REVIEW ARTICLE

Hyaluronan and synovial joint: function, distribution and healing

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ABSTRACT

Synovial fluid is a viscous solution found in the cavities of synovial joints. The principal role of synovial fluid is to reduce friction between the articular cartilages of synovial joints during movement. The presence of high molar mass hyaluronan (HA) in this fluid gives it the required viscosity for its function as lubricant solution. Inflammation oxidation stress enhances normal degradation of hyaluronan causing several diseases related to joints.

This review describes hyaluronan properties and distribution, applications and its function in synovial joints, with short review for using thiol compounds as antioxidants preventing HA degradations under inflammation conditions.

KEY WORDS: synovial joint fluid; hyaluronan; antioxidant; thiol compound

Introduction

The human skeleton consists of both fused and individual bones supported and supplemented by ligaments, tendons, and skeletal muscles. Articular ligaments and tendons are the main parts holding together the joint(s). In respect of movement, there are freely moveable, partially moveable, and immovable joints. Synovial joints (Figure 1), the freely moveable ones, allow for a large range of motion and encompass wrists, knees, ankles, shoulders, and hips (Kogan, 2010).

Structure of synovial joints

Cartilage

In a healthy synovial joint, heads of the bones are encased in a smooth (hyaline) cartilage layer. These tough slippery layers – e.g. those covering the bone ends in the knee joint – belong to mechanically highly stressed tissues in the human body. At walking, running, or sprinting the strokes frequency attain approximately 0.5, 2.5 or up to 10 Hz.

Cartilage functions also as a shock absorber. This property is derived from its high water entrapping capacity as well as from the structure and intermolecular interactions among polymeric components that constitute the

Figure 1. Normal, healthy synovial joint (adapted from Kogan, 2010).

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cartilage tissue (Servaty *et al.*, 2000). Figure 2 sketches a section of the cartilage – a chondrocyte cell that permanently restructures/rebuilds its extracellular matrix. Three classes of proteins exist in articular cartilage: collagens (mostly type II collagen); proteoglycans (primarily aggrecan); and other noncollagenous proteins (including link protein, fibronectin, COMP – cartilage oligomeric matrix protein) and the smaller proteoglycans (biglycan, decorin, and fibromodulin). The interaction between highly negatively charged cartilage proteoglycans and type II collagen fibrils is responsible for the compressive and tensile strength of the tissue, which resists applied load *in vivo*.

Synovium/synovial membrane

Each synovial joint is surrounded by a fibrous, highly vascular capsule/envelope called synovium, whose internal surface layer is lined with a synovial membrane. Inside this membrane, type B synoviocytes (fibroblast-like cell lines) are localized/embedded. Their primary function is to continuously extrude high-molar-mass hyaluronans (HAs) into synovial fluid.

Synovial fluid

The synovial fluid (SF) of natural joints normally functions as a biological lubricant as well as a biochemical

pool through which nutrients and regulatory cytokines traverse. SF contains molecules that provide low-friction and low-wear properties to articulating cartilage surfaces.

Molecules postulated to play a key role in lubrication alone or in combination, are proteoglycan 4 (PRG4) (Swann *et al.*, 1985) present in SF at a concentration of 0.05–0.35 mg/ml (Schmid *et al.*, 2001), hyaluronan (HA) (Ogston & Stanier, 1953) at 1–4 mg/ml (Mazzucco *et al.*, 2004), and surface-active phospholipids (SAPL) (Schwarz & Hills, 1998) at 0.1 mg/ml (Mazzucco *et al.*, 2004). Synoviocytes secrete PRG4 (Jay *et al.*, 2000; Schumacher *et al.*, 1999) and are the major source of SAPL (Dobbie *et al.*, 1995; Hills & Crawford, 2003; Schwarz & Hills, 1996), as well as HA (Haubeck *et al.*, 1995; Momberger *et al.*, 2005) in SF. Other cells also secrete PRG4, including chondrocytes in the superficial layer of articular cartilage (Schmid *et al.*, 2001b; Schumacher *et al.*, 1994) and, to a much lesser extent, cells in the meniscus (Schumacher *et al.*, 2005).

