions and are anti-apoptotic and able to induce the production of heat shock proteins [30, 31].

HA as a large polymer is also present at elevated levels in many malignant tumors and, in some cases, is an accurate predictor of patient morbidity. For example, experimental overexpression of HA synthases in human tumor cells results in elevated HA production, causes increased tumor growth *in vivo* [32, 33]. On the other hand, oligosaccharides of HA may inhibit growth of tumors *via* competing for endogenous polymeric HA, replacing high-affinity, multivalent receptor interactions with low affinity, low-valency interactions. HA oligosaccharides significantly inhibit cell proliferation, motility, invasiveness in LM-8 murine osteosarcoma cells and MG-63 human osteoblastic osteosarcoma cells by suppressing cell-associated ECM formation. In the studies, these effects were due to the presence of oligosaccharides longer than octasaccharides [34].

Taken together, it appears that HA length, despite the fact that it is a simple unadorned polymer, is an information-rich system [35].

RECENT INCREASED INTEREST IN HA

Interest in HA has been increased steadily. There has been an increase in the number of HA-related publications (Fig. **2**). Further increases are certain to occur following the dramatic observations made recently with

the naked mole rat, *Heterocephalus glaber*. These rodents (Fig. **1b**, [36]) have a remarkable life span that exceeds 30 years, when that of rats and mice rarely exceeds 2-3 years. Cells from these animals also have an unusual resistance to malignant transformation [37]. Naked mole rats have a molar mass of HA in their circulation and embedded in their tissues five-times that of other rodents. These results are reflected also in their isolated fibroblasts. They secrete high-molar-mass HA, resulting from the decreased activity of the hyaluronidase enzymes that degrade HA and a unique sequence of the key enzyme involved in HA synthesis, the hyaluronan synthase 2 (HAS 2) [8, 9]. With genetic deletion of HAS 2 or over-expression of the hyaluronidase 2 enzyme, these animals cease being resistant to malignant transformation and become readily able to form tumors [37].

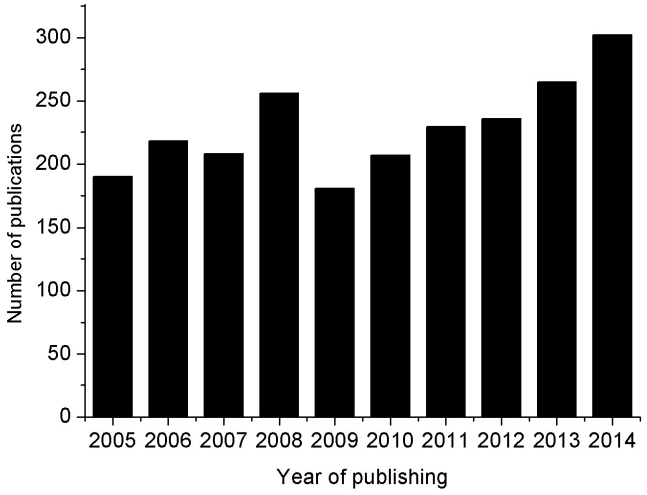

Fig. (2). Publishing activity regarding HA research during the past 10 years, as established using the ScienceDirect database [24].

Human milk contains HA and its concentration is highest immediately after delivery. Treatment of epithelium with physiologic levels of milk-derived HA increases intracellular expression of β-defensin and resistance to *Salmonella* [38]. Based on these results, milk HA enhances functional antimicrobial defense mechanisms of the intestinal epithelium being a mediator of maternal protection of newborns.

It appears that HA is associated with important phenomena critical for the quality of human life, such as protection of newborns, cancer resistance and increased longevity. This accounts for the recent increased interest in HA. Pharmaceutical uses for HA and its derivatives will certainly become key reagents for enhancing these parameters that have the potential for improving human life.

BIOMEDICAL APPLICATIONS

As a component of normal human tissues, HA has been promoted as a biomaterial scaffold for applications for regenerative medicine and in tissue engineering [2, 39-41]. Solutions of HA have remarkable properties. Among these are viscoelasticity, biocompatibility, bioreactivity, non-immunogenicity, non-thrombogenicity, biodegradability, and hygroscopicity. These characteristics permit their use in a wide range of applications, including biomedical, cosmetic, and food industries [40].

However, important properties of HA such as high water solubility at room temperature and very rapid rates of turnover and elimination can also be a barrier for its application as a scaffold for the lack of integrity of structure. To eliminate these hindrances, techniques of crosslinking and HA modifications were developed [39-42]. The properties of HA are greatly enhanced with crosslinking, which produces a hydrogel. This facilitates formation of desired molecular orientations and shape, as well as techniques that allow delivery of therapeutic molecules directly into the host [43, 44]. HA can be cross-linked by covalently linking thiols (Extracel, HyStem), methacrylates and with tyramines (Corgel). Crosslinking of HA can be performed with formaldehyde (Hylan-A) or divinylsulfone (Hylan-B). HA biopolymers can be exposed to chemical modifications to all its functional groups (the carboxylacidic, hydroxyl and the *N*-acetyl groups) [2, 41]. Currently, water-soluble carbodiimide-, polyvalent hydrazide-, divinyl sulfone-, disulfide-, glutaraldehyde-, auto- and photo-crosslinking, crosslinking with polyfunctional epoxides of hydrogels, have been developed [2, 45, 46]. Such chemical crosslinking of HA inhibits degradation of the polymer *in vivo* and allows prolonged structural stability.

