# Hyaluronic Acid

Subjects: Polymer Science | Biochemistry & Molecular Biology

Contributor: Petr Snetkov , Kseniia Zakharova , Svetlana Morozkina , Roman Olekhnovich , Mayya Uspenskaya

Hyaluronic acid, as a natural linear polysaccharide, has attracted researchers' attention from its initial detection and isolation from tissues in 1934 until the present day. Due to biocompatibility and a high biodegradation of hyaluronic acid, it finds wide application in bioengineering and biomedicine: from biorevitalizing skin cosmetics and endoprostheses of joint fluid to polymeric scaffolds and wound dressings. However, the main properties of aqueous polysaccharide solutions with different molecular weights are different. Moreover, the therapeutic effect of hyaluronic acid-based preparations directly depends on the molecular weight of the biopolymer. The present entry collects the information about hyaluronic acid and its original properties. Particular emphasis is placed on the structural, physical and physico-chemical properties of hyaluronic acid in water solutions, as well as their degradability.

biocompatibility degradability hyaluronic acid molecular weight structure viscosity water polymer solution

## 1. Introduction

Hyaluronic acid (HA), as a member of hyaluronan family, was first discovered by K. Meyer and John W. Palmer in 1934 [1], and nowadays continues to attract careful attention on the part of chemists, biochemists, bioengineers, and other investigators from various scientific areas. HA is an essential component of the extracellular and pericellular matrixes, and can also be found inside cells [2]. The occurrence of hyaluronic acid in tissues varies: for instance, rooster's combs contain 7.50 mg/mL, human navel cords (gelatin of Wharton)—4.10 mg/mL, human joint synovial fluid—1.50–3.60 mg/mL, vitreous humor—0.14–0.34 mg/g, human dermis and epidermis—0.20–0.50 and 0.10 mg/g, respectively [3]. The turnover of hyaluronic acid in vertebrate tissues on average is equal to 5 g per day, and is provided by biosynthesis and enzymatic degradation [4]. Meanwhile, the turnover of hyaluronic acid in the blood-flow reaches 30–100 mg per day [5].

Apart from the animal of origin, hyaluronic acid can be separated based on bacteria, for example, from Streptococcus genus (uberis, equisimilis, zooepidermicus, pyogenes, equi), Pasteurella multocida [6][7][8][9][10], and Corynebacterium glutamicum [11]; from the green algae Chlorella purposely infected by the Chlorovirus [6][7][12]; Saccharomycetes (Cryptococcus neoformans [6][7]); and from molluscan shellfish, such as the bivalve mollusc Mytilus galloprovincialis [6][13]. At the same time, hyaluronic acid has not been disclosed in fungus, insects, or plants [6][14].

Note that hyaluronic acid is usually obtained from bovine vitreous humors, rooster combs, the skin of sharks, and human umbilical cords [15]. However, animal origin hyaluronic acid contains endotoxin and protein residuals, which possess immunogenic effects [14][15]. Thus, 1 mg of hyaluronic acid from human navel cord and from bovine vitreous humor could include >100.0 EU endotoxin and approximately 47.7 and 36.2 µg protein, respectively. By contrast, hyaluronic acid from rooster comb contains 23.0 EU endotoxin and 1.0 µg protein per 1 mg of polymer [16]. At the same time, bacterial technology makes it possible to obtain high-purity hyaluronic acid with low protein and endotoxin levels [15]. Therefore, bacterially derived hyaluronic acid purchased from Sigma and Genzyme includes only 1.0–1.6 µg protein and approximately 0.02 EU endotoxin per 1 mg hyaluronic acid [16]. Nevertheless, the level of immunogenic effect of protein residuals in bacterial hyaluronic acid could be greater than in animal hyaluronic acid despite the low summary protein content [17].

It is obvious that molecular weight of hyaluronic acid depends on the source. Consequently, hyaluronic acid from animal materials has a very high molecular weight (up to 20,000 kDa). For example, rooster combs contain hyaluronic acid with 1200 kDa, the navel cords—3400 kDa, bovine vitreous humors—770–1700 kDa. By contrast, bacterial hyaluronic acid has a molecular weight between 1000 and 4000 kDa; however, the enzymatic technique makes it possible to obtain polysaccharides with a range of molecular weight between 550 kDa and 2500 kDa [18]. The molecular weight of hyaluronic acid also depends of some other conditions: for instance, in human normal synovial fluid, it is equal to 6000–7000 kDa, while in rheumatoid fluid, the molecular weight is less, and is equal to 3000–5000 kDa [19][20].

The biological effects of hyaluronic acid depend heavily on molecular weight. Hyaluronic acid with molecular weights from 0.4 to 4.0 kDa acts as an inducer of heat shock proteins, and has a non-apoptotic property. Polysaccharides with a molecular weight equal to 6–20 kDa possess immunostimulatory, angiogenic, and phlogotic activities. Hyaluronic acid with a molecular weight of 20–200 kDa takes part in biological processes such as embryonic development, wound healing and ovulation. By contrast, high molecular weight hyaluronic acid (>500 kDa) has anti-angiogenic activity, and can function as a space filler and a natural immunologic depressant [21].

The fact that the molecular weight of hyaluronic acid may vary its biological properties is the current subject of interest. The drastic difference in its functions is the reason that for medical applications, preference is given to low-polydispersity or monodisperse HA. Preparation of monodisperse hyaluronic acid is achieved by successive cycles of degradation and subsequent assembly of HA chains [22]. Additionally, the mechanisms of interaction of hyaluronic acid of various molecular weights with receptors on the cell surface are currently being actively studied. It has previously been suggested that HA of different MW may affect the same receptors differently; however, recent study refutes this theory [23]. On the other hand, it has been shown that HA of very high molecular weight (6000 kDa) produced by naked mole-rat suppresses the signaling of CD44, which results in altered expression of a subset of p53 target genes, thereby suggesting that HMWHA has the properties of a cytoprotective molecule [24], but there are differences in the genes regulated by p53 between different species so this investigation is restricted to human cells.