As a biochemical depot, SF is an ultra filtrate of blood plasma that is concentrated by virtue of its filtration through the synovial membrane. The synovium is a thin lining (~50 µm in humans) comprised of tissue macrophage A cells, fibroblast-like B cells (Athanasou & Quinn, 1991; Revell, 1989; Wilkinson *et al.*, 1992), and fenestrated capillaries (Knight & Levick, 1984). It is backed

Figure 2. Articular cartilage main components and structure (adapted from Chen *et al.*, 2006).

by a thicker layer (~100 µm) of loose connective tissue called the subsynovium (SUB) that includes an extensive system of lymphatics for clearance of transported molecules. The cells in the synovium form a discontinuous layer separated by intercellular gaps of several microns in width (Knight & Levick, 1984; McDonald & Levick, 1988). The extracellular matrix in these gaps contains collagen types I, III, and V (Ashhurst *et al.*, 1991; Rittig *et al.*, 1992), hyaluronan (Worrall *et al.*, 1991), chondroitin sulphate (Price *et al.*, 1996; Worrall *et al.*, 1994), biglycan and decorin proteoglycans (Coleman *et al.*, 1998), and fibronectin (Poli *et al.*, 2004). The synovial matrix provides the permeable pathway through which exchange of molecules occurs (Levick, 1994), but also offers sufficient outflow resistance (Coleman *et al.*, 1998; Scott *et al.*, 1998) to retain large solutes of SF within the joint cavity. Together, the appropriate reflection of secreted lubricants by the synovial membrane and the appropriate lubricant secretion by cells are necessary for development of a mechanically functional SF (Blewis *et al.*, 2007).

In the joint, HA plays an important role in the protection of articular cartilage and the transport of nutrients to cartilage. In patients with rheumatoid arthritis (RA), (Figure 3) it has been reported that HA acts as an anti-inflammatory substance by inhibiting the adherence of immune complexes to neutrophils through the Fc receptor (Brandt, 1970), or by protecting the synovial tissues from the attachment of inflammatory mediators (Miyazaki *et al.*, 1983; Mendichi & Soltes, 2002).

Reactive oxygen species (ROS) ($O_2^{\bullet-}$, H_2O_2 , $\bullet OH$) are generated in abundance by synovial neutrophils from RA patients, as compared with synovial neutrophils of osteoarthritis (OA) patients and peripheral neutrophils of both RA and OA patients (Niwa *et al.*, 1983).

McCord (1973) demonstrated that HA was susceptible to degradation by ROS *in vitro*, and that this could be protected by superoxide dismutase (SOD) and/or catalase, which suggests the possibility that there is pathologic oxidative damage to synovial fluid components in RA patients. Dahl *et al.* (1985) reported that there are reduced HA concentrations in synovial fluids from RA patients. It has also been reported that ROS scavengers inhibit the degradation of HA by ROS (Soltes, 2010; Blake *et al.*, 1981; Betts & Cleland, 1982; Soltes *et al.*, 2004).

These findings appear to support the hypothesis that ROS are responsible for the accelerated degradation of HA in the rheumatoid joint. In the study of Juranek and Soltes (2012) the oxygen radical scavenging activities of synovial fluids from both RA and OA patients were assessed, and the antioxidant activities of these synovial fluids were analyzed by separately examining HA, D-glucuronic acid, and N-acetyl-D-glucosamine.

Hyaluronan

In 1934, Karl Meyer and his colleague John Palmer isolated a previously unknown chemical substance from the vitreous body of cows' eyes. They found that the substance

NORMAL JOINT

JOINT AFFECTED BY RHEUMATOID ARTHRITIS

Figure 3. Normal, (healthy) and rheumatoid arthritis synovial joint.

contained two sugar molecules, one of which was uronic acid. For convenience, therefore, they proposed the name “hyaluronic acid”. The popular name is derived from “hyalos”, which is the Greek word for glass + uronic acid (Meyer & Palmer, 1934). At the time, they did not know that the substance which they had discovered would prove to be one of the most interesting and useful natural macromolecules. HA was first used commercially in 1942

when Endre Balazs applied for a patent to use it as a substitute for egg white in bakery products (Necas *et al.*, 2008).