Currently, the most widespread HA derived products on the market are benzyl esters of HA [45]. Burdick and Prestwich [2] have reviewed the preparation of thiol-, haloacetate-, hydrazide-, aldehyde-, and tyramine-modified HA and their applications in cartilage tissue engineering, cardiac repair, engineering of cardiac valves, molecule delivery, control of stem cells behavior. Moreover, hydrogels derived from HA may serve as biological activators to cells, whereas changes in cellular behavior are observed, including differentiation of stem cells.

Cross-linked HA polymers as well as HA-ester derivatives are anticipated to become available for clinical application in dermatology [47-49]. Reitinger and

Lepperdinger [50] report the development of a new composite biomaterial based on HA-hydrogel containing cross-linked fibronectin. A well-established system is thiolated HA (*e.g.* Extracel™, Glycosan BioSystems, Inc.). Köwitsch *et al.* [51] have examined the bioactivity of HA derivatives such as aldehyde-HA and thiol-HA immobilized on model substrata such as amino-terminated surfaces or on gold. The major drug delivery applications of HA, both biomedical applications and HA-based treatments are summarized in Table **3** [18].

VISCOSEPARATION

Cross-linked HA at various concentrations has applications in a number of fields of medicine [21, 39, 41]. Modified HA-based solutions, *e.g.* Seprafilm Adhesion Barrier (Genzyme Corp.), are routinely applied to minimize postsurgical adhesion formation as an anti-adhesion material. Concerning to the design of cell non-adhesive surfaces, the mechanism of cell and surface interaction should be considered. In case of adhering cells to the surface of a material, several physico-chemical reactions occur between the cells and the material interface. Immediately after implanting a biomaterial into an organism or into contact with cell culture environments, protein adsorption to its surface occurs, which mediates the cell adhesion and also provides signals to the cell through the cell adhesion receptors, mainly integrins [52]. Modifications of HA structure lead to lowered water solubility. Such materials thus become more stable in a physiological environment [53].

VISCOSUPPLEMENTATION

Osteoarthritis is a chronic, degenerative disease that commonly afflicts tissues such as joints, cartilages, bones, synovium, ligaments and muscle [54, 55]. The concentration of HA in the SF of the normal human articular joint is 2.5-4.0 mg/mL. Under pathological conditions, the concentration of HA can fall precipitously to values below 1.2 mg/mL. The molar mass of HA is also reduced resulting in solutions that are markedly decreased in elasticity and viscosity.

Intra-articular applications of sterile HA solutions, termed viscosupplementation, provide analgesic, anabolic, chondro-protective and anti-inflammatory effects that diminish pain and disability and thus enhance function of joints, decrease cartilage degradation, and promote cartilage matrix biosynthesis. The use of viscosupplementation for other joints, such as shoulder, hip, and ankle that are currently under investigation, has introduced the term "visco-induction", indicating that clinical efficacy can be long-term observed (several months), even though the mean-residence time for such intra-articularly introduced HA being only a few days [54-63].

Japan and Italy were the first countries, where viscosupplementation was applied in clinical practice (1980s), followed by Canada, Europe and the USA (1990s) [24]. Commercially available products for viscosupplementation contain sodium HA of different molar masses. Synvisc®, also known as Hylan G-F 20 (Genzyme Corp, Cambridge, MA, USA), is a preparation containing cross-linked HA (Hylan-B), while Hyalgan® (Fidia, Abano Terme, Italy) and Artz Dispo (Seikagaku, Tokyo, Japan) are the products containing HA of molar mass of 1 MDa. Other products are Monovisc (biscarbodiimide XL, Anika) and Gel-One (cinnamate XL, Seikagaku).

The proposed mechanism underlying the therapeutic effects of intra-articularly introduced HA derivatives on symptomatic osteoarthritic joints are as follows: 1)

Table 3. Summary of the drug delivery applications of HA [18].

Route	Efficacy	Therapeutic agents
Ophthalmic	Elevated ocular persistence of drug, which can lead to increased bioavailability	Pilocarpine, tropicamide, timolol, gentimicin, tobramicin, arecaidine polyester, (S)-aceclidine
Nasal	Bioadhesion resulting in increased bioavailability	Xylometazoline, vasopressin, gentamicin
Pulmonary	Absorption enhancer and dissolution rate modification	Insulin
Parenteral	Drug carrier and facilitator of liposomal entrapment	Taxol, superoxide dismutase, human recombinant insulin-like growth factor, doxorubicin
Implant	Dissolution rate modification	Insulin
Gene	Dissolution rate modification and protection	Plasmid DNA/monoclonal antibodies

maintenance of elasticity and viscous properties of the SF, 2) anti-inflammatory and anti-nociceptive effects and 3) normalization of HA synthesis by synoviocytes. The material acceptable for visco-supplementation should fulfill the following criteria: 1) tissue and blood compatibility, 2) permeability to low- and high-molar-mass substances, 3) rheological properties similar to those of normal SF and 4) slowing elimination rates in order to maintain extended protection [54, 60].

VISCOSURGERY

Ophthalmology. In the eye, HA is present in lacrimal glands, the vitreous body, human tear fluid, corneal epithelium and also in conjunctiva [24]. HA is used as a viscoelastic gel to support healing and regeneration of surgical wounds, to prevent damage of the endothelial layer of the cornea, to function as a lubricant, to eliminate physical damage of other tissues, to maintain operative space and depth of the anterior chamber. This is particularly important in anterior segment surgery, glaucoma surgery, and during corneal transplantation [42]. During implant surgery, when the intraocular pressure is high, HA reduces this pressure. Furthermore, HA increases corneal humidity due to elevated water retention on the corneal surface. Also, HA is used to treat syndrome of dry eye [24, 63].