Evidently, the structural, physical, physicochemical and degradable properties of hyaluronic acid also depend on its molecular weight. For example, the increase in the molecular weight and concentration of hyaluronic acid in polymer solutions leads to the reinforcement of the three-dimensional network of the polymer. Consequently, it results in an increase in the solution viscosity and viscoelasticity [6]. In some cases, for example, in the electrospinning process, molecular weight, concentration, and viscosity are the key parameters providing the nanofibers obtaining [25].

There are a lot of studies dedicated to the properties of hyaluronic acid. Unfortunately, the vast majority of such papers touch upon one of several groups of polymer characteristics. Particular interest is aroused by the biological properties of materials based on hyaluronic acid. Still, for the development and technology of advanced wound healing [26], drug delivery systems with controlled release [27], and polymer scaffolds [28], knowledge of the molecular weight dependency on the abovementioned properties is necessary. This entry collects brief data on the structure, viscosity, density, surface tension, cohesive/adhesive, hydrodynamic and degradable properties of hyaluronic acid.

## 2. Aqueous Hyaluronic Acid Solutions and Their Properties

Hyaluronic acid is a linear heteropolysaccharide (glucosaminoglycan, mucopolysaccharide) with high molecular weight formed by regularly repeating residues of N-acetyl-D-glucosamine and D-glucuronic acid [1][29]. In a hyaluronic acid molecule, the D-glucuronic acid is associated with amino-sugar by β-(1 → 3)-glycosidic linking, and amino-sugar is connected with the D-glucuronic acid by a β-(1 → 4)-glycoside tieup [17].

The existence of polar and apolar segments in the polymer structure affords hyaluronic acid the capability to chemically interact with various chemical agents [17], for instance, with metachromatic dyes, which find application in clinical examinations [30], and chitosan, which makes it possible to obtain a new class of materials based on polyelectrolyte complexes [31][32].

Hyaluronic acid forms hydrogen bonds, which, on the one hand, could poise the macromolecule in solutions, but, on the other hand, give rise to rigidity in the polymer system, which, finally, specify the properties of hyaluronic acid solutions. Note that an aqueous molecule could be a bridge between the two connected functional groups [17][33]. Eventually, such primary structure and hydrogen bonds help to form secondary and tertiary structures [33][34].

Hyaluronic acid and its salt, with ammonium ions, magnesium, and alkaline metals, have good solubility in water and possess a high level of viscosity even at low polymer concentrations [17]. Moreover, hyaluronic acid in solution could organize a three-dimensional cellular structure with enormous dimensions at concentrations of less than 1 μg/mL [34]. By contrast, biopolymers can organize pseudo-gels when concentrations are equal to or above 1.0 wt.% [17][19][20]. However, hyaluronic acid with high molecular weight equal to 5.0 MDa at concentrations greater than 0.1 mg/mL forms entangled polymer networks, but hyaluronic acid solutions do not have prolonged mechanical integrity [12]. Salts of hyaluronic acid with cations possessing two and more valence numbers have substantial

insolubility in water. Additionally, if such ions are introduced into hyaluronic acid solutions, intermolecular cross-links are constituted, resulting in the development of a gel with great water content [17].

It is known that the macromolecule of hyaluronic acid in solution could organize the left-oriented individual or twin spiral [34]. Study confirms that hyaluronic acid K and Na salts demonstrate a twin helix structure in solution [35]; moreover, that helix has antiparallel left-oriented strands [17][36].

Obviously, that structure of hyaluronic acid in general specifies the other properties of biopolymer. Additional information about key hyaluronic acid properties is listed in Table 1.

Table 1. Summary of structural, physical, and physico-chemical properties of hyaluronic acid.

| Property | HA MW, kDa | Authors | Reference |
|---|---|---|---|
| *Structure* | 2 000 – 8000 | Ribitsch et al. | [37] |
| | 30 – 1700 | Cleland and Wang | [38] |
| | - | Almond et al. | [39] |
| | 100, 500, 1000, 3000, 6000 | Cowman et al. | [40] |
| | - | Lapcík et al. | [41] |
| | 1900 | Maleki et al. | [42] |
| | - | Ghosh et al. | [43] |
| | 1600, 1700, 4000 | Morris et al. | [44] |

| | | | |
|---|---|---|---|
| *Rheological properties* | | | |
| | 125, 241, 390, 598, 800, 961, 1270, 1430, 1620, 1770, 2040, 2150 | Yanaki and Yamaguchi | [45] |
| | 1000 | Scott and Heatley | [33] |
| | - | Lapcík et al. | [41] |
| | 1900 | Maleki et al. | [42] |
| | - | Ghosh et al. | [43] |
| | 1600, 1700, 4000 | Morris et al. | [44] |
| | 2000 | Rwei et al. | [46] |
| | > 1000 | Gura et al. | [47] |
| | 1500 | Pisárčik et al. | [48] |
| | 10, 100, 1000, 2000 | Kim et al. | [49] |
| | 350, 680, 1800 | Falcone et al. | [50] |
| | 1000, 2000, 3000, 4000 | Bothner and Wik | [51] |

| | | | |
|---|---|---|---|
| | | | |
| | 560, 760, 780, 1040, 1700, 1930, 1970 | Kobayashi et al. | [52] |
| | 77, 640, 1060, 2010 | Rebenda et al. | [53] |
| | 125, 241, 390, 598, 800, 961, 1270, 1430, 1620, 1770, 2040, 2150 | Yanaki and Yamaguchi | [45] |
| | 1500 | Krause et al. | [54] |
| *Surface tension* | 100, 500, 4000 | Knepper et al. | [55] |
| | 1630 | Ribeiro et al. | [56] |
| | 807, 4280, 5560 | Silver et al. | [57][58] |
| | 1000, 5000 | Nepp et al. | [59] |
| *Cohesive and adhesive properties* | 350, 680, 1800 | Falcone et al. | [50] |
| | 132, 1500, 2000 | Vorvolakos et al. | [60] |
| | - | Liao et al. | [61] |

| | | | |
|---|---|---|---|
| *Density* | 134, 620 | Saettone et al. | [62] |
| | 134, 620, 2200 | Durrani et al. | [63] |
| | 1500 | Gómez-Alejandre et al. | [64] |
| | 1430 | García-Abuín et al. | [65] |
| *Ultrasound velocity* | 10–30, 110–130, 300–500, 1500–1750 | Kargerová and Pekař | [66] |
| | 1430 | García-Abuín et al. | [65] |
| | 10–30, 110–130, 300–500, 1500–1750 | Kargerová and Pekař | [66] |
| *Osmolality and colloid osmotic pressure* | 1000, 2000, 3000, 4000 | Bothner et al. | [51] |
| | 750 (eye drops) | Aragona et al. | [67] |
| | From 500 to 7900 (eye drops) | Dick et al. | [68] |

| | | | |
|---|---|---|---|
| *Hydraulic conductivity and fluid absorption rate* | From 500 to 7900 (eye drops) | Dick | [69] |
| | 85, 280, 500, 4000 | Wang et al. | [70] |
| | 45.4, 81.9, 165, 196, 699, 844, 1110 | Lam | [71] |
| | 45.4, 81.9, 165, 196, 699, 844, 1110 | Lam and Bert | [72] |