The term “hyaluronan” was introduced in 1986 to conform to the international nomenclature of polysaccharides and is attributed to Endre Balazs (Balazs *et al.*, 1986) who coined it to encompass the different forms the molecule can take, e.g, the acid form, hyaluronic acid, and the salts, such as sodium hyaluronate, which forms at physiological pH (Laurent, 1989). HA was subsequently isolated from many other sources and the physicochemical structure properties and biological role of this polysaccharide were studied in numerous laboratories (Kreil, 1995). This work has been summarized in a Ciba Foundation Symposium (Laurent, 1989) and a recent review (Laurent & Fraser, 1992; Chabrecek *et al.*, 1990; Orvisky *et al.*, 1992).

Hyaluronan (Figure 4) is a unique biopolymer composed of repeating disaccharide units formed by *N*-acetyl- β -D-glucosamine and β -D-glucuronic acid. Both sugars are spatially related to glucose which in the β -configuration allows all of its bulky groups (the hydroxyls, the carboxylate moiety, and the anomeric carbon on the adjacent sugar) to be in sterically favorable equatorial positions while all of the small hydrogen atoms occupy the less sterically favorable axial positions. Thus, the structure of the disaccharide is energetically very stable. HA is also unique in its size, reaching up to several million Daltons and is synthesized at the plasma membrane rather than in the Golgi, where sulfated glycosaminoglycans are added to protein cores (Itano & Kimata, 2002; Weigel *et al.*, 1997; Kogan *et al.*, 2007a).

In a physiological solution, the backbone of a HA molecule is stiffened by a combination of the chemical structure of the disaccharide, internal hydrogen bonds, and interactions with the solvent. The axial hydrogen atoms form a non-polar, relatively hydrophobic face while the equatorial side chains form a more polar, hydrophilic face, thereby creating a twisting ribbon structure. Solutions of hyaluronan manifest very unusual rheological properties and are exceedingly lubricious and very hydrophilic. In solution, the hyaluronan polymer chain takes on the form of an expanded, random coil. These chains entangle with each other at very low concentrations, which may contribute to the unusual rheological properties. At higher concentrations, solutions have an extremely high but shear-dependent viscosity. A 1% solution is like jelly, but when it is put under pressure it moves easily and can be administered through a small-bore needle. It has therefore been called a “pseudo-plastic” material. The extraordinary rheological properties of hyaluronan solutions make them ideal as lubricants. There is evidence

that hyaluronan separates most tissue surfaces that slide along each other. The extremely lubricious properties of hyaluronan have been shown to reduce postoperative adhesion formation following abdominal and orthopedic surgery. As mentioned, the polymer in solution assumes a stiffened helical configuration, which can be attributed to hydrogen bonding between the hydroxyl groups along the chain. As a result, a coil structure is formed that traps approximately 1000 times its weight in water (Chabrecek *et al.*, 1990; Cowman & Matsuoka, 2005; Schiller *et al.*, 2011)

Properties of hyaluronan

Hyaluronan networks

The physico-chemical properties of hyaluronan were studied in detail from 1950 onwards (Comper & Laurent, 1978).

The molecules behave in solution as highly hydrated randomly kinked coils, which start to entangle at concentrations of less than 1 mg/mL. The entanglement point can be seen both by sedimentation analysis (Laurent *et al.*, 1960) and viscosity (Morris *et al.*, 1980). More recently Scott and his group have given evidence that the chains when entangling also interact with each other and form stretches of double helices so that the network becomes mechanically more firm (Scott *et al.*, 1991).

Rheological properties

Solutions of hyaluronan are viscoelastic and the viscosity is markedly shearing dependent (Morris *et al.*, 1980; Gibbs *et al.*, 1968). Above the entanglement point the viscosity increases rapidly and exponentially with concentration ($\sim c^{3.3}$) (Morris *et al.*, 1980) and a solution of 10 g/l may have a viscosity at low shear of $\sim 10^6$ times the viscosity of the solvent. At high shear the viscosity may drop as much as $\sim 10^3$ times (Gibbs *et al.*, 1968). The elasticity of the system increases with increasing molecular weight and concentration of hyaluronan as expected for a molecular network. The rheological properties of hyaluronan have been connected with lubrication of joints and tissues and hyaluronan is commonly found in the body between surfaces that move along each other, for example cartilage surfaces and muscle bundles (Bothner & Wik, 1987).