Ocular tissue deterioration can occur on a heredity basis or as a result of other pathophysiological processes. Currently increased attention is being paid to diabetic cataracts because of the rapid worldwide increase of diabetes mellitus. Healon® (sodium hyaluronate, a former product of the Swedish company Pharmacia) was for a long time the only available material for visco-surgical procedures. Today, similar products are used world-wide in surgery as a soft instrument to remove cataract, glaucoma surgery, for posterior segment surgery, intraocular lens implantation and keratoplasty. Furthermore, it can be used for repositioning and unrolling of the retina following detachment, lysis of anterior synechiae and for separation of tissues and adhesions mechanically. The use of HA with contact lenses in several types of applications have been reported in several studies [24]. Viscoat® by Cilco (USA) is a product applied for extraction of cataract and intraocular lens implantation and it is produced as a combination of sodium HA and sodium chondroitin sulphate. Hyalistil®, a 0.2% HA solution produced by Sifi (Italy), is used to stabilize the tear film, to hydrate and lubricate the cornea and is desirable in increasing the comfort when applying contact lenses. Blink Contacts, eye drops produced by AMO (USA), are for users of contact lenses containing 0.15% HA [46, 64].

VISCO-AUGMENTATION

Otolaryngology. Properties of HA such as osmosis, viscoelasticity and space-filling ability are critical for voice production. HA directly affects the thickness and viscosity of vocal folds. Moreover, HA derivatives can be applied in visco-augmentation of vocal cords, for the treatment of glottal insufficiency and the repair of injured or scarred vocal cords. A short residence time of HA is however a main disadvantage of applying HA as a lamina propria bioimplant for the treatment of vocal fold disorders. The HA mean-residence-time in the rabbit vocal fold is only 3-5 days. The solution is to modify the molecular structure of HA to prolong the material residence time [40]. One of the simplest hydrogels used for vocal fold augmentation is the divinylsulfone cross-linked HA derivative Hylan-B which, in animal models, is found to be anti-inflammatory, non-antigenic and non-toxic.

To date, Hylan-B and its derivatives serve as a space filling hydrogel, whereas their only possible application is by injection. Several clinical studies involving rheological studies and animal models support the mechanism of HA derivatives to be beneficial to replace a lamina propria for vocal fold scars and sulci [65, 66].

COSMETIC APPLICATIONS

About 50% of the total HA in humans occurs in skin [67]. HA therefore is a reasonable matrix component for enhancing dermal regeneration and augmentation [68]. The excellent moisturizing properties of HA are the basis of its inclusion in skin-care products [24, 40, 47].

Materials for deep augmentation injection into skin are temporary and permanent/long-term. Long-term or permanent injectable materials include calcium hydroxyapatite, autologous fat, (Radiesse™, Merz Pharma GmbH, Germany), polydimethylsiloxane (or particulate silicone), and polytef paste (Teflon™). Among temporary injection materials rank collagen-based products (Cymetra™, Zyplast™, Cosmoplast/Cosmoderm™) bovine gelatin (Gelfoam™, Surgifoam™), and 1-carboxymethylcellulose (Radiesse Voice Gel™, Merz Aestetics, Germany). These materials differ in the endurance of integration, in their biocompatibility and specific viscoelastic properties. The injectable materials based on HA include Hylaform® (Genzyme

Corp., MA, USA) and Restylane™ (Q-med, Sweden) [45, 65].

VISCOPROTECTION

Wound healing. HA stimulates wound healing as can be demonstrated for tendon, bone, corneal, diabetic foot, nasal mucosal and venous leg ulcers [21]. HA participates in several stages of wound healing [69]. In an early stage, high-molar-mass HA is bound to fibrinogen during clot formation. In the adult, wound healing frequently results in scar formation. However, in fetal wounds, scar formation is inhibited by the presence of high-molar-mass HA [70]. This was demonstrated in experimental fetal rabbit and fetal sheep models as well as in term infants following mid-gestation *in utero* surgery [71].

In 2011, Cutting [72] reported that in spite of positive effects of several HA-containing products on wound healing, some adverse effects were also expressed. This may be explained by possible wound infections (empirical observations) associated with application of some HA-based dressings. For this reason, the current author [72] reports application of a newly developed wound preparation that includes HA associated with an antimicrobial iodine-containing compound. This is a novel and innovative solution that has the potential to provide improved clinical outcomes for complex wound-healing situations. Fragmentation of high-molar-mass HA occurs naturally within the wound. This leads to altered properties of the healing material that affects the potential benefits of topical applications. One option, in order to overcome this problem is esterification of the HA moieties. The product thus becomes more resistant to its degradation by hyaluronidases. However, such esterification increases the hydrophobicity of the HA-based material.

Another approach also uses the aforementioned HA-iodine complex. Contipro Pharma Inc. (Czech Republic) produces Hyiodine®, a wound-healing product for a wide range of chronic and acute wounds. Its efficacy is based on the synergistic effect of HA and iodine [73].

Combinations of HA with other biomaterials for particular wound healing situations have been studied in the past few years. HA conjugated with gelatin or collagen in the presence or absence of growth factors such as EGF (epidermal growth factor) or bFGF (basic fibroblast growth factor) showed promising results in experimental wound-healing models [74-81]. The antimicrobial HA-based wound dressings based on HA are formulated combining HA with antibiotics, nano-silver or mixed with antimicrobial polymers such as chitosan [82-85].