## 3. Degradable Properties

The presence of hyaluronic acid in many tissues and fluids determines its widespread use in medicine and cosmetology. The biological activity of HA depends on its molecular weight [73]. It has been shown that high molecular weight HA has anti-inflammatory properties, and its rheological characteristics determine its use as a synovial fluid prosthesis in the treatment of various joint diseases, in cosmetology, and in aesthetic medicine as dermal fillers and in ophthalmology as artificial tears [6][74]. Degradation of HA leads to a decrease in the molecular weight and, consequently, to a decrease in viscosity, which is detrimental to the use of HA [40][75].

Hyaluronic acid undergoes degradation under the influence of ultrasound [76][77][78]. This happens as a result of a cleavage of the glycosidic bonds between GlcA and GlcNAc units by the free radicals OH and H, which can be generated by the action of ultrasonic waves in water and the collapse of cavitation bubbles, which causes the breakage of the macromolecule backbone in the solutions [77]. Interestingly, sonication leads to the degradation of HA in a non-random fashion. It is assumed that high molecular weight HA degrades more slowly than low molecular weight HA [76] and exposure to ultrasound does not lead to complete degradation.

Exposure to alkali and acid also leads to the degradation of hyaluronic acid [43][79][80]. This method leads to the complete hydrolysis of HA to oligosaccharide-hyalobiuronic acid [79]. With the presence of acid, hydrolysis randomly occurs on glucuronic acid, and under the action of alkali, it randomly occurs on acetylglycosamine [80]. It is hard to assume that there is any cohesion between the rate of degradation and molecular weight of HA; however, it is suggested that the pH value, as along with the concentration of HA, may affect the rate of hydrolysis [42].

Thermal degradation mechanism is presumably a random chain scission that occurs in the HA chain [81][82][83][84]. With increasing temperature, the decrease in molecular weight was more rapid for both the sample in solution and the powder. During the first three hours of heating at a temperature of 90 °C (powder and solution) and 120 °C (powder), the decrease in molecular weight was much more instantaneous than with a longer exposure to lower temperature. In general, it was concluded that degradation of HA with a lower MW occurs more quickly than with a higher MW at a moderate temperature [81].

It is known that HA degrades when exposed to reactive oxygen species. The impact of various oxidizing agents such as ozone, UV light, hydrogen peroxide and others on HA was studied [85][86][87][88][89][90][91]. Unfortunately, there is no information available about the dependence of the rate of oxidative degradation of HA on its molecular weight as only one sample of HA was studied in most articles.

Hyaluronic acid undergoes degradation under normal conditions. To minimize molecular weight loss during long-term storage, HA can be put in the refrigerator. Studies [92][93] showed that storage conditions have a greater effect on degradation than the initial molecular weight of the sample.

The study of the biodegradation of hydrogels of various compositions based on HA is currently receiving attention. Hydrogels of HA can be applied in different fields, including tissue engineering, drug delivery, wound dressings and regenerative medicine due to its biodegradability, biocompatibility and versatility [94]. To obtain hydrogel from HA, the latter might be crosslinked by chemical modification. In addition to creating a three-dimensional structure, chemical modification makes it possible to achieve better physicochemical characteristics in hydrogels, thereby increasing their resistance to biodegradation [95].

More detailed information about the degradation dependence on the MW of hyaluronic acid is presented in the Table 2.

Table 2. Summary of degradable properties of hyaluronic acid.

| Type of Degradation | HA MW, kDa | Author | Reference |
|---|---|---|---|
| *Ultrasound* | 400, 1000, 1200 | Kubo et al. | [78] |
| | - | Vercruysse et al. | [76] |
| *Temperature* | 1670, 1800 | Mondek et al. | [81] |

| | | | |
|---|---|---|---|
| *Long-term (caused by storage time)* | 17, 267, 752, 1000 | Simulescu et al. | [92] |
| | 14.3, 267.2, 1160.6 | Simulescu et al. | [93] |
| *Enzymatic* | 10, 50 | Kim et al. | [96] |
| | 200, 2000 | Xue et al. | [97] |
| | 50, 350, 1100 | Burdick et al. | [98] |
| | 100, 1000, 2000 | Cao et al. | [99] |

## 4. Conclusions and Perspectives

Hyaluronic acid, as a hydrophilic biopolymer with a unique set of structural, physical, physicochemical, and biodegradable properties, attracts a great deal of attention. This entry demonstrates the key properties of hyaluronic acid and gives the references on original studies. Firstly, the hyaluronic acid structure and coil overlap were discussed. However, despite comprehensive studies, this field requires more detailed analysis, for example, with respect to structural dimensions such as the diameter of the coil, etc.

Secondly, viscosity, surface tension, and density, as the key parameters, were investigated in detail. Further analysis is viable for aqueous-organic solutions of hyaluronic acid or for aqueous HA solutions with additional polymers, which are applied for electrospinning to obtain nano- and microfibers.

Thirdly, knowledge of the cohesion and adhesion properties of hyaluronic acid is necessary for the development of biomedical applications, especially for surgery, ambustial therapy, wound healing, and cell growing. Such parameters were extensively analyzed, but it is interesting to evaluate the influence of the biologically active agents used in the abovementioned applications on the cohesion and adhesion properties of the compound formed.

