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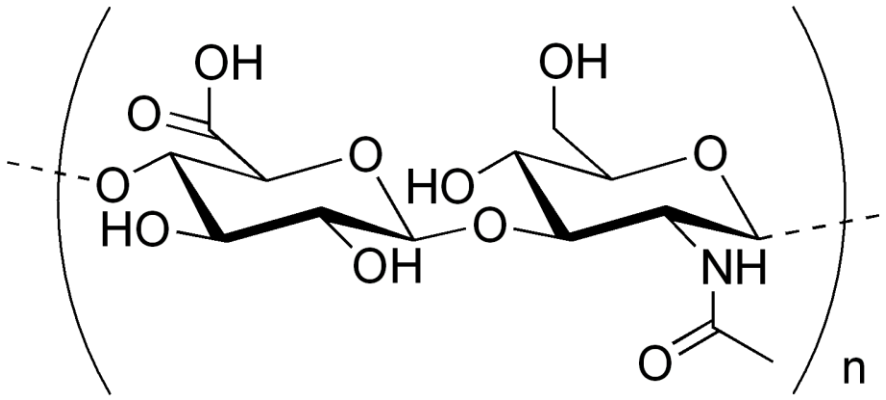

Hyaluronic acid

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ITMO University

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HYALURONIC ACID AS A POLYMER MATRIX FOR PERSPECTIVE BIOMATERIALS

< STUDY GUIDE >

RECOMMENDED FOR USE AT ITMO UNIVERSITY

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ITMO University is the leading Russian university in the field of information and photonic technologies, one of the few Russian universities that received the status of a national research university in 2009. Since 2013, ITMO University has been a participant in the program to improve the competitiveness of Russian universities among the world's leading scientific and educational centers, known as the "5 in 100" project. The goal of ITMO University is to become a world-class research university, entrepreneurial in type, focused on the internationalization of all areas of activity.

Table of content

INTRODUCTION

Nowadays biopolymers and materials based on them play an important role in bioengineering, biomedicine, drug delivery systems, tissue engineering, food industry, etc. There are many practical applications based on biopolymers, for example eye drops, contact lenses, scaffolds, wound healings, surgical sutures, sprays, medications, and many others.

Hyaluronic acid, being a prominent representative of biopolymers, is the main component of intracellular, extracellular, and pericellular matrices. Moreover, hyaluronic acid can bind with the specific receptors located on the cell surface and cause various biological effects, which is critical for modern drug delivery systems. Interestingly, hyaluronic acid with different molecular weight is a remarkable marker of different diseases - from rheumatoid arthritis to cancer.

Due to its excellent biological, chemical, physico-chemical properties, hyaluronic acid is a topical basis for perspective biomaterials. Firstly, the biopolymer is totally biocompatible to living organisms due to its presence in their tissues. Secondly, exogenic hyaluronic acid can biodegrade under the action of enzymes, mainly by hyaluronidases. Thirdly, the biopolymer has no allergenicity, which highlights the possibility of its wide application.

Thus, hyaluronic acid may be used as the matrix for various biomaterials, which are attractive for the application in biomedicine and bioengineering. This study guide describes in detail the key properties of hyaluronic acid, explains the possibility of its use for drug delivery, as well as demonstrates the topical applications of biomaterials based on this biopolymer.

CHAPTER 1. HYALURONIC ACID: STRUCTURE, SOURCES, BIOLOGICAL PROPERTIES

1.1. Structure of hyaluronic acid

Hyaluronic acid (HA) is a key molecule representative of hyaluronans. It was discovered by Karl Meyer and John Palmer in 1934 [1] and still attracts the attention of researchers in various fields of science and technology, such as chemistry, biochemistry, bioengineering, and related sciences. Hyaluronic acid is a high molecular weight linear hetero polysaccharide consisting of regularly alternating residues of D-glucuronic acid and N-acetyl-D-glucosamine [1,2]. In the hyaluronic acid molecule, D-glucuronic acid is linked to aminosaccharide via a β-(1→3)-glycosidic bond, while aminosaccharide is linked to D-glucuronic acid via a β-(1→4)-glycosidic bond [3,4]. The structure of the monomeric unit (primary structure) of hyaluronic acid is shown in Figure 1.1.

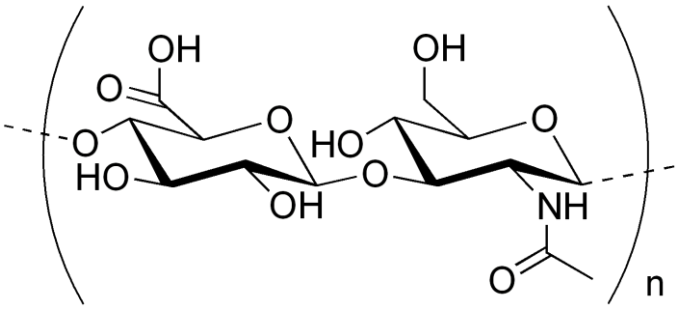

Figure 1.1 – Hyaluronic acid primary structure

Due to the presence of polar and non-polar segments in the biopolymer structure, hyaluronic acid can chemically interact with various agents [3], such as metachromatic dyes, which are widely used in clinical research [5]; and chitosan, which allows to obtain promising materials based on polyelectrolyte complexes [6,7]. In this case, functional groups of HA (hydroxyl and carboxyl groups, acetylated amino group), as well as glycoside bonds with carbohydrate residues, are arranged in a spatially preferable equatorial position. In contrast, hydrogen atoms occupy less sterically hindered axial positions [8].

Hyaluronic acid in aqueous solutions forms hydrogen bonds, which both provide energy stability of the disaccharide structure in solutions and increase the rigidity of the polymer matrix, which ultimately determines the rheological properties of hyaluronic acid solutions. Figure 1.2 shows hydrogen bonds occurring both within one macromolecule and between neighboring molecules. The water molecule can act as a “bridge” between the functional groups [3,9]. Thus, the primary structure of HA and the resulting hydrogen bonds contribute to the formation of secondary and tertiary structures [9,10,11].