Hunt and Grover [86] report on the application of biopolymer gel encapsulation in regenerative medicine. Properties and applications of biopolymer gels based on alginate, fibrin, collagen, agarose, gelatin and HA are described. Table **4** summarizes some of the functions of HA in the several steps of wound healing [69].

HYALURONAN LABELING FOR DIAGNOSTIC PURPOSES

Lindqvist *et al.* [87] developed a HA-loading test for assessment of HA kinetics to use it in patients with liver and joint diseases. The test describes the metabolism of HA but cannot define the specific contribution of different organs. A method for labelling of HA with the short-lived positron-emitting radionuclide ^{11}C was applied in healthy individuals and in patients suffering from liver diseases. The finding is that positron electron topography with [^{11}C] HA may be an accurate method to assess liver dysfunction, when endothelial cell function is impaired.

Near-infrared (NIR) fluorescence was used for functional lymphatic imaging in the abdomen and anterior hindlimb of anesthetized, intact Yorkshire swine [88]. The results show the capability to image the immediate trafficking of indocyanine green from the plexus, through the vessels and lymphangions, and to the superficial mammary, sub iliac, and middle iliac lymph nodes, which were located as deep as 3 cm beneath the tissue surface. The results suggest that microgram quantities of NIR optical imaging agents and their conjugates have a potential to image lymph function in patients suffering from lymph-related disorders.

Jadhav *et al.* [89] synthesized a macrocyclic ^{68}Ga-NOTA-chelated oligonucleotide-HA conjugates using a solid supported technique to introduce NOTA-chelator (NOTA - 1,4,7-triazacyclononane-*N,N′,N″*-triacetic acid) into the 3′-terminus and a copper-free strain promoted azide alkyne cycloaddition to HA/oligonucleotide conjugation. As a method positron emission tomography was used in healthy rats to monitor distribution kinetic studies and a potential of HA-induced targeting of oligonucleotides into rats afflicted with myocardial infarction was determined.

DRUG DELIVERY

A number of studies report that HA facilitates the prolonged residence and the increased bioavailability of certain drug molecules. Typical examples are pilocarpine and vasopressin. HA of higher size rather than

Table 4. Functions of HA during wound healing [69].

Phases	Effects
Inflammatory phase	Binding to fibrinogen to initiate clotting pathway
	Ensuring inflammatory cell migration
	Creating edema to allow cell infiltration
	Inhibiting neutrophil migration to dampen inflammatory response
Proliferative phase	Accumulating of fibroblasts to wound site
	Filling in gaps of newly formed extracellular matrix, creating cushioning and structural organization
	Stimulating matrix metaloproteinases for angiogenesis
	Promoting keratinocyte migration and proliferation
Remodeling phase	Contribution to normal and pathological scarring

Table 5. Practical applications of visco-elastic solutions of HA or its gels [18, 40].

Application	Treatment
Viscosupplementation	To replace or supplement tissue fluids, such as replacement of SF in painful arthritis, and to relieve pain
Viscosurgery	To protect delicate tissues and provide space during surgical manipulations, as in ophthalmological surgeries
Viscoaugmentation	To fill and augment tissue spaces, as in skin, sphincter muscles, vocal and pharyngeal tissues
Viscoseparation	To separate connective tissue surfaces traumatized by surgical procedures or injury, in order to prevent adhesions and excessive scar formation
Viscoprotection	To protect healthy, wounded, or injured tissue surfaces from dryness or noxious environmental agents, and to promote the healing of such surfaces
Drug delivery	Matrix and tissue engineering

the low-molar-mass HA species supports increased drug bioavailability. HA as a non-immunogenic substance is used in parental/pulmonary drug delivery systems to allow constant release and longer retention of the therapeutic agents. For example, the anti-cancer drug, taxol, when linked to HA was observed to target breast cancer, human colon and ovarian cell lines *in vitro* selectively. Furthermore, HA was used to discover an implantable delivery system for long-term delivery of anti-inflammatory and antibiotic drugs *in vivo* [21].

HA can be also included in nasal, ophthalmic and parenteral drug delivery. Novel applications, including pulmonary implantation and gene delivery, are summarized in Table **5** [18, 40]. Kogan *et al.* [40] reported that HA can be either directly conjugated to drugs or used for preparation of microcapsules to enhance drug delivery. HA is also involved in improving biocompatibility of chitosan microspheres, which function as capsules for drug delivery. It is apparent that HA has multiple uses, both direct and indirect, in the pharmaceutical industry.

CONCLUSION

New insights into mammalian longevity and malignancy were discovered recently in research on the naked mole rat. These observations can play key roles in future treatment of human cancer. They will also facilitate approaches to improve quality of life while extending human longevity. The new treatment modalities and therapeutic applications will involve the production of high-molar-mass HAs. These considerations prompted the current overview, anticipating opportunities for new techniques for preparation and isolation of HA, for modifications of structure that improve availability and resistance to degradation. Such materials will stimulate many new investigations.

In the future, more attention will be devoted to enzymatic production of HA. A huge number of modifications of HA can be performed with the aim of en-

hancing the quality of the material for medicine, pharmaceuticals, cosmetics as well as for industrial production. We are only now beginning to appreciate the large numbers of applications for this versatile molecule as well as the innumerable modifications possible with this multifaceted, highly ionic polymer.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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REFERENCES

[1] Menaa, T.; Menaa, A.; Menaa, B. Hyaluronic acid and derivatives for tissue engineering. *J. Biotechnol. Biomater.,* **2011**, *51*, 17.