The next parameter, ultrasound velocity, is not important in itself. Moreover, it has been discovered that this parameter is not dependent on the molecular weight of the polymer. Nonetheless, measuring the ultrasound velocity is useful for the determination of the structural and physical properties of the polymer and their alterations.

Osmolality and colloid osmotic pressure are very important parameters of body liquids. Moreover, during the development of any kind of artificial fluids (tears, synovial fluid, etc.), it is critical to choose an osmolality that is approximately equal to the natural one. Osmolality was analyzed using eye drops, while the colloid osmotic pressure was investigated based on three fractions of hyaluronic acid. Future studies with a wider range of molecular weights could expand the fundamental scientific data in this field.

One more key parameter is hydraulic conductivity, which is as important for peritoneal fluid as it is for transport solutes. Furthermore, this parameter must be considered for the development of medical applications. Unfortunately, there are only a small number of studies in this field, and this area requires additional examination.

Study of the degradation processes of hyaluronic acid under the influence of oxidants and enzymes is necessary for assessing the half-life of drugs based on hyaluronic acid.

It was shown that HA does not degrade to oligosaccharides under the influence of ultrasound; this is in contradistinction to the action of pH and oxidants, which lead to complete hydrolysis of HA.

Moreover, long-term degradation and thermal degradation were discussed. The dependence of these parameters on the molecular weight of the HA makes it possible to choose the optimal period and temperature for storing the sample in order to avoid loss of molecular weight.

Study of the time and degree of enzymatic hydrolysis is necessary for assessing the duration of drug efficacy. It has been shown that hydrogels consisting of HA with a higher molecular weight are less susceptible to enzymatic hydrolysis, although the molecular weight of the sample is not the only factor to affect the degree of decomposition.

However, scientific studies call for further investigations for a better understanding of the relation between the degradable properties and the molecular weight of hyaluronic acid. Such investigations may create a background for development of topical and complicated drug delivery systems, scaffolds and wound dressings, which take biomedicine and bioengineering to a new level.

## References

1. Meyer, K.; Palmer, J.; The polysaccharide of the vitreous humor. *J. Biol. Chem.* **1934**, *107*, 629–634.
2. J. Nečas; L. Bartošíková; P. Brauner; J. Kolář; Hyaluronic acid (hyaluronan): a review. *Veterinární Medicína* **2008**, *53*, 397-411, 10.17221/1930-vetmed.

3. Kogan, G.; Šolté, L.; Stern, R.; Mendichi, R.. Hyaluronic acid: A biopolymer with versatile physico-chemical and biological properties. In Handbook of Polymer Research: Monomers, Oligomers, Polymers and Composites; Pethrick, R.A., Ballada, A., Zaikov, G.E., Eds.; Nova Science Publishers: Hauppauge, NY, USA, 2007; pp. 393–439.
4. Nicola Volpi; Juergen Schiller; Robert Stern; Ladislav Soltés; Role, metabolism, chemical modifications and applications of hyaluronan.. *Current Medicinal Chemistry* **2009**, *16*, 1718-1745, 10.2174/092986709788186138.
5. J R Fraser; T C Laurent; Turnover and metabolism of hyaluronan.. *Ciba Foundation symposium* **1989**, *143*, 41–59.
6. Arianna Fallacara; Erika Baldini; Stefano Manfredini; Silvia Vertuani; Hyaluronic Acid in the Third Millennium. *Polymers* **2018**, *10*, 701, 10.3390/polym10070701.
7. Juliana D. Oliveira; Lucas Silva Carvalho; Antonio Milton Vieira Gomes; Lucio Rezende Queiroz; Beatriz Simas Magalhães; Nádia S. Parachin; Genetic basis for hyper production of hyaluronic acid in natural and engineered microorganisms.. *Microb Cell Fact* **2016**, *15*, 119, 10.1186/s12934-016-0517-4.
8. Schiraldi, C.; La Gatta, A.; De Rosa, M.. Biotechnological Production and Application of Hyaluronan. In Biopolymers; Elnashar, M., Eds.; IntechOpen: London, UK, 2010; pp. 387–412.
9. Balazs, E.A.; Leshchiner, E.; Larsen, N.E.; Band, P.. Applications of hyaluronan and its derivatives. In Biotechnological Polymers; Gebelein, C.G., Eds.; Technomic: Lancaster, UK, 1993; pp. 41–65.
10. Paul L. DeAngelis; Wei Jing; Richard R. Drake; Ann Mary Achyuthan; Identification and Molecular Cloning of a Unique Hyaluronan Synthase fromPasteurella multocida. *Journal of Biological Chemistry* **1998**, *273*, 8454-8458, 10.1074/jbc.273.14.8454.
11. Yang Wang; Litao Hu; Hao Huang; Hao Wang; Tianmeng Zhang; Jian Chen; Guocheng Du; Zhen Kang; Eliminating the capsule-like layer to promote glucose uptake for hyaluronan production by engineered Corynebacterium glutamicum. *Nature Communications* **2020**, *11*, 3120, 10.1038/s41467-020-16962-7.
12. Paul L DeAngelis; Hyaluronan synthases: fascinating glycosyltransferases from vertebrates, bacterial pathogens, and algal viruses.. *Cellular and Molecular Life Sciences* **1999**, *56*, 670-682, 10.1007/s000180050461.
13. Nicola Volpi; Francesca Maccari; Purification and characterization of hyaluronic acid from the mollusc bivalve Mytilus galloprovincialis.. *Biochimie* **2003**, *85*, 619-625, 10.1016/s0300-9084(03)00083-x.
14. Grigorij Kogan; Ladislav Šoltés; Robert Stern; Peter Gemeiner; Hyaluronic acid: a natural biopolymer with a broad range of biomedical and industrial applications. *Biotechnology Letters*