Water homeostasis

A fixed polysaccharide network offers a high resistance to bulk flow of solvent (Comper & Laurent, 1978). This was demonstrated by Day (1950) who showed that hyaluronidase treatment removes a strong hindrance to water flow through a fascia. Thus HA and other polysaccharides prevent excessive fluid fluxes through tissue compartments. Furthermore, the osmotic pressure of a hyaluronan solution is non-ideal and increases exponentially with the concentration. In spite of the high molecular weight of the polymer the osmotic pressure of a 10 g/l hyaluronan solution is of the same order as an 10 g/l albumin solution. The exponential relationship makes hyaluronan and other polysaccharides excellent osmotic buffering substances – moderate changes in concentration lead

Figure 4. Structural formula of hyaluronan – the acid form.

to marked changes in osmotic pressure. Flow resistance together with osmotic buffering makes hyaluronan an ideal regulator of the water homeostasis in the body.

Network interactions with other macromolecules

The hyaluronan network retards the diffusion of other molecules (Comper & Laurent, 1978; Simkovic *et al.*, 2000). It can be shown that it is the steric hindrance which restricts the movements and not the viscosity of the solution. The larger the molecule the more it will be hindered. *In vivo* hyaluronan will therefore act as a diffusion barrier and regulate the transport of other substances through the intercellular spaces. Furthermore, the network will exclude a certain volume of solvent for other molecules; the larger the molecule the less space will be available to it (Comper & Laurent, 1978). A solution of 10 g/l of hyaluronan will exclude about half of the solvent to serum albumin. Hyaluronan and other polysaccharides therefore take part in the partition of plasma proteins between the vascular and extravascular spaces. The excluded volume phenomenon will also affect the solubility of other macromolecules in the interstitium, change chemical equilibria and stabilize the structure of, for example, collagen fibers.

Medical applications of hyaluronic acid

The viscoelastic matrix of HA can act as a strong bio-compatible support material and is therefore commonly used as growth scaffold in surgery, wound healing and embryology. In addition, administration of purified high molecular weight HA into orthopaedic joints can restore the desirable rheological properties and alleviate some of the symptoms of osteoarthritis (Balazs & Denlinger, 1993; Balazs & Denlinger, 1989; Kogan *et al.*, 2007). The success of the medical applications of HA has led to the production of several successful commercial products, which have been extensively reviewed previously.

Table 1 summarizes both the medical applications and the commonly used commercial preparations containing HA used within this field. HA has also been extensively studied in ophthalmic, nasal and parenteral drug delivery. In addition, more novel applications including pulmonary, implantation and gene delivery have also been suggested. Generally, HA is thought to act as either a mucoadhesive and retain the drug at its site of action/absorption or to modify the *in vivo* release/absorption rate of the therapeutic agent. A summary of the drug delivery applications of HA is shown in Table 2.

Table 1. Summary of the medical applications of hyaluronic acid (Brown & Jones, 2005).

Disease state	Applications	Commercial products	Publications
Osteoarthritis	Lubrication and mechanical support for the joints	Hyalgan® (Fidia, Italy) Artz® (Seikagaku, Japan) ORTHOVISC® (Anika, USA) Healon®, Opegan® and Opelead®	Hochburg, 2000; Altman, 2000; Dougados, 2000; Guidolin <i>et al.</i> , 2001; Maheu <i>et al.</i> , 2002; Barrett & Siviero, 2002; Miltner <i>et al.</i> , 2002; Tascioglu and Oner, 2003; Uthman <i>et al.</i> , 2003; Kelly <i>et al.</i> , 2003; Hamburger <i>et al.</i> , 2003; Kirwan, 2001; Ghosh & Guidolin, 2002; Mabuchi <i>et al.</i> , 1999; Balazs, 2003; Fraser <i>et al.</i> , 1993; Zhu & Granick, 2003.
Surgery and wound healing	Implantation of artificial intraocular lens, viscoelastic gel	Bionect®, Connettivina® and Jossalind®	Ghosh & Jassal, 2002; Risbert, 1997; Inoue & Katakami, 1993; Miyazaki <i>et al.</i> , 1996; Stiebel-Kalish <i>et al.</i> , 1998; Tani <i>et al.</i> , 2002; Vazquez <i>et al.</i> , 2003; Soldati <i>et al.</i> , 1999; Ortonne, 1996; Cantor <i>et al.</i> , 1998; Turino & Cantor, 2003.
Embryo implantation	Culture media for the use of <i>in vitro</i> fertilization	EmbryoGlue® (Vitrolife, USA)	Simon <i>et al.</i> , 2003; Gardner <i>et al.</i> , 1999; Vanos <i>et al.</i> , 1991; Kemmann, 1998; Suchanek <i>et al.</i> , 1994; Joly <i>et al.</i> , 1992; Gardner, 2003; Lane <i>et al.</i> , 2003; Figueiredo <i>et al.</i> , 2002; Miyano <i>et al.</i> , 1994; Kano <i>et al.</i> , 1998; Abeydeera, 2002; Jaakma <i>et al.</i> , 1997; Furnus <i>et al.</i> , 1998; Jang <i>et al.</i> , 2003.