[2] Burdick, J.A.; Prestwich, G.D. Hyaluronic acid hydrogels for biomedical applications. *Adv. Mater.,* **2011**, *23*(12), H41-H56.

[3] Lee, J.Y.; Spicer, A.P. Hyaluronan: a multifunctional, megaDalton, stealth molecule. *Curr. Opin. Cell Biol.,* **2000**, *12*(5), 581-586.

[4] Davies, A.; Gormally, J.; Wyn-Jones, E.; Wedlock, D.J.; Phillips, G.O. A study of factors influencing hydration of sodium hyaluronate from compressibility and high-precision densimetric measurements. *Biochem. J.,* **1983**, *213*(2), 363-369.

[5] Almond, A.; Sheehan, J.K. Predicting the molecular shape of polysaccharides from dynamic interactions with water. *Glycobiology,* **2003**, *13*(4), 255-264.

[6] Granger, H.J.; Laine, G.A.; Barnes, G.E.; Lewis, R.E. Cardiovascular responses to elevation of intra-abdominal hydrostatic pressure. *Am. J. Physiol.,* **1985**, *248,* R208-13.

[7] Stern, R.; Jedrzejas, M.J. Hyaluronidases: their genomics, structures, and mechanisms of action. *Chem. Rev.,* **2006**, *106*(3), 818-839.

[8] Stern, R. Devising a pathway for hyaluronan catabolism: are we there yet? *Glycobiology,* **2003**, *13*(12), 105R-115R.

[9] Stern, R. Hyaluronan catabolism: a new metabolic pathway. *Eur. J. Cell Biol.,* **2004**, *83*(7), 317-325.

[10] Cowman, M.K.; Matsuoka, S. Experimental approaches to hyaluronan structure. *Carbohydr. Res.,* **2005**, *340*(5), 791-809.

[11] Meyer, K.; Palmer, J. The polysaccharide of the vitreous humor. *J. Biol. Chem.,* **1935**, *107*, 629-634.

[12] Csoka, A.B.; Stern, R. Hypotheses on the evolution of hyaluronan: a highly ironic acid. *Glycobiology,* **2013**, *23*(4), 398-411.

[13] Margolis, R.U.; Margolis, R.K.; Chang, L.B.; Preti, C. Glycosaminoglycans of brain during development. *Biochemistry,* **1975**, *14*(1), 85-88.

[14] Jenkins, H.G.; Bachelard, H.S. Developmental and age-related changes in rat brain glycosaminoglycans. *J. Neurochem.,* **1988**, *51*(5), 1634-1640.

[15] Fraser, J.R.; Laurent, T.C.; Laurent, U.B. Hyaluronan: its nature, distribution, functions and turnover. *J. Intern. Med.,* **1997**, *242*(1), 27-33.

[16] Price, R.D.; Berry, M.G.; Navsaria, H.A. Hyaluronic acid: the scientific and clinical evidence. *J. Plast. Reconstr. Aesthet. Surg.,* **2007**, *60*(10), 1110-1119.

[17] Hascall, V.C.; Majors, A.K.; De La Motte, C.A. Intracellular hyaluronan: A new frontier for inflammation? *Biochim. Biophys. Acta.,* **2004**, *1673*, 3-12.

[18] Tamer, T.M. *Engineering of Polymer and Chemical Complexity*; Frocke, W.W., Ed.; Apple Academic Press: Watertown, **2014**, pp. 109-143.

[19] Turley, E.A. Hyaluronan and cell locomotion. *Cancer Metastasis Rev.,* **1992**, *11*(1), 21-30.

[20] Turley, E.A.; Noble, P.W.; Bourguignon, L.Y. Signaling properties of hyaluronan receptors. *J. Biol. Chem.,* **2002**, *277*(7), 4589-4592.

[21] Girish, K.S.; Kemparaju, K. The magic glue hyaluronan and its eraser hyaluronidase: a biological overview. *Life Sci.,* **2007**, *80*(21), 1921-1943.

[22] Papakonstantinou, E.; Roth, M.; Karakiulakis, G. Hyaluronic acid: A key molecule in skin aging. *Dermatoendocrinol,* **2012**, *4*(3), 253-258.

[23] Erickson, M.; Stern, R. Chain gangs: New aspects of hyaluronan metabolism. *Int. Biochem. Res.,* **2012**, *2012*, 19.

[24] Necas, J.; Bartosikova, L.; Brauner, P. Hyaluronic acid (hyaluronan): A review. *Vet. Med.,* **2008**, *53*, 397-411.

[25] Jiang, D.; Liang, J.; Noble, P.W. Hyaluronan in tissue injury and repair. *Annu. Rev. Cell Dev. Biol.,* **2007**, *23*, 435-461.

[26] Jiang, D.; Liang, J.; Noble, P.W. Hyaluronan as an immune regulator in human diseases. *Physiol. Rev.,* **2011**, *91*(1), 221-264.

[27] Itano, N.; Kimata, K. Altered hyaluronan biosynthesis in cancer progression. *Semin. Cancer Biol.,* **2008**, *18*(4), 268-274.

[28] Itano, N.; Zhuo, L.; Kimata, K. Impact of the hyaluronan-rich tumor microenvironment on cancer initiation and progression. *Cancer Sci.,* **2008**, *99*(9), 1720-1725.

[29] Zhang, L.; Underhill, C.B.; Chen, L. Hyaluronan on the surface of tumor cells is correlated with metastatic behavior. *Cancer Res.,* **1995**, *55*(2), 428-433.