**2006**, *29*, 17-25, 10.1007/s10529-006-9219-z.

15. Xian Xu; A. K. Jha; Daniel A. Harrington; Mary C. Farach-Carson; Xinqiao Jia; Hyaluronic acid-based hydrogels: from a natural polysaccharide to complex networks. *Soft Matter* **2012**, *8*, 3280-3294, 10.1039/c2sm06463d.
16. Aviva Shiedlin; Russell Bigelow; William Christopher; Saman Arbabi; Laura Yang; Ronald V. Maier; Norman Wainwright; Alice Childs; Robert J. Miller; Evaluation of Hyaluronan from Different Sources: Streptococcuszooepidemicus, Rooster Comb, Bovine Vitreous, and Human Umbilical Cord. *Biomacromolecules* **2004**, *5*, 2122-2127, 10.1021/bm0498427.
17. Selyanin, M.A.; Khabarov, V.N.; Boykov, P.Y.. Hyaluronic Acid: Production, Properties, Application in Biology and Medicine; John Wiley & Sons, Ltd.: Chichester, UK, 2015; pp. 215.
18. Carmen G. Boeriu; Jan Springer; Floor K. Kooy; Lambertus A. M. Van Den Broek; Gerrit Eggink; Production Methods for Hyaluronan. *International Journal of Carbohydrate Chemistry* **2013**, *2013*, 1-14, 10.1155/2013/624967.
19. Torvard C Laurent; Ulla Bg Laurent; J Robert E Fraser; The structure and function of hyaluronan: An overview. *Immunology & Cell Biology* **1996**, *74*, a1-a7, 10.1038/icb.1996.32.
20. J. R. E. Fraser; T. C. Laurent; Hyaluronan: its nature, distribution, functions and turnover. *Journal of Internal Medicine* **1997**, *242*, 27-33, 10.1046/j.1365-2796.1997.00170.x.
21. Robert Stern; Akira A. Asari; Kazuki N. Sugahara; Hyaluronan fragments: An information-rich system. *European Journal of Cell Biology* **2006**, *85*, 699-715, 10.1016/j.ejcb.2006.05.009.
22. Jingmin Li; Meng Qiao; Yuan Ji; Lei Lin; Xing Zhang; Robert J. Linhardt; Chemical, enzymatic and biological synthesis of hyaluronic acids. *International Journal of Biological Macromolecules* **2020**, *152*, 199-206, 10.1016/j.ijbiomac.2020.02.214.
23. Kim, S.J.; Owen, S.C.; Hyaluronic acid binding to CD44S is indiscriminate of molecular weight. *BBA Biomembr.* **2020**, *1862*, 183348, 10.1016/j.bbamem.2020.183348.
24. Masaki Takasugi; Denis Firsanov; Gregory Tombline; Hanbing Ning; Julia Ablaeva; Andrei Seluanov; Vera Gorbunova; Naked mole-rat very-high-molecular-mass hyaluronan exhibits superior cytoprotective properties. *Nature Communications* **2020**, *11*, 2376, 10.1038/s41467-020-16050-w.
25. Petr Snetkov; Svetlana Morozkina; Mayya Uspenskaya; Roman Olekhnovich; Hyaluronan-Based Nanofibers: Fabrication, Characterization and Application. *Polymers* **2019**, *11*, 2036, 10.3390/polym11122036.
26. Mariana F.P. Graça; Sónia P. Miguel; Cátia S.D. Cabral; Ilídio J. Correia; Hyaluronic acid—Based wound dressings: A review. *Carbohydrate Polymers* **2020**, *241*, 116364, 10.1016/j.carbpol.2020.116364.

27. Ilker Sefik Bayer; Hyaluronic Acid and Controlled Release: A Review. *Molecules* **2020**, *25*, 2649, 10.3390/molecules25112649.
28. Arshia Ehsanipour; Tommy Nguyen; Tasha Aboufadel; Mayilone Sathialingam; Phillip Cox; Weikun Xiao; Christopher M. Walthers; Stephanie K. Seidlits; Injectable, Hyaluronic Acid-Based Scaffolds with Macroporous Architecture for Gene Delivery. *Cellular and Molecular Bioengineering* **2019**, *12*, 399-413, 10.1007/s12195-019-00593-0.
29. Alfred Linker; K Mayer; Karl Meyer; Production of Unsaturated Uronides by Bacterial Hyaluronidases. *Nature* **1954**, *174*, 1192-1194, 10.1038/1741192a0.
30. Gokul Sridharan; Akhil A Shankar; Toluidine blue: A review of its chemistry and clinical utility. *Journal of Oral and Maxillofacial Pathology* **2012**, *16*, 251-255, 10.4103/0973-029X.99081.
31. Guiping Ma; Yang Liu; Dawei Fang; Jie Chen; Cheng Peng; Xu Fei; Jun Nie; Hyaluronic acid/chitosan polyelectrolyte complexes nanofibers prepared by electrospinning. *Materials Letters* **2012**, *74*, 78-80, 10.1016/j.matlet.2012.01.012.
32. Ramona C. Polexe; T. Delair; Elaboration of Stable and Antibody Functionalized Positively Charged Colloids by Polyelectrolyte Complexation between Chitosan and Hyaluronic Acid. *Molecules* **2013**, *18*, 8563-8578, 10.3390/molecules18078563.
33. John E. Scott; Frank Heatley; Hyaluronan forms specific stable tertiary structures in aqueous solution: A 13C NMR study. *Proceedings of the National Academy of Sciences USA* **1999**, *96*, 4850-4855, 10.1073/pnas.96.9.4850.
34. J. E. Scott; C. Cummings; A. Brass; Y. Chen; Secondary and tertiary structures of hyaluronan in aqueous solution, investigated by rotary shadowing-electron microscopy and computer simulation. Hyaluronan is a very efficient network-forming polymer.. *Biochemical Journal* **1991**, *274*, 699-705, 10.1042/bj2740699.
35. E. D. T. Atkins; J. K. Sheehan; Hyaluronates: Relation between Molecular Conformations. *Science* **1973**, *179*, 562-564, 10.1126/science.179.4073.562.
36. I. C. M. Dea; R. Moorhouse; D. A. Rees; S. Arnott; J Mitchell Guss; E. A. Balazs; Hyaluronic Acid: A Novel, Double Helical Molecule. *Science* **1973**, *179*, 560-562, 10.1126/science.179.4073.560.
37. G. Ribitsch; J. Schurz; V. Ribitsch; Investigation of the solution structure of hyaluronic acid by light scattering, SAXS, and viscosity measurements. *Colloid and Polymer Science* **1980**, *258*, 1322-1334, 10.1007/bf01668780.
38. Robert L. Cleland; John L. Wang; Ionic polysaccharides. III. Dilute solution properties of hyaluronic acid fractions. *Biopolymers* **1970**, *9*, 799-810, 10.1002/bip.1970.360090706.
39. Andrew Almond; Andrew Brass; J.K. Sheehan; Deducing polymeric structure from aqueous molecular dynamics simulations of oligosaccharides: predictions from simulations of hyaluronan

tetrasaccharides compared with hydrodynamic and X-ray fibre diffraction data 1 1Edited by R. Huber. *Journal of Molecular Biology* **1998**, *284*, 1425-1437, 10.1006/jmbi.1998.2245.