Table 2. Summary of the drug delivery applications of hyaluronic acid.

Route	Justification	Therapeutic agents	Publications
Ophthalmic	Increased ocular residence of drug, which can lead to increased bioavailability	Pilocarpine, tropicamide, timolol, gentimycin, tobramycin, arecaidine polyester, (S) aceclidine	Jarvinen <i>et al.</i> , 1995; Sasaki <i>et al.</i> , 1996; Gurny <i>et al.</i> , 1987; Camber <i>et al.</i> , 1987; Camber & Edman, 1989; Saettone <i>et al.</i> , 1994; Saettone <i>et al.</i> , 1991; Bucolo <i>et al.</i> , 1998; Bucolo & Mangiafico, 1999; Herrero-Vanrell <i>et al.</i> , 2000; Moreira <i>et al.</i> , 1991; Bernatchez <i>et al.</i> , 1993; Gandolfi <i>et al.</i> , 1992; Langer <i>et al.</i> , 1997.
Nasal	Bioadhesion resulting in increased bioavailability	Xylometazoline, vasopressin, gentamycin	Morimoto <i>et al.</i> , 1991; Lim <i>et al.</i> , 2002.
Pulmonary	Absorption enhancer and dissolution rate modification	Insulin	Morimoto <i>et al.</i> , 2001; Surendrakumar <i>et al.</i> , 2003.
Parenteral	Drug carrier and facilitator of liposomal entrapment	Taxol, superoxide dismutase, human recombinant insulin-like growth factor, doxorubicin	Drobnik, 1991; Sakurai <i>et al.</i> , 1997; Luo and Prestwich, 1999; Luo <i>et al.</i> , 2000; Prisell <i>et al.</i> , 1992; Yerushalmi <i>et al.</i> , 1994; Yerushalmi & Margalit, 1998; Peer & Margalit, 2000; Eliaz & Szoka, 2001; Peer <i>et al.</i> , 2003.
Implant	Dissolution rate modification	Insulin	Surini <i>et al.</i> , 2003; Takayama <i>et al.</i> , 1990.
Gene	Dissolution rate modification and protection	Plasmid DNA/monoclonal antibodies	Yun <i>et al.</i> , 2004; Kim <i>et al.</i> , 2003.

Cosmetic uses of hyaluronic acid

HA has been extensively utilized in cosmetic products because of its viscoelastic properties and excellent biocompatibility. Application of HA containing cosmetic products to the skin is reported to moisturize and restore elasticity, thereby achieving an antiwrinkle effect, albeit so far no rigorous scientific proof exists to substantiate this claim. HA-based cosmetic formulations or sunscreens may also be capable of protecting the skin against ultraviolet irradiation due to the free radical scavenging properties of HA (Manuskiatti & Maibach, 1996).

HA, either in a stabilized form or in combination with other polymers, is used as a component of commercial dermal fillers (*e.g.* Hylaform®, Restylane® and Dermalive®) in cosmetic surgery. It is reported that injection of such products into the dermis, can reduce facial lines and wrinkles in the long term with fewer side-effects and better tolerability compared with the use of collagen (Duranti *et al.*, 1998; Bergeret-Galley *et al.*, 2001; Leyden *et al.*, 2003). The main side-effect may be an allergic reaction, possibly due to impurities present in HA (Schartz, 1997; Glogau, 2000).

Biological function of hyaluronan

Naturally, hyaluronan has essential roles in body functions according to organ type in which it is distributed (Laurent *et al.*, 1996).