[30] Soltés, L.; Kogan, G.; Stankovská, M.; Mendichi, R.; Rychlý, J.; Schiller, J.; Gemeiner, P. Degradation of high-molar-mass hyaluronan and characterization of fragments. *Biomacromolecules,* **2007**, *8*(9), 2697-2705.

[31] Xu, H.; Ito, T.; Tawada, A.; Maeda, H.; Yamanokuchi, H.; Isahara, K.; Yoshida, K.; Uchiyama, Y.; Asari, A. Effect of hyaluronan oligosaccharides on the expression of heat shock protein 72. *J. Biol. Chem.,* **2002**, *277*(19), 17308-17314.

[32] Schwertfeger, K.L.; Cowman, M.K.; Telmer, P.G.; Turley, E.A.; McCarthy, J.B. Hyaluronan, inflammation, and breast cancer progression. *Front. Immunol.,* **2015**, *6*, 236.

[33] Ghatak, S.; Misra, S.; Toole, B.P. Hyaluronan oligosaccharides inhibit anchorage-independent growth of tumor cells by suppressing the phosphoinositide 3-kinase/Akt cell survival pathway. *J. Biol. Chem.,* **2002**, *277*(41), 38013-38020.

[34] Urakawa, H.; Nishida, Y.; Knudson, W.; Knudson, C.B.; Arai, E.; Kozawa, E.; Futamura, N.; Wasa, J.; Ishiguro, N. Therapeutic potential of hyaluronan oligosaccharides for bone metastasis of breast cancer. *J. Orthop. Res.,* **2012**, *30*(4), 662-672.

[35] Stern, R.; Asari, A.R.; Sugahara, K.N. Size- specific fragments of hyaluronan: An information-rich system. *Eur. J. Cell Biol.,* **2006**, *85*, 699-715.

[36] *http://quotesgram.com/mole-rat-quotes-quotes/,*

[37] Tian, X.; Azpurua, J.; Hine, C.; Vaidya, A.; Myakishev-Rempel, M.; Ablaeva, J.; Mao, Z.; Nevo, E.; Gorbunova, V.; Seluanov, A. High-molecular-mass hyaluronan mediates

the cancer resistance of the naked mole rat. *Nature,* **2013**, *499*(7458), 346-349.

[38] Hill, D.R.; Rho, H.K.; Kessler, S.P.; Amin, R.; Homer, C.R.; McDonald, C.; Cowman, M.K.; de la Motte, C.A. Human milk hyaluronan enhances innate defense of the intestinal epithelium. *J. Biol. Chem.,* **2013**, *288*(40), 29090-29104.

[39] Collins, M.N.; Birkinshaw, C. Hyaluronic acid based scaffolds for tissue engineering--a review. *Carbohydr. Polym.,* **2013**, *92*(2), 1262-1279.

[40] Kogan, G.; Soltés, L.; Stern, R.; Gemeiner, P. Hyaluronic acid: a natural biopolymer with a broad range of biomedical and industrial applications. *Biotechnol. Lett.,* **2007**, *29*(1), 17-25.

[41] Fakhari, A.; Berkland, C. Applications and emerging trends of hyaluronic acid in tissue engineering, as a dermal filler and in osteoarthritis treatment. *Acta Biomater.,* **2013**, *9*(7), 7081-7092.

[42] Volpi, N.; Schiller, J.; Stern, R.; Soltés, L. Role, metabolism, chemical modifications and applications of hyaluronan. *Curr. Med. Chem.,* **2009**, *16*(14), 1718-1745.

[43] Krüger, J.P.; Ketzmar, A.K.; Endres, M.; Pruss, A.; Siclari, A.; Kaps, C. Human platelet-rich plasma induces chondrogenic differentiation of subchondral progenitor cells in polyglycolic acid-hyaluronan scaffolds. *J. Biomed. Mater. Res. B Appl. Biomater.,* **2014**, *102*(4), 681-692.

[44] Mathews, S.; Bhonde, R.; Gupta, P.K.; Totey, S. Novel biomimetic tripolymer scaffolds consisting of chitosan, collagen type 1, and hyaluronic acid for bone marrow-derived human mesenchymal stem cells-based bone tissue engineering. *J. Biomed. Mater. Res. B Appl. Biomater.,* **2014**, *102*(8), 1825-1834.

[45] Allison, D.D.; Grande-Allen, K.J. Review. Hyaluronan: a powerful tissue engineering tool. *Tissue Eng.,* **2006**, *12*(8), 2131-2140.

[46] Prestwich, G.D.; Kuo, J.W. Chemically-modified HA for therapy and regenerative medicine. *Curr. Pharm. Biotechnol.,* **2008**, *9*(4), 242-245.

[47] Stern, R.; Maibach, H.I. Hyaluronan in skin: aspects of aging and its pharmacologic modulation. *Clin. Dermatol.,* **2008**, *26*(2), 106-122.

[48] Mineo, A.; Suzuki, R.; Kuroyanagi, Y. Development of an artificial dermis composed of hyaluronic acid and collagen. *J. Biomater. Sci. Polym. Ed.,* **2013**, *24*(6), 726-740.

[49] Sawa, M.; Kuroyanagi, Y. Potential of a cryopreserved cultured dermal substitute composed of hyaluronic acid and collagen to release angiogenic cytokine. *J. Biomater. Sci. Polym. Ed.,* **2013**, *24*(2), 224-238.