40. Mary K. Cowman; Tannin A. Schmidt; Preeti Raghavan; Antonio Stecco; Viscoelastic Properties of Hyaluronan in Physiological Conditions. *F1000Research* **2015**, *4*, 622, 10.12688/f1000research.6885.1.
41. Ľ. Lapčík; Stefaan C. De Smedt; Joseph Demeester§; Peter Chabreček; Hyaluronan: Preparation, Structure, Properties, and Applications†. *Chemical Reviews* **1998**, *98*, 2663-2684, 10.1021/cr941199z.
42. Atoosa Maleki; Anna-Lena Kjøniksen; Bo Nyström; Effect of pH on the Behavior of Hyaluronic Acid in Dilute and Semidilute Aqueous Solutions. *Macromolecular Symposia* **2008**, *274*, 131-140, 10.1002/masy.200851418.
43. Snehasish Ghosh; Ivan Kobal; Dino Zanette; Wayne F. Reed; Conformational contraction and hydrolysis of hyaluronate in sodium hydroxide solutions. *Macromolecules* **1993**, *26*, 4685-4693, 10.1021/ma00069a042.
44. Edwin R. Morris; David A. Rees; E.Jane Welsh; Conformation and dynamic interactions in hyaluronate solutions. *Journal of Molecular Biology* **1980**, *138*, 383-400, 10.1016/0022-2836(80)90294-6.
45. Toshio Yanaki; Toshijiro Yamaguchi; Temporary network formation of hyaluronate under a physiological condition. 1. Molecular-weight dependence. *Biopolymers* **1990**, *30*, 415-425, 10.1002/bip.360300319.
46. Syang-Peng Rwei; Saint-Wei Chen; Ching-Feng Mao; Hsu-Wei Fang; Viscoelasticity and wearability of hyaluronate solutions. *Biochemical Engineering Journal* **2008**, *40*, 211-217, 10.1016/j.bej.2007.12.021.
47. E. Gura; M. Hückel; P.-J. Müller; Specific degradation of hyaluronic acid and its rheological properties. *Polymer Degradation and Stability* **1998**, *59*, 297-302, 10.1016/s0141-3910(97)00194-8.
48. Martin Pisárčik; Dušan Bakoš; Michal Čeppan; Non-Newtonian properties of hyaluronic acid aqueous solution. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* **1995**, *97*, 197-202, 10.1016/0927-7757(95)03097-w.
49. Jihoon Kim; Ji-Youn Chang; Yoon-Young Kim; Moon-Jong Kim; H.-S. Kho; Effects of molecular weight of hyaluronic acid on its viscosity and enzymatic activities of lysozyme and peroxidase. *Archives of Oral Biology* **2018**, *89*, 55-64, 10.1016/j.archoralbio.2018.02.007.
50. Samuel J. Falcone; David M. Palmeri; Richard A. Berg; Rheological and cohesive properties of hyaluronic acid. *Journal of Biomedical Materials Research Part A* **2006**, *76*, 721-728, 10.1002/jbm.a.30623.

51. Hege Bothner; Ove Wik; Rheology of Hyaluronate. *Acta Oto-Laryngologica* **1987**, *104*, 25-30, 10.3109/00016488709102834.
52. Y. Kobayashi; A. Okamoto; K. Nishinari; Viscoelasticity of hyaluronic acid with different molecular weights. *Biorheology* **1994**, *31*, 235-244, 10.3233/bir-1994-31302.
53. David Rebenda; Martin Vrbka; Pavel Čípek; Evgeniy Toropitsyn; David Nečas; Martin Pravda; Martin Hartl; On the Dependence of Rheology of Hyaluronic Acid Solutions and Frictional Behavior of Articular Cartilage. *Materials* **2020**, *13*, 2659, 10.3390/ma13112659.
54. Wendy E. Krause; Enrico G. Bellomo; Ralph H. Colby; Rheology of sodium hyaluronate under physiological conditions.. *Biomacromolecules* **2001**, *2*, 65-69, 10.1021/bm0055798.
55. Paul A. Knepper; Steven Covici; James R. Fadel; Chandra S. K. Mayanil; Robert Ritch; Surface-Tension Properties of Hyaluronic Acid. *Journal of Glaucoma* **1995**, *4*, 194-199, 10.1097/00061198-199506000-00009.
56. Walkiria Ribeiro; José Luís Mata; Benilde De Jesus Vieira Saramago; Effect of Concentration and Temperature on Surface Tension of Sodium Hyaluronate Saline Solutions. *Langmuir* **2007**, *23*, 7014-7017, 10.1021/la700269k.
57. Frederick H. Silver; Joseph Librizzi; George D. Pins; Ming-Che Wang; Dominick Benedetto; Physical properties of hyaluronic acid and hydroxypropylmethylcellulose in solution: Evaluation of coating ability. *Journal of Applied Biomaterials* **1994**, *5*, 89-98, 10.1002/jab.770050111.
58. Frederick H. Silver; Joseph J. Librizzi; Dominick Benedetto; Physical properties of model viscoelastic materials. *Journal of Applied Biomaterials* **1994**, *5*, 227-234, 10.1002/jab.770050308.
59. Johannes Nepp; Joerg Schauersberger; Gebtraud Schild; Kerstin Jandrasits; Jinus Haslinger-Akramian; Agnes Derbolav; Andreas Wedrich; The clinical use of viscoelastic artificial tears and sodium chloride in dry-eye syndrome.. *Biomaterials* **2001**, *22*, 3305-3310, 10.1016/s0142-9612(01)00167-3.
60. Katherine Vorvolakos; James C. Coburn; David M. Saylor; Dynamic interfacial behavior of viscoelastic aqueous hyaluronic acid: effects of molecular weight, concentration and interfacial velocity. *Soft Matter* **2014**, *10*, 2304-2312, 10.1039/c3sm52372a.
61. Yong-Hong Liao; S. E. Jones; Ben Forbes; Gary P. Martin; Marc B. Brown; Hyaluronan: Pharmaceutical Characterization and Drug Delivery. *Drug Delivery* **2005**, *12*, 327-342, 10.1080/10717540590952555.
62. Marco F. Saettone; Patrizia Chetoni; Maria Tilde Torracca; Susi Burgalassi; Boris Giannaccini; Evaluation of muco-adhesive properties and in vivo activity of ophthalmic vehicles based on hyaluronic acid. *International Journal of Pharmaceutics* **1989**, *51*, 203-212, 10.1016/0378-5173(89)90193-2.