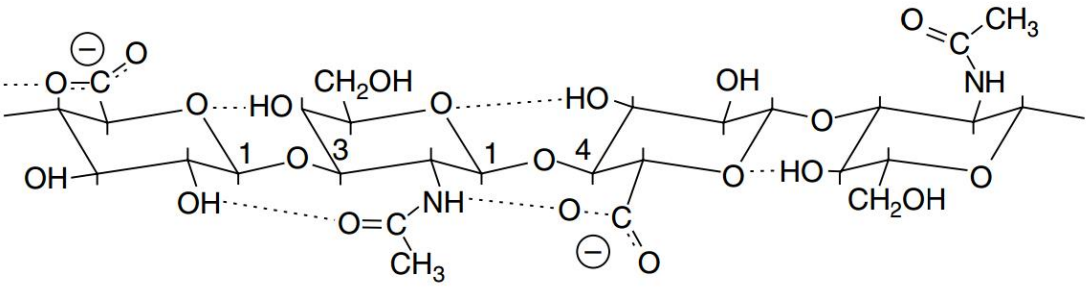

Figure 1.2 – Schematic representation of hydrogen bonds formed inside one HA macromolecule. Reproduced from [9], with permission from John Wiley and Sons, 2022.

Hyaluronic acid, as well as hyaluronates of ammonium, sodium and other alkali metals, have good solubility in water and forms solutions with high viscosity even at low concentrations of biopolymer [3]. Moreover, hyaluronic acid in solution can organize a three-dimensional net structure at concentrations below 0.0001 wt.% [10]. In addition, HA can form pseudogels at concentrations equal to 0.1 wt.% or higher [3,12,13]. Hyaluronic acid with high molecular weight (5.0 MDa) already at solution concentration higher than 0.01 wt.% forms an interwoven polymer network [14]. HA salts containing two or more valence cations are insoluble in water, except of magnesium hyaluronate. Moreover, when such cations are introduced into hyaluronic acid solutions, cross-molecular linking occurs, which leads to the formation of a gel with high water content [3].

As known, the hyaluronic acid macromolecule in water solution can form a left-oriented single or double helix [10,11]. Current studies prove that potassium and sodium hyaluronates in the solution form a double helix [15]; moreover, this helix has antiparallel left-oriented chains [3,16]. Figure 1.3 demonstrates the anti-parallel double helix structure of hyaluronic acid.

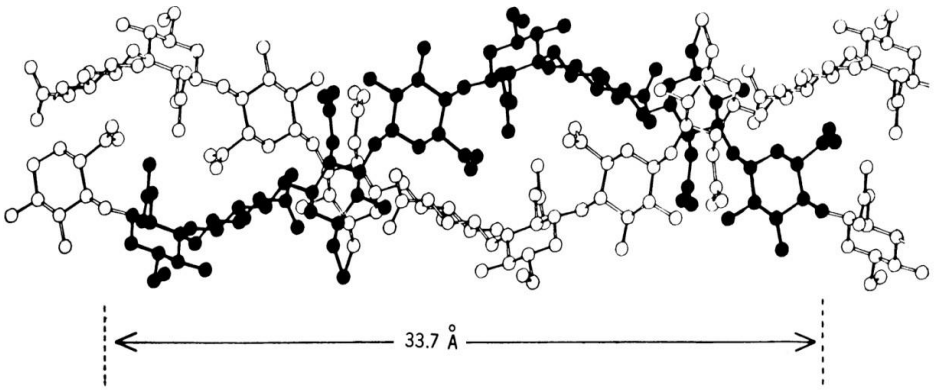

Figure 1.3 – Schematic representation of the antiparallel helix structure of hyaluronic acid. Reproduced from [16], with permission from The American Association for the Advancement of Science, 2022.

In addition to the double helical structure, hyaluronic acid can have a superspiral organization [17]. As mentioned above, an intramolecular polymer network is formed with the increase of HA concentration. In the diluted HA solution, the biopolymer is an independent macromolecule. However, increasing the HA concentration results in the formation of three-dimensional network structures because of the limited volume and intertwining of the polymer macromolecules. Hyaluronic acid macromolecules can be coiled into a globule [3].

The secondary structure of hyaluronic acid resembles a flat belt transformed into a helix or twisted into a sheet [9,10] (Figure 1.4). Intermolecular hydrogen bonds drastically influence the structure of hyaluronic acid [2,3,10,15,16]. In the diluted solutions of hyaluronic acid, macromolecules have semi-rigid helical chains and can form spiral belts and even helical rings. Due to the formation of a rigid helix, HA macromolecules bind a large amount of water and form volumetric tertiary structures, “domains” [3,15,16,18,19].