[50] Reitinger, S.; Lepperdinger, G. Hyaluronan, a ready choice to fuel regeneration: a mini-review. *Gerontology,* **2013**, *59*(1), 71-76.

[51] Köwitsch, A.; Yang, Y.; Ma, N.; Kuntsche, J.; Mäder, K.; Groth, T. Bioactivity of immobilized hyaluronic acid derivatives regarding protein adsorption and cell adhesion. *Biotechnol. Appl. Biochem.,* **2011**, *58*(5), 376-389.

[52] Liha, E.; Ohb, S.H.; Jounga, Y.K.; Lee, J.H.; Han, D.K. Polymers for cell/tissue anti-adhesion. *Prog. Polym. Sci.,* **2015**, *44*, 28-61.

[53] Diamond, M.P.; Burns, E.L.; Accomando, B.; Mian, S.; Holmdahl, L. Seprafilm(®) adhesion barrier: (2) a review of the clinical literature on intraabdominal use. *Gynecol. Surg.,* **2012**, *9*(3), 247-257.

[54] McArthur, B.A.; Dy, C.J.; Fabricant, P.D.; Valle, A.G. Long term safety, efficacy, and patient acceptability of hyaluronic acid injection in patients with painful osteoarthritis of the knee. *Patient Prefer. Adherence,* **2012**, *6*, 905-910.

[55] Gigante, A.; Callegari, L. The role of intra-articular hyaluronan (Sinovial) in the treatment of osteoarthritis. *Rheumatol. Int.,* **2011**, *31*(4), 427-444.

[56] Soltes, L.; Mendichi, R.; Kogan, G.; Mach, M. Associating hyaluronan derivatives: a novel horizon in viscosupplementation of osteoarthritic joints. *Chem. Biodivers.,* **2004**, *1*(3), 468-472.

[57] Soltes, L.; Steiner, B.; Machova, E.; Kogan, G.; Bystricky, S.; Mendichi, R.; Bauer, V.; Mach, M.; Alfoldi, J.; Stratilova, E. Clathrate complexes formed by hyaluronic acid derivatives and use tehreof as pharmaceuticals. Australian Patent, 2001252180, 2005. B1 European Patent, EP 1272530, 2006.7563824 B2 US Patent, 2009. Canadian Patent, CA 2402421 2009.

[58] Rodell, C.B.; Kaminski, A.L.; Burdick, J.A. Rational design of network properties in guest-host assembled and shear-thinning hyaluronic acid hydrogels. *Biomacromolecules,* **2013**, *14*(11), 4125-4134.

[59] Strauss, E.J.; Hart, J.A.; Miller, M.D.; Altman, R.D.; Rosen, J.E. Hyaluronic acid viscosupplementation and osteoarthritis: current uses and future directions. *Am. J. Sports Med.,* **2009**, *37*(8), 1636-1644.

[60] Van Den Bekerom, M.P.; Mylle, G.; Rys, B.; Mulier, M. Viscosupplementation in symptomatic severe hip osteoarthritis: a review of the literature and report on 60 patients. *Acta Orthop. Belg.,* **2006**, *72*(5), 560-568.

[61] Volpi, N.; Schiller, J.; Stern, R.; Soltés, L. Role, metabolism, chemical modifications and applications of hyaluronan. *Curr. Med. Chem.,* **2009**, *16*(14), 1718-1745.

[62] Migliore, A.; Giovannangeli, F.; Bizzi, E.; Massafra, U.; Alimonti, A.; Laganà, B.; Diamanti Picchianti, A.; Germano, V.; Granata, M.; Piscitelli, P. Viscosupplementation in the management of ankle osteoarthritis: a review. *Arch. Orthop. Trauma Surg.,* **2011**, *131*(1), 139-147.

[63] Schiller, J.; Volpi, N.; Hrabárová, E. Hyaluronic acid: a natural biopolymer. In: *Biopolymers: Biomedical and Environmental Applications* Scrivener, S.; Eds.; *Publishing,* **2008**, LLC, 334.

[64] Williams, D.L.; Mann, B.K. Efficacy of a crosslinked hyaluronic acid-based hydrogel as a tear film supplement: a masked controlled study. *PLoS One,* **2014**, *9*(6), e99766.

[65] Gaston, J.; Thibeault, S.L. Hyaluronic acid hydrogels for vocal fold wound healing. *Biomatter,* **2013**, *3*(1), 17.

[66] Mallur, P.S.; Rosen, C.A. Vocal fold injection: review of indications, techniques, and materials for augmentation. *Clin. Exp. Otorhinolaryngol.,* **2010**, *3*(4), 177-182.

[67] Reed, R.K.; Lilja, K.; Laurent, T.C. Hyaluronan in the rat with special reference to the skin. *Acta Physiol. Scand.,* **1988**, *134*(3), 405-411.

[68] Meyer, L.J.; Stern, R. Age-dependent changes of hyaluronan in human skin. *J. Invest. Dermatol.,* **1994**, *102*(3), 385-389.

[69] Frenkel, J.S. The role of hyaluronan in wound healing. *Int. Wound. J.,* **2013**, *2012*, 17.

[70] Longaker, M.T.; Chiu, E.S.; Adzick, N.S.; Stern, M.; Harrison, M.R.; Stern, R. Studies in fetal wound healing. V. A prolonged presence of hyaluronic acid characterizes fetal wound fluid. *Ann. Surg.,* **1991**, *213*(4), 292-296.