63. A.M. Durrani; S.J. Farr; I.W. Kellaway; Influence of molecular weight and formulation pH on the precorneal clearance rate of hyaluronic acid in the rabbit eye. *International Journal of Pharmaceutics* **1995**, *118*, 243-250, 10.1016/0378-5173(94)00389-m.

64. S Gómez-Alejandre; E Sánchez De La Blanca; Cristina Abradelo; M. Fernanda Rey-Stolle; I Hernández-Fuentes; Partial specific volume of hyaluronic acid in different media and conditions.. *International Journal of Biological Macromolecules* **2000**, *27*, 287-290, 10.1016/s0141-8130(00)00130-6.

65. A. García-Abuín; ‡ Diego Gómez-Díaz; J. M. Navaza; L. Regueiro; I. Vidal-Tato; Viscosimetric behaviour of hyaluronic acid in different aqueous solutions. *Carbohydrate Polymers* **2011**, *85*, 500-505, 10.1016/j.carbpol.2011.02.028.

66. A. Kargerová; Miloslav Pekař; Densitometry and ultrasound velocimetry of hyaluronan solutions in water and in sodium chloride solution. *Carbohydrate Polymers* **2014**, *106*, 453-459, 10.1016/j.carbpol.2014.01.020.

67. P Aragona; G Di Stefano; F Ferreri; R Spinella; A Stilo; Sodium hyaluronate eye drops of different osmolarity for the treatment of dry eye in Sjögren's syndrome patients. *British Journal of Ophthalmology* **2002**, *86*, 879-884, 10.1136/bjo.86.8.879.

68. H B Dick; A J Augustin; N Pfeiffer; Osmolality of various viscoelastic substances: comparative study.. *Journal of Cataract and Refractive Surgery* **2000**, *26*, 1242-1246.

69. 69. Dick, H.B. Viscoelastics in Ophthalmic Surgery; Dick, H.B., Schwenn, O., Eds.; Springer Science & Business Media: Berlin/Heidelberg, Germany; New York, NY, USA; Barcelona, Spain; Hong Kong, China; Milano, Italy; Paris, France; Singapore; Tokyo, Japan, 2000.

70. Tao Wang; Hui-Hong Cheng; Olof Heimbürger; Chi Chen; Jacek Waniewski; Jonas Bergström; B. Lindholm; Hyaluronan decreases peritoneal fluid absorption: Effect of molecular weight and concentration of hyaluronan. *Kidney International* **1999**, *55*, 667-673, 10.1046/j.1523-1755.1999.00279.x.

71. Lam, L.S. Flow Conductivity of Solutions of Hyaluronic Acid: Effects of Concentration and Molecular Weight. Master’s Thesis, University of British Columbia, Vancouver, BC, Canada, 1988. DOI:10.14288/1.0058822

72. Luk S. Lam; Joel L. Bert; Hydraulic flow conductivity of hyaluronic acid solutions: Effects of concentration and molecular weight. *Biorheology* **1990**, *27*, 789-795, 10.3233/bir-1990-27514.

73. Ramesh C. Gupta; Rajiv Lall; Ajay Srivastava; Anita Sinha; Hyaluronic Acid: Molecular Mechanisms and Therapeutic Trajectory. *Frontiers in Veterinary Science* **2019**, *6*, 192, 10.3389/fvets.2019.00192.

74. Jaime M. Cyphert; Carol S. Trempus; Stavros Garantziotis; Size Matters: Molecular Weight Specificity of Hyaluronan Effects in Cell Biology. *International Journal of Cell Biology* **2015**, *2015*,

1-8, 10.1155/2015/563818.

75. Amir Fakhari; Cory J. Berkland; Applications and emerging trends of hyaluronic acid in tissue engineering, as a dermal filler and in osteoarthritis treatment.. *Acta Biomaterialia* **2013**, *9*, 7081-7092, 10.1016/j.actbio.2013.03.005.
76. K. P. Vercruysse; A. R. Lauwers; J. M. Demeester; Absolute and empirical determination of the enzymatic activity and kinetic investigation of the action of hyaluronidase on hyaluronan using viscosimetry. *Biochemical Journal* **1995**, *306*, 153-160, 10.1042/bj3060153.
77. Jawhar Hafsa; Mohamed Aymen Chaouch; Bassem Charfeddine; Christophe Rihouey; Khalifa Limem; Didier Le Cerf; Sonia Rouatbi; Hatem Majdoub; Effect of ultrasonic degradation of hyaluronic acid extracted from rooster comb on antioxidant and antiglycation activities. *Pharmaceutical Biology* **2016**, *55*, 156-163, 10.1080/13880209.2016.1232740.
78. Kohmei Kubo; Toshiya Nakamura; Keiichi Takagaki; Yutaka Yoshida; Masahiko Endo; Depolymerization of hyaluronan by sonication. *Glycoconjugate Journal* **1993**, *10*, 435-439, 10.1007/bf00737963.
79. Roger W. Jeanloz; Dorothy A. Jeanloz; The Degradation of Hyaluronic Acid by Methanolysis*. *Biochemistry* **1964**, *3*, 121-123, 10.1021/bi00889a019.
80. Y. Tokita; A. Okamoto; Hydrolytic degradation of hyaluronic acid. *Polymer Degradation and Stability* **1995**, *48*, 269-273, 10.1016/0141-3910(95)00041-j.
81. Jakub Mondek; Michal Kalina; Vasile Simulescu; Miloslav Pekař; Thermal degradation of high molar mass hyaluronan in solution and in powder; comparison with BSA. *Polymer Degradation and Stability* **2015**, *120*, 107-113, 10.1016/j.polymdegradstab.2015.06.012.
82. H. Bothner; T. Waaler; O. Wik; Limiting viscosity number and weight average molecular weight of hyaluronate samples produced by heat degradation. *International Journal of Biological Macromolecules* **1988**, *10*, 287-291, 10.1016/0141-8130(88)90006-2.
83. Milena Rehákova; Dušan Bakoš; Maroš Soldán; Katarína Vizárová; Depolymerization reactions of hyaluronic acid in solution. *International Journal of Biological Macromolecules* **1994**, *16*, 121-124, 10.1016/0141-8130(94)90037-x.
84. Karen M. Lowry; Ellington M. Beavers; Thermal stability of sodium hyaluronate in aqueous solution. *Journal of Biomedical Materials Research* **1994**, *28*, 1239-1244, 10.1002/jbm.820281014.
85. Sylwia Grabska-Zielińska; Alina Sionkowska; The influence of UV-radiation on hyaluronic acid and its blends with addition of collagen and chitosan. *International Journal of Polymer Analysis and Characterization* **2019**, *24*, 285-294, 10.1080/1023666x.2019.1592899.