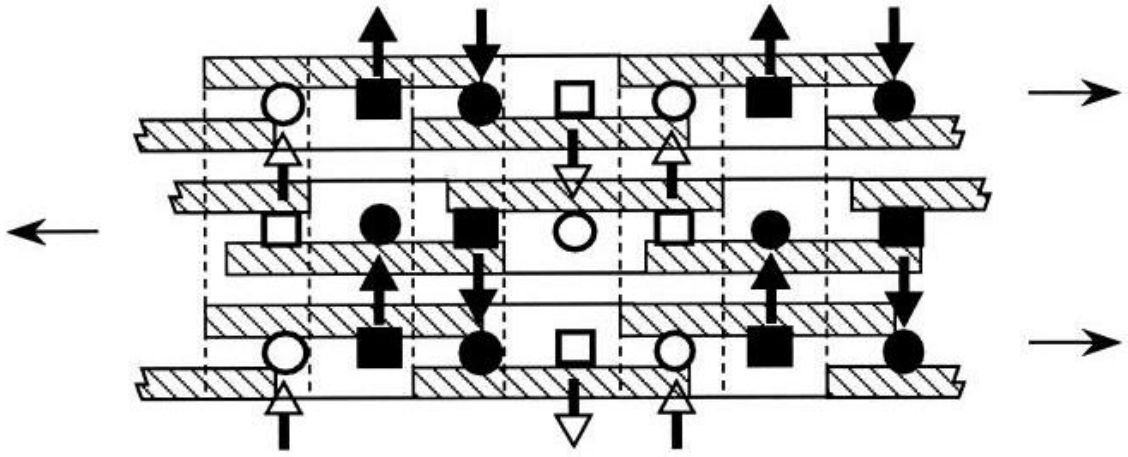

Figure 1.4 – Schematic representation of the secondary structure of hyaluronic acid. Reproduced from [9], with permission from John Wiley and Sons, 2022.

The hydrodynamic volume of hyaluronic acid is usually analyzed in solutions with an ionic strength equal to the physiological level. In this case, the charges of the carboxyl groups of HA are almost completely isolated from each other, so the repulsive interaction between them does not lead to a noticeable increase in the volume of the polymer globule. In the presence of sodium chloride with the concentration equal to 0.15 M or less, electrostatic repulsion increases the hydrodynamic volume of the hyaluronic acid macromolecule.

The hydrodynamic volume of hyaluronic acid directly depends on the polymer molecular weight (Table 1.1) and affects the maximum possible concentration of the biopolymer as the polymer networks begin to intertwine with each other.

Table 1.1 - Dependence of characteristic viscosity and hydrodynamic volume of hyaluronic acid from molecular weight [20].

MW, kDa	**L, nm**	**[η], cm^3/g**	**V_s, cm^3/g**	**$\langle r^2 \rangle^{1/2}$, nm**	**C, µg/cm^3**
100	250	290	120	52	8 600
500	1 250	1 100	420	140	2 400
1000	2 500	1 800	730	210	1 400
3000	7 500	4 400	1 800	400	570
6000	15 000	7 700	3 100	600	320

Notes:
MW – Hyaluronic acid molecular weight
L – macromolecular chain length
[η] – intrinsic viscosity
V_s – specific volume of the polymer
$\langle r^2 \rangle^{1/2}$ – Chain hydrodynamic diameter
C – coil overlap concentration

The structure of hyaluronic acid in solution significantly depends on the solvent pH: the addition of acids or alkalis shifts the balance between the repulsive and attractive forces of the polymer chains [21,22]. When pH is greater than 11.0 and less than 4.0, hyaluronic acid undergoes depolymerization [22,23]. However, in alkaline solutions, the degradation effect is more active due to the breaking of hydrogen bonds that play an important role in the structural organization of hyaluronic acid [21,22,23].

Obviously, the rheological properties of hyaluronic acid solutions depend on its three-dimensional structure and polyelectrolyte character [9,26-29]. Hyaluronic acid solutions are non-Newtonian fluids with a shear-thinning effect and viscoelastic behavior [22,28]. The decrease of viscosity of hyaluronic acid solutions with increasing shear rate may be caused by the breakdown of intramolecular hydrogen bonds and the hydrophobic effect with increasing shear rate. The latter effect is caused by the deformation of the molecular chains of hyaluronic acid and their ordering in the flow direction, which cumulatively leads to a decrease in solution viscosity [21,29]. Hyaluronic acid solutions exhibit no thixotropic properties; if the shear rate becomes less and finally achieves almost zero, the molecular chains of hyaluronic acid take the original conformation. In this case, the viscosity curve goes through the same points in the opposite direction, there is no hysteresis [27]. Thus, the breakdown of the three-dimensional polymer network is reversible. As viscosity of HA solutions plays an important role in physiological and biochemical processes, this characteristic should be considered in medical, cellular, bioengineering, cosmetic, food and other applications of hyaluronic acid.

1.2. HA sources

HA is an important component of intracellular, extracellular, and pericellular matrices [8]. The content of hyaluronic acid in various tissues is different: rooster combs contain 7.50 mg/mL, umbilical cords (Wharton's jelly) - 4.10 mg/mL, human intra-articular fluid - 1.50-3.60 mg/mL, vitreous humour - 0.14-0.34 mg/g, human dermis and epidermis - 0.20-0.50 and 0.10 mg/g, respectively [30]. The metabolism of hyaluronic acid in vertebrate tissues is equal to 5.0 g per day and controlled by biosynthesis (hyaluronidase activity) and enzymatic degradation [31]. Meanwhile, the hyaluronic acid metabolism in the bloodstream achieves 30-100 mg per day [32].

Apart from the animal sources, hyaluronic acid can be also obtained by biosynthesis using:

- bacteria of the genus *Streptococcus* (*uberis, equisimilis, zooepidermicus, pyogenes, equi*), *Pasteurella multocida* [22,33-36] and *Corynebacterium glutamicum* [37];
- green algae *Chlorella* pre-infected with chlorovirus [22,34,38];
- saccharomycetes *Cryptococcus neoformans* [22,34];
- molluscs (e.g. *Mytilus galloprovincialis*) [22,39].

Interestingly, hyaluronic acid is not found in the tissues of fungi, insects or plants [22,40].

On an industrial scale hyaluronic acid is usually obtained from cattle vitreous humor, rooster combs, sharkskin, and human umbilical cord [15]. Hyaluronic acid of animal origin contains endotoxin and protein residues that have high immunogenic effects [14,40]. For example, 1.0 mg of hyaluronic acid from human umbilical cord and bovine vitreous can contain over 100.0 EU of endotoxin and about 47.7 μg and 36.2 μg of protein, respectively. Hyaluronic acid from rooster combs contains only 23.0 EU of endotoxin and 1.0 μg of protein per 1.0 mg of biopolymer [41]. At the same time, bacterial technology can produce high purity hyaluronic acid with low protein and endotoxin levels [14]. Hyaluronic acid of bacterial origin produced by Sigma and Genzyme contains only 1.0-1.6 μg of protein and about 0.02 EU of endotoxin per 1.0 mg of hyaluronic acid [41]. However, the level of immunogenic effects of protein in bacterial hyaluronic acid may be higher than in animal hyaluronic acid despite the lower total protein content [3].