[71] Longaker, M.T.; Whitby, D.J.; Adzick, N.S.; Crombleholme, T.M.; Langer, J.C.; Duncan, B.W.; Bradley, S.M.; Stern, R.; Ferguson, M.W.; Harrison, M.R. Studies in fetal wound healing, VI. Second and early third trimester fetal wounds demonstrate rapid collagen deposition without scar formation. *J. Pediatr. Surg.,* **1990**, *25*(1), 63-68.

[72] Cutting, K.F. Wound healing through synergy of hyaluronan and an iodine complex. *J. Wound Care,* **2011**, *20*(9), 424-430, 426, 428-430.

[73] Hyiodine®. http://www.contipro.com/woundhealing/ healing-products, **2013**.

[74] Cai, S.; Liu, Y.; Zheng Shu, X.; Prestwich, G.D. Injectable glycosaminoglycan hydrogels for controlled release of human basic fibroblast growth factor. *Biomaterials,* **2005**, *26*(30), 6054-6067.

[75] Liu, Y.; Cai, S.; Shu, X.Z.; Shelby, J.; Prestwich, G.D. Release of basic fibroblast growth factor from a crosslinked glycosaminoglycan hydrogel promotes wound healing. *Wound Repair Regen.,* **2007**, *15*(2), 245-251.

[76] Matsumoto, Y.; Arai, K.; Momose, H.; Kuroyanagi, Y. Development of a wound dressing composed of a hyaluronic acid sponge containing arginine. *J. Biomater. Sci. Polym. Ed.,* **2009**, *20*(7-8), 993-1004.

[77] Matsumoto, Y.; Kuroyanagi, Y. Development of a wound dressing composed of hyaluronic acid sponge containing arginine and epidermal growth factor. *J. Biomater. Sci. Polym. Ed.,* **2010**, *21*(6-7), 715-726.

[78] Kondo, S.; Niiyama, H.; Yu, A.; Kuroyanagi, Y. Evaluation of a wound dressing composed of hyaluronic acid and collagen sponge containing epidermal growth factor in diabetic mice. *J. Biomater. Sci. Polym. Ed.,* **2011**, 3.

[79] Kondo, S.; Kuroyanagi, Y. Development of a wound dressing composed of hyaluronic acid and collagen sponge with epidermal growth factor. *J. Biomater. Sci. Polym. Ed.,* **2012**, *23*(5), 629-643.

[80] Yamamoto, A.; Shimizu, N.; Kuroyanagi, Y. Potential of wound dressing composed of hyaluronic acid containing epidermal growth factor to enhance cytokine production by fibroblasts. *J. Artif. Organs,* **2013**, *16*(4), 489-494.

[81] Yu, A.; Niiyama, H.; Kondo, S.; Yamamoto, A.; Suzuki, R.; Kuroyanagi, Y. Wound dressing composed of hyaluronic acid and collagen containing EGF or bFGF: comparative culture study. *J. Biomater. Sci. Polym. Ed.,* **2013**, *24*(8), 1015-1026.

[82] Correia, C.R.; Moreira-Teixeira, L.S.; Moroni, L.; Reis, R.L.; van Blitterswijk, C.A.; Karperien, M.; Mano, J.F. Chitosan scaffolds containing hyaluronic acid for cartilage tissue engineering. *Tissue Eng. Part C Methods,* **2011**, *17*(7), 717-730.

[83] Lu, H.D.; Zhao, H.Q.; Wang, K.; Lv, L.L. Novel hyaluronic acid-chitosan nanoparticles as non-viral gene delivery vectors targeting osteoarthritis. *Int. J. Pharm.,* **2011**, *420*(2), 358-365.

[84] Mathews, S.; Bhonde, R.; Gupta, P.K.; Totey, S. A novel tripolymer coating demonstrating the synergistic effect of chitosan, collagen type 1 and hyaluronic acid on osteogenic differentiation of human bone marrow derived mesenchymal stem cells. *Biochem. Biophys. Res. Commun.,* **2011**, *414*(1), 270-276.

[85] Park, H.; Choi, B.; Hu, J.; Lee, M. Injectable chitosan hyaluronic acid hydrogels for cartilage tissue engineering. *Acta Biomater.,* **2013**, *9*(1), 4779-4786.

[86] Hunt, N.C.; Grover, L.M. Cell encapsulation using biopolymer gels for regenerative medicine. *Biotechnol. Lett.,* **2010**, *32*(6), 733-742.

[87] Lindqvist, U.; Westerberg, G.; Bergström, M.; Torsteindottir, I.; Gustafson, S.; Sundin, A.; Lööf, L.; Långström, B. [11C]Hyaluronan uptake with positron emission tomography in liver disease. *Eur. J. Clin. Invest.,* **2000**, *30*(7), 600-607.

[88] Sharma, R.; Wang, W.; Rasmussen, J.C.; Joshi, A.; Houston, J.P.; Adams, K.E.; Cameron, A.; Ke, S.; Kwon, S.; Mawad, M.E.; Sevick-Muraca, E.M. Quantitative imaging of lymph function. *Am. J. Physiol. Heart Circ. Physiol.,* **2007**, *292*(6), H3109-H3118.

[89] Jadhav, S.; Käkelä, M.; Mäkilä, J.; Kiugel, M.; Liljenbäck, H.; Virta, J.; Poijärvi-Virta, P.; Laitala-Leinonen, T.; Kytö, V.; Jalkanen, S.; Saraste, A.; Roivainen, A.; Lönnberg, H.; Virta, P. Synthesis and *in vivo* PET imaging of hyaluronan conjugates of oligonucleotides. *Bioconjug. Chem.,* **2016**, *27*(2), 391-403.