86. Yue Wu; Preparation of low-molecular-weight hyaluronic acid by ozone treatment. *Carbohydrate Polymers* **2012**, *89*, 709-712, 10.1016/j.carbpol.2012.03.081.

87. Robert Stern; Grigorij Kogan; Mark J. Jedrzejas; Ladislav Šoltés; The many ways to cleave hyaluronan. *Biotechnology Advances* **2007**, *25*, 537-557, 10.1016/j.biotechadv.2007.07.001.

88. L. Šoltés; Grigorij Kogan; M. Stankovská; R. Mendichi; J. Schiller; P. Gemeiner; Degradation of High-Molar-Mass Hyaluronan and Characterization of Fragments. *Biomacromolecules* **2007**, *8*, 2697-2705, 10.1021/bm070309b.

89. Ľ. Lapčík; J. Schurz; L. Lapčík Jr.; Photochemical degradation of hyaluronic acid by singlet oxygen. *Colloid and Polymer Science* **1991**, *269*, 633-635, 10.1007/bf00659919.

90. Hongyue Chen; Jing Qin; Yi Hu; Efficient Degradation of High-Molecular-Weight Hyaluronic Acid by a Combination of Ultrasound, Hydrogen Peroxide, and Copper Ion. *Molecules* **2019**, *24*, 617, 10.3390/molecules24030617.

91. Clare L. Hawkins; Michael J. Davies; Degradation of Hyaluronic Acid, Poly- and Mono-Saccharides, and Model Compounds by Hypochlorite: Evidence for Radical Intermediates and Fragmentation. *Free Radical Biology and Medicine* **1998**, *24*, 1396-1410, 10.1016/s0891-5849(98)00009-4.

92. Vasile Simulescu; Jakub Mondek; Michal Kalina; Miloslav Pekař; Kinetics of long-term degradation of different molar mass hyaluronan solutions studied by SEC-MALLS. *Polymer Degradation and Stability* **2015**, *111*, 257-262, 10.1016/j.polymdegradstab.2014.12.005.

93. Vasile Simulescu; Michal Kalina; Jakub Mondek; Miloslav Pekař; Long-term degradation study of hyaluronic acid in aqueous solutions without protection against microorganisms. *Carbohydrate Polymers* **2016**, *137*, 664-668, 10.1016/j.carbpol.2015.10.101.

94. Sonia Trombino; Camilla Servidio; Federica Curcio; Roberta Cassano; Strategies for Hyaluronic Acid-Based Hydrogel Design in Drug Delivery.. *Pharmaceutics* **2019**, *11*, 407, 10.3390/pharmaceutics11080407.

95. Xiao Wang; Wan-Wei Dai; Ya-Long Dang; Ying Hong; Chun Zhang; Five Years’ Outcomes of Trabeculectomy with Cross-linked Sodium Hyaluronate Gel Implantation for Chinese Glaucoma Patients. *Chinese Medical Journal* **2018**, *131*, 1562-1568, 10.4103/0366-6999.233655.

96. JungJu Kim; Yongdoo Park; Giyoong Tae; Kyu Back Lee; Chang Mo Hwang; Soon Jung Hwang; In Sook Kim; Insup Noh; Kyung Sun; Characterization of low-molecular-weight hyaluronic acid-based hydrogel and differential stem cell responses in the hydrogel microenvironments. *Journal of Biomedical Materials Research Part A* **2009**, *88*, 967-975, 10.1002/jbm.a.31947.

97. Yu Xue; Hongyue Chen; Chao Xu; Dinghua Yu; Huajin Xu; Yi Hu; Synthesis of hyaluronic acid hydrogels by crosslinking the mixture of high-molecular-weight hyaluronic acid and low-molecular-

weight hyaluronic acid with 1,4-butanediol diglycidyl ether. *RSC Advances* **2020**, *10*, 7206-7213, 10.1039/c9ra09271d.

98. † Jason A. Burdick; † Cindy Chung; † Xinqiao Jia; Mark A. Randolph; Robert Langer; Controlled Degradation and Mechanical Behavior of Photopolymerized Hyaluronic Acid Networks. *Biomacromolecules* **2005**, *6*, 386-391, 10.1021/bm049508a.
99. Wanxu Cao; Junhui Sui; Mengcheng Ma; Yang Xu; Weimin Lin; Yafang Chen; Yi Man; Yong Sun; Yujiang Fan; Xingdong Zhang; et al. The preparation and biocompatible evaluation of injectable dual crosslinking hyaluronic acid hydrogels as cytoprotective agents. *J. Mater. Chem. B* **2019**, *7*, 4413-4423, 10.1039/c9tb00839j.