The molecular weight (MW) of hyaluronic acid significantly depends on the source. HA of animal origin has a very high molecular weight (up to 20,000 kDa). For example, rooster combs contain HA with a MW equal to 1200 kDa, umbilical cords with 3400 kDa, and the cattle vitreous humor with 770-1700 kDa. Bacterial HA has a molecular weight from 1000 to 4000 kDa; however, the enzymatic method allows the production of a biopolymer with a wide range of MW: from 550 kDa to 2500 kDa [42]. The molecular weight of HA also depends

on health conditions. For example, in the synovial fluid of a healthy human it is equal to 6000-7000 kDa, while in rheumatoid arthritis tissues the MW is lower: 3000-5000 kDa [12,13].

1.3. Biological properties of hyaluronic acid

The biological effects of hyaluronic acid are directly dependent on its molecular weight. HA with a molecular weight between 0.4 and 4.0 kDa acts as an inducer of heat shock proteins and has non-apoptotic properties. The biopolymer with a molecular weight of 6.0-20.0 kDa has immunostimulatory, angiogenic, and inflammatory activities. HA with molecular weight 20.0-200.0 kDa is involved in several biological processes, such as embryonic development, wound healing, and ovulation. Hyaluronic acid with a high molecular weight (> 500.0 kDa) has anti-angiogenic activity and can act as a space filler (for example, between collagen fibers) and as a natural immunosuppressant [43].

The functions of biopolymers with different molecular weights vary and require the use of monodispersed (low-polydispersed) HA for medical applications. The production of monodispersed hyaluronic acid is achieved by chemical or enzymatic depolymerization, and subsequent assembly of hyaluronic acid chains [44]. In addition, there is active current research on the mechanisms of interaction between cell surface receptors and hyaluronic acid of different molecular weights. Previously, it was assumed that HA with different molecular weights could have different effects on the same receptors; however, a recent study refutes this theory [45]. Hyaluronic acid with a molecular weight equal to 6.0 MDa formed in the tissues of the naked mole rat (*Heterocephalus glaber*) suppresses CD44 signaling, which leads to altered expression of a subset of p53 target genes. This proves the fact that high-molecular weight HA has the properties of an effective cytoprotective agent [46].

CHAPTER 2. HYALURONIC ACID AS A DRUG CARRIER

2.1. Binding ability of hyaluronic acid

As stated above, HA is involved in various biochemical and biological processes. The ability to retain water allows HA to act as a “living” barrier to changes in fluid content in tissues. HA acts as a filler of the cellular and intercellular matrices, and, considering its rheological properties along with the specific glycoprotein lubricin [47,48], it also acts as a joint lubricant [49-51]. Hyaluronic acid does not only perform the structure-forming and barrier functions, but it also plays an important role in inflammation, immune response, and tissue regeneration [22]. Due to its unique biological and physicochemical properties, HA is actively investigated as a promising biomaterial for various medical, bioengineering, pharmaceutical, food, and cosmetic applications. One of the current applications of HA is its use as a drug carrier in drug and vaccine delivery systems [52] as well as in diagnostic systems [53]. Apart from the pharmaceutical agents and biologically active substances (BAS), transplantable cells can be encapsulated into the hyaluronic acid matrix [52], that increases the relevance of research in this area and opens the possibility for personalized medicine.

Hyaluronic acid binds to receptors and causes various biological effects, some of which are shown in Figure 2.1.

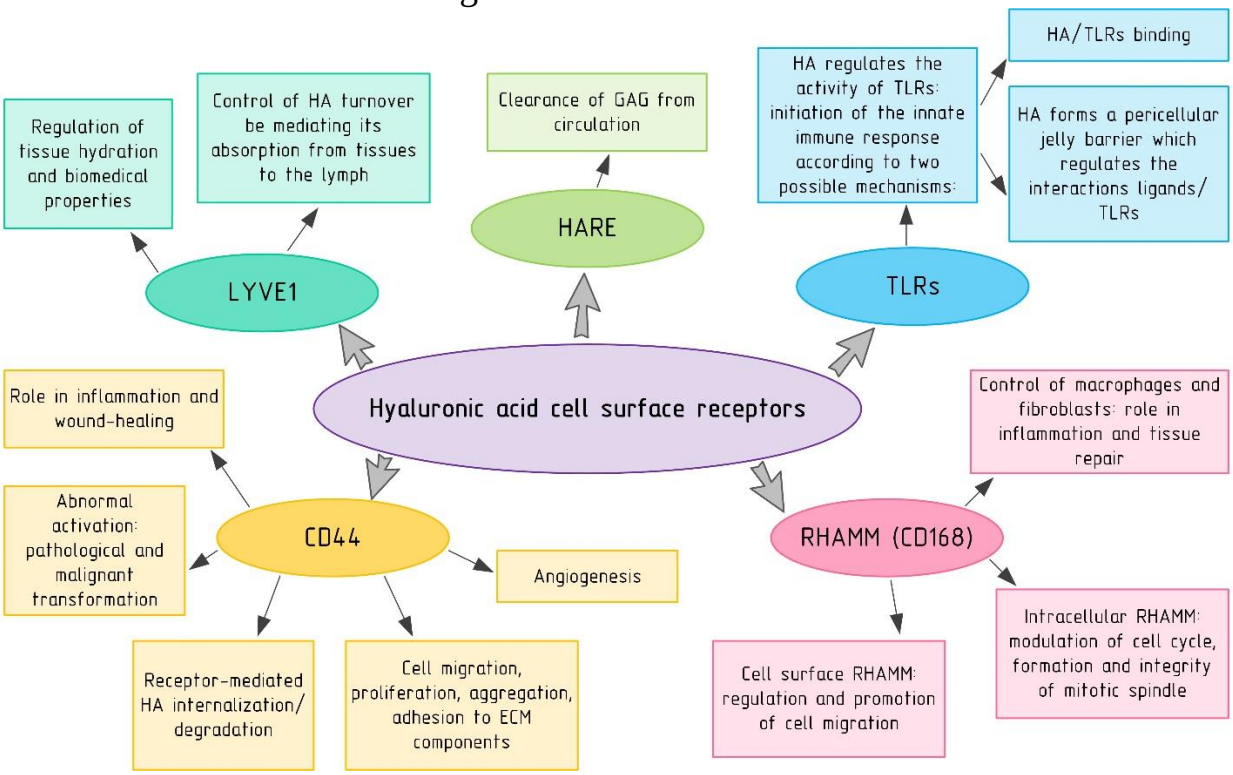

Figure 2.1 – HA cell receptors and its key functions

The use of hyaluronic acid as a drug delivery carrier for tumour treatment is based on the fact that, by binding to specific receptors, it is involved in the activation of intracellular signaling cascades associated with tumour growth and tumour cell adhesion [54]. It has been previously studied that overexpression of CD44 receptor correlates with metastasis increase [55]. CD44 is extensively overexpressed in various cancer types, for instance, gallbladder, prostate, lymphoma, breast, colon and endometrial cancer [56]. The mechanism of action of drug-loaded HA delivery systems was suggested by P. Gibbs et al. [57]. The efficacy of hyaluronic acid as a drug delivery matrix of irinotecan for the treatment of metastatic colorectal cancer was confirmed in a randomized clinical trial (phase II) [57].

2.2. Drug delivery systems based on hyaluronic acid

There are many varieties of drug delivery systems such as liposomes, dendrimers, hydrogels, magnetic nanoparticles, quantum dots, polymeric nanoparticles, etc. [58]. Polymeric nanoparticles are the most interesting and topical drug delivery systems; they are particles derived from natural or synthetic polymer, ranging in size from 10 to 1000 nm [59]. The nanoscale of particles promotes their better cell uptake (less than 1.0 μm) and provides a prolonged biological effect [60]. Biodegradability, non-toxicity, and biocompatibility of natural polymers determine the efficacy and safety of such nanoparticles as a carrier for drug delivery, which has a reduced systemic effect and decreased side effects compared to unencapsulated drugs.

Particles containing hyaluronic acid are known to have a negative electrokinetic or zeta potential, ζ-potential, [61-63] with a value of -30 mV or lower. The negative ζ-potential ensures particle stability and prevents aggregation, which allows the use of such particles for direct injection without the risk of vascular and capillary blockage.

Polymer nanoparticles can be obtained by electrohydrodynamic jetting of liquids [64]. A polymer solution with a given volume flow rate is fed through a needle (nozzle). Under the influence of electrostatic forces, an initially continuous, stationary, accelerated and thinning flow is generated, its axis coincides with the general direction of the electric field. As a result, the jet is formed in the form of a cone extending downwards [65]. Depending on the process parameters and solution properties, the jet may split into many smaller jets, resulting in the formation of fibers (electrospinning), or polymer solution droplets may occur, resulting in the formation of particles (electrospraying) [66].

The advantages of electrospinning technology are the simple hardware implementation, the variety of nanostructures produced, and the possibility of scaling up to industrial production [69]. The process of electrohydrodynamic jetting (electrospinning and electrospraying) was described in detail [67,68].

A constant high voltage source is required to support the electrohydrodynamic jetting. The electrical voltage depends on the equipment and on average ranges from 1.0 to 50 kV and above. The second key element in such process is the feeding system with a metal needle (die, nozzle). The third key element is the metal collecting electrode, which can have different shapes: flat plate, drum, disk, tube, etc. In the electrohydrodynamic jetting, high voltage is used to produce an electrically charged jet of polymer solution (or melt) coming out of the forming nozzle. The first electrode is connected to a container with the spinning solution (or a needle at the end of the feeding system), and the second electrode is connected to a precipitation electrode usually grounded [70]. Before reaching the deposition electrode, the solvent evaporates from the formed fibers, the fibers interweave a mesh on the electrode surface[71]. Varying the technological parameters of the electrohydrodynamic jetting may result in both a change in the morphology of the individual fibers and a change in the degree of fiber orientation (when using a drum collector).

The presence of a high voltage source requires a stable and uninterrupted power supply, special equipment and adherence to safety precautions. During the electrohydrodynamic jetting, the working chamber must be closed and constant air circulation through the chamber is required. Additionally, the air exiting the spinning (spraying) box must be filtered to avoid the environment pollution by organic solvent vapors. Despite these difficulties, electrohydrodynamic jetting can easily be scaled up from a laboratory level to an industrial one. Moreover, existing semi-industrial electrospinning kits can produce fibrous materials in widths of 1000 to 2000 mm in quantities of up to 5000 m^2 per day.

Magnetospinning is a new method to obtain polymeric fibers from ferromagnetic liquids by alternating magnetic field produced by rotating magnet [72]. Remarkably, the stability of fiber formation does not depend on the type of polymer used, the solvent used or the electrical conductivity of the polymer solution. The organization of magnetospinning is simple and requires no special safety rules (compared with the case of electrospinning) and can be easily scaled up to an industrial scale.

Electrospinning of magnetic nanofibers, accompanied by exposure to a magnetic field, was originally proposed by Yang et al. [73] for the purpose of “straightening” the fibers after electrospinning.

A polymer solution containing magnetic nanoparticles is fed through a needle at a constant speed to a rotating magnet. The magnet is at a fixed distance from the needle in its outermost position. Thus, a drop of polymer solution is attracted to the magnet, which then moves away from the needle, pulling the polymer “string” behind it. The needles on the rotating disc serve to wind the fiber. Therefore, the characteristics of the produced fibers depend on the polymer solution recipe, the type of magnetic nanoparticles, the speed of the disc rotation, the solution volume flow, the distance between the needle and the magnet, and the external parameters: ambient temperature and humidity.

Currently, there are several methods to obtain micro- and nanoscale fibers and particles, which are very important for modern biomedicine and bioengineering. Despite their diversity, electrospinning/electrospraying is still one of the most effective and promising methods. This method allows to obtain finished (without post processing, for example, weaving) polymeric materials for various fields of science and industry, including multilayer coatings. In comparison with other methods, electrospinning is highly efficient, cost-effective and technologically most convenient method for the fabrication of micro- and nanoscale fibers.

CHAPTER 3. TOPICAL APPLICATIONS OF POLYMERIC FIBROUS BIOMATERIALS

As promising polymer systems, nanofibers-based materials are widely used for scaffolds, as wound and burn coatings, for the targeted delivery of pharmacologically active substances (including transdermal drug transfer), for improving engraftment of implants and endoprostheses, as well as for other biomedical applications. The versatility of nanofibrous materials is due to the nature of the biopolymers used; they possess high porosity, and possibilities of adjusting their physical, mechanical and morphological properties, chemical modifying and "loading" biologically active substances into a polymer matrix. Loading of Pharmaceutical agents can be loaded both into particles and fibers. Thus, fibrous materials based on hyaluronic acid and water-soluble antibiotic kanamycin [74] and ibuprofen [75], as well as fibers containing natural bioactive substances - curcumin [76] and a mixture of curcumin and usnic acid [77] were successfully obtained and characterized.

Moreover, fibrous materials have some advantages over films based on the same polymer, as they possess better cell adhesion to the material and their proliferation, increased water and air permeability, etc. [78].

The most frequent applications of biopolymer fibrous materials are:

- Wound and burn coatings
- Targeted delivery systems
- Tissue engineering

Each area will be discussed in detail below.

3.1. Wound and burn coatings

Skin is the largest organ that protects the living organism from physical, mechanical, bacteriological, chemical, and thermal factors. The skin consists of three layers with blood vessels and nerve fibers: the epidermis, the dermis and the subcutaneous tissue (hypodermis). The outer layer of skin (epidermis) is thin, but tough and thick enough to provide a barrier function to the skin. The undamaged epidermis is waterproof and able to protect the body from bacteria and viruses. The dermis is located underneath the epidermis layer and consists of collagen with some amount of elastin and polysaccharides (mainly hyaluronic acid). Fibroblasts, being the main cell type of the dermis, are able to produce enzymes (e.g. protease, collagenase), which play an important role in the healing of wounds and burns. The dermis is also the "supplier" of cells for epidermal renewal. The hypodermis is the layer of skin beneath the dermis that contains a significant number of fat cells providing mechanical and thermoregulatory properties in the skin [79].

Wound healing is a complex biochemical process involving the interaction of various cells and matrix components. Damaged skin is regenerated by the formation of scar tissue rather than by complete skin regeneration. Covering the wound with epithelial cells is one of the most important factors of healing as it restores the intact epidermal barrier and protects the underlying tissue. Dermal cells are easier to repair, but the scar tissue formed during the healing process has less elasticity, flexibility and strength than the normal dermis. Consequently, scar tissue significantly restricts movement and causes pain. On the other hand, under some pathological conditions (e.g. diabetes) the controlled migration of keratinocytes after their division at the wound edges (re-epithelialization) does not occur, resulting in non-healing wounds. Currently available skin substitutes are unable to fully reproduce the anatomical, physiological, biological, barrier and aesthetic characteristics of intact skin. However, materials have been developed to accelerate the healing process of wounds and burns [80]. Figure 3.1 represents different stages of wound healing process.

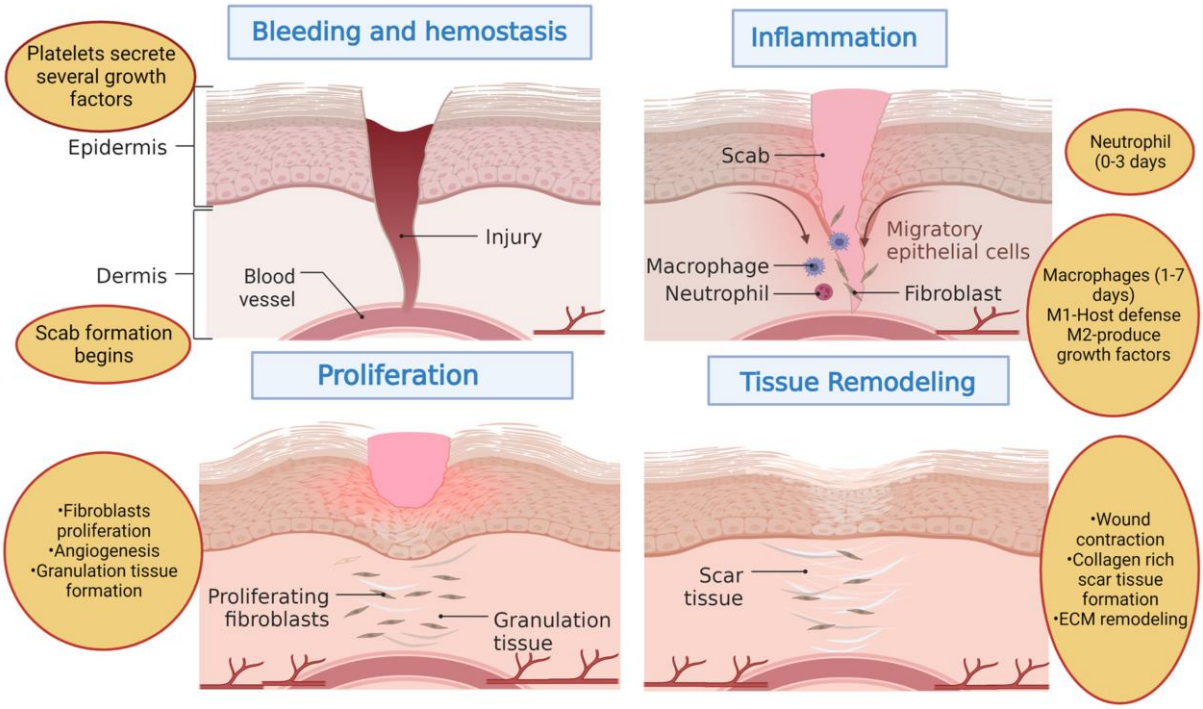

Figure 3.2 – Schematic representation of wound healing process. Reproduced from [81], with permission from MDPI, 2022.

Such materials have to satisfy certain requirements [79,82,83]:
- high biocompatibility and biodegradability;
- simplicity of application to damaged areas;
- sterility, nontoxicity, absence of allergic reactions;
- protection of the wound from mechanical factors, dust, and bacteria;
- water- and air permeability;

- stimulation of skin regeneration;
- high absorption activity in relation to an exudate;
- high adhesion to the wound.

Biocompatible synthetic polymers as well as polymers of natural origin can be used for the production of wound dressings. The latter, especially water-soluble ones, are preferred for fibrous wound healing materials due to their better adhesion and cell proliferation [83,84]. Moreover, the extensive area and porous structure of fibrous materials provides an optimal level of air exchange between the wound and the surrounding environment, control of the evaporation rate of fluid from the wound, the possibility of exudate adhesion, and a barrier function against exogenous microorganisms.

Currently, electrospun biopolymeric materials based on collagen [85-87], silk fibroin [82,88,89], chitosan [90,91], hyaluronic acid [92], alginate [93], etc. have been developed.

3.2. Drug delivery systems

Electrospinning/electrospraying of fibers and particles allows encapsulating drugs into a polymeric matrix. Liquid and solid biologically active agents can be directly loaded (immobilized) into nanofibers and nanoparticles. Thus, versatile scaffolds (shells) for the incorporation of various drugs, vaccines, and even cells could be created. In addition to pharmaceutical agents, fluorescent tags can be included for creating advanced diagnostic systems and biochemical sensors [94].

The use of hydrophobic and hydrophilic polymers helps to regulate the release rate of loaded BAS, which guarantees the possibility of prolonged and dosed release of pharmaceutical agents, vaccines, antibiotics, etc. [95-98].

The following effects can be achieved by the encapsulation of biologically active agents into polymeric matrices:

- local effect of the drug directly in the affected area;
- prolonged action of the drug;
- control of the release rate;
- minimization of side effects;
- reduction in drug dosage compared to unencapsulated forms;
- reduced effect on the immune system.

The high specific surface area of nanofibrous materials ensures efficient drug delivery and the desired therapeutic effect. The porosity of polymeric nanostructured materials enables rapid drug diffusion. Furthermore, depending on the carrier polymer, nanofibrous materials can be chemically and/or physically modified, resulting in changes in porosity, morphology, swelling rate in biological media, the rate of BAS release, etc. [99].

The polymer-drug system can be obtained through the formation of chemical compounds and the presence of hydrogen, ionic, van der Waals bonds between the functional groups of BAS and the biopolymer. Such polymers should be water-soluble, non-toxic, biodegradable and contain functional groups, have a defined hydrophilic-hydrophobic balance and molecular weight distribution (MWD), etc.

The incorporation of a drug into polymeric fibers can be achieved by the using of several approaches. Firstly, the resulting polymeric fibers can be further modified after the formation. Secondly, BAS-loaded fibers may be produced from polymer solutions containing BAS (mixture of BASs). Thirdly, core-shell systems can be created so that one of the polymers provides physical and mechanical properties, and the other one is a drug carrier [100]. The latter method is the most interesting because it allows varying the properties of both polymers, thus, nanofibrous systems with different porosity, release rates, and biodegradability can be produced [101].

A graphical representation of the currently widely used strategies for manufacturing of nanofibers containing drugs and biologically active substances is shown in Figure 3.2.

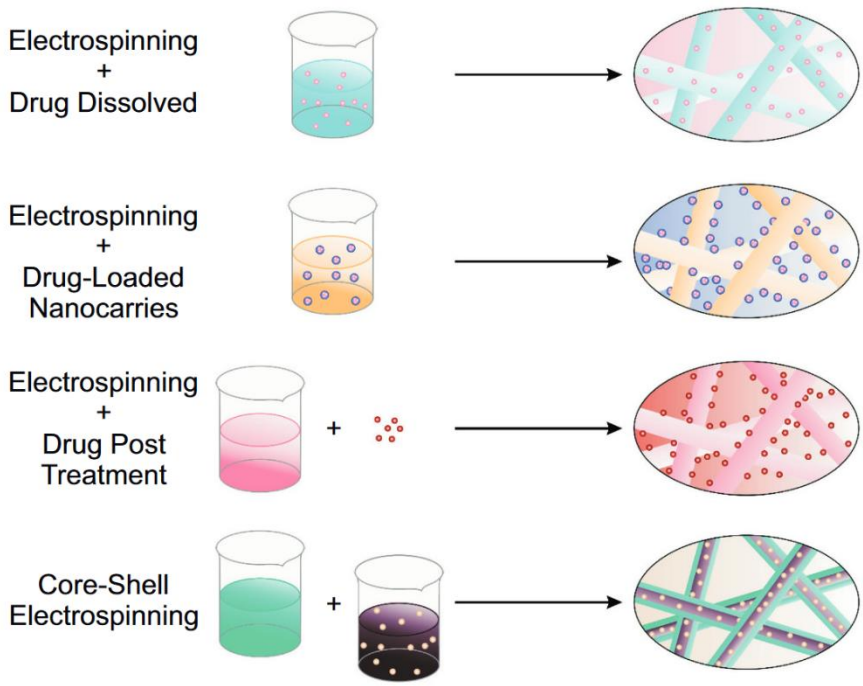

Figure 3.2 – Schematic representation of some methods for producing of drug-loaded nanofibers: (a) electrospinning + dissolved drug; (b) electrospinning + drug nanocapsules; (c) electrospinning + drug immobilization; (d) electrospinning core-shell fibers. Reproduced from [102], with permission from Bentham Science Publishers, 2022.

As mentioned above, the electrohydrodynamic jetting allows obtaining both nanofibers and nanoparticles. The size of nanoparticles plays a key role in their properties. Nanoparticles with diameters smaller than 10 nm are removed by rapid renal clearance. Nanoparticles with diameters between from 10 and 70 nm have a high penetrating capacity into capillaries with a minimum diameter of 7 μm [103]. Nanoparticles between 70 and 200 nm in diameter have the longest duration of circulation, while nanoparticles larger than 200 nm in diameter are usually metabolized by the spleen and are eventually eliminated by phagocytosis [78].

Several natural, synthetic and artificial polymers can be used as drug carriers. Natural polymers such as collagen [104], alginates [105-108], chitosan and hyaluronic acid [109-113] are preferred due to their high biocompatibility and biodegradability.

3.3. Tissue Engineering

Injuries, damages, diseases of various genesis or ageing processes in certain tissues and organs can lead to losing their ability to perform their functions, which can cause significant limitations in life. In this case, damaged tissues can be repaired/replaced by tissue engineering, which makes it possible to grow artificial tissues and organs using so-called scaffolds, i.e. cell cages.

Cell scaffolds are substrates (templates) made from porous natural or synthetic material that provide adhesion and proliferation of immobilized cells. The choice of a scaffold nanofiber structure is not accidental - these substrates have a three-dimensional porous surface and their properties are close to the extracellular matrix, which facilitates cell proliferation. Moreover, the improved physical and mechanical properties, biodegradability, controlled swelling in the model and biological media, and other specific features give nanofibrous polymeric materials a number of advantages over film-based systems. Nanoscale polymeric materials allow to control the orientation of the cell division plane and cell proliferation and limit cell growth zones.

A fully functional skin substitute can be created by using polymeric fibrous materials based on natural and/or synthetic origin as a scaffold (matrix) and various cell types in the presence of growth factors and cytokines [79]. A schematic representation of the components for the creation of a fully functional skin substitute is shown in Figure 3.3.

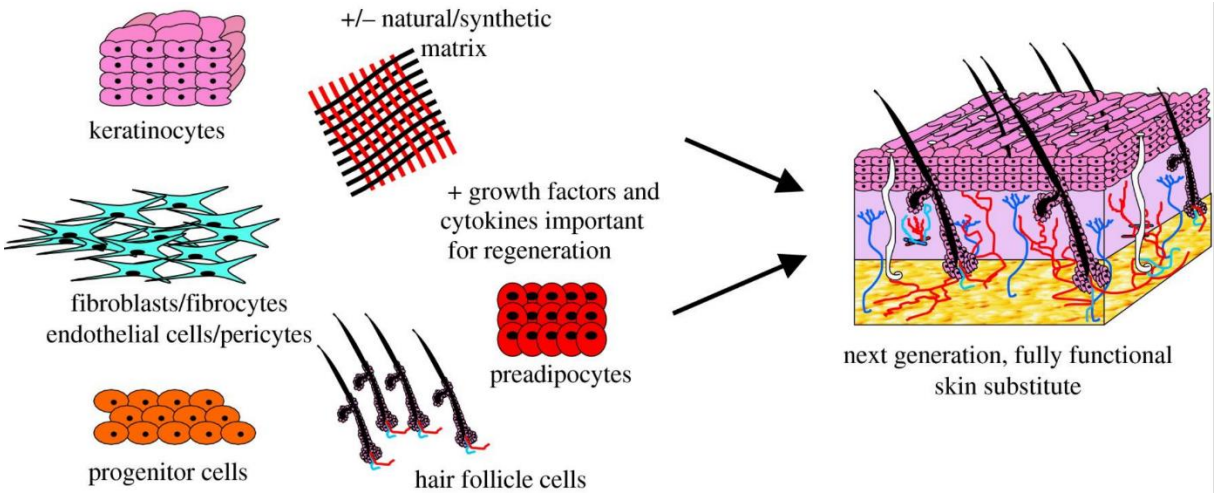

Figure 3.3 – Schematic representation of the components for making a complete skin substitute. Reproduced from [79], with permission from The Royal Society Publishing, 2022.

Electrospinning is successfully applied to scaffolds for growing artificial skin [114], blood vessels [115], cartilage and ligaments [116], bones [117], muscles [118], nerve cells and nerve fibers [119]. Nanofibrous scaffolds can be made from both synthetic polymers and natural ones; however, the latter have a number of advantages over synthetic ones, in particular, due to high biocompatibility and biodegradation. Studies show that such fibers provide the required level of adhesion, migration, proliferation and differentiation of certain cellular phenotypes. Biopolymers including collagen, chitosan, silk fibroin, hyaluronic acid, etc. significantly increase the rate of cell adhesion and proliferation and demonstrate better compatibility with the recipient's living tissues [78].

Among various natural polymers, the most promising direction is the creation and use of fibrous materials based on hyaluronic acid, which has a unique complex of structural, physical, physicochemical and biological characteristics, as well as biocompatibility and biodegradability.

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