Space filler

The specific functions of hyaluronan in joints are still essentially unknown. The simplest explanation for its presence would be that a flow of hyaluronan through the joint is needed to keep the joint cavity open and thereby allow extended movements of the joint. Hyaluronan is constantly secreted into the joint and removed by the synovium. The total amount of hyaluronan in the joint cavity is determined by these two processes. The half-life of the polysaccharide at steady-state is in the order of 0.5–1 day in rabbit and sheep (Brown *et al.*, 1991; Fraser *et al.*, 1993). The volume of the cavity is determined by the pressure conditions (hydrostatic and osmotic) in the cavity and its surroundings. Hyaluronan could, by its osmotic contributions and its formation of flow barriers in the limiting layers, be a regulator of the pressure and flow rate (McDonald & Leviek, 1995). It is interesting that in fetal development the formation of joint cavities is parallel with a local increase in hyaluronan (Edwards *et al.*, 1994).

Lubrication

Hyaluronan has been regarded as an ideal lubricant in the joints due to its shear-dependent viscosity (Ogston & Stanier, 1953) but its role in lubrication has been refuted by others (Radin *et al.*, 1970). However, there are now reasons to believe that the function of hyaluronan is to form a film between the cartilage surfaces. The load on the joints may press out water and low-molecular solutes from the hyaluronan layer into the cartilage matrix. As a

result, the concentration of hyaluronan increases and a gel structure of micrometric thickness is formed which protects the cartilage surfaces from frictional damage (Hlavacek, 1993). This mechanism to form a protective layer is much less effective in arthritis when the synovial hyaluronan has both a lower concentration and a lower molecular weight than normal. Another change in the arthritic joint is the protein composition of the synovial fluid. Fraser *et al.* (1972) showed more than 40 years ago that addition of various serum proteins to hyaluronan substantially increased the viscosity and this has received a renewed interest in view of recently discovered hyaladherins (see above). TSG-6 and inter- α -trypsin inhibitor and other acute phase reactants such as haptoglobin are concentrated to arthritic synovial fluid (Hutadilok *et al.*, 1988). It is not known to what extent these are affecting the rheology and lubricating properties.

Scavenger functions

Hyaluronan has also been assigned scavenger functions in the joints. It has been known since the 1940s that hyaluronan is degraded by various oxidizing systems and ionizing irradiation and we know today that the common denominator is a chain cleavage induced by free radicals, essentially hydroxy radicals (Myint *et al.*, 1987). Through this reaction hyaluronan acts as a very efficient scavenger of free radicals. Whether this has any biological importance in protecting the joint against free radicals is unknown. The rapid turnover of hyaluronan in the joints has led to the suggestion that it also acts as a scavenger for cellular debris (Laurent *et al.*, 1995). Cellular material could be caught in the hyaluronan network and removed at the same rate as the polysaccharide (Stankovska *et al.*, 2007; Rapt, *et al.*, 2009).

Regulation of cellular activities

As discussed above, more recently proposed functions of hyaluronan are based on its specific interactions with hyaladherins. One interesting aspect is the fact that hyaluronan influences angiogenesis but the effect is different depending on its concentration and molecular weight (Sattar *et al.*, 1992). High molecular weight and high concentrations of the polymer inhibit the formation of capillaries, while oligosaccharides can induce angiogenesis. There are also reports of hyaluronan receptors on vascular endothelial cells by which hyaluronan could act on the cells (Edwards *et al.*, 1995). The avascularity of the joint cavity could be a result of hyaluronan inhibition of angiogenesis.

Another interaction of some interest in the joint is the binding of hyaluronan to cell surface proteins. Lymphocytes and other cells may find their way to joints through this interaction. Injection of high doses of hyaluronan intra-articularly could attract cells expressing these proteins. Cells can also change their expression of hyaluronan-binding proteins in states of disease, whereby hyaluronan may influence immunological reactions and cellular traffic in the path of physiological processes in cells (Edwards *et al.*, 1995). The observation often

reported that intra-articular injections of hyaluronan alleviate pain in joint disease (Adams, 1993) may indicate a direct or indirect interaction with pain receptors.

Hyaluronan and synovial fluid

In normal/healthy joint, the synovial fluid, which consists of an ultrafiltrate of blood plasma and glycoproteins contains HA macromolecules of molar mass ranging between 6–10 mega Daltons (Praest *et al.*, 1997). SF serves also as a lubricating and shock absorbing boundary layer between moving parts of synovial joints. SF reduces friction and wear and tear of the synovial joint playing thus a vital role in the lubrication and protection of the joint tissues from damage during motion (Oates *et al.*, 2002).

As SF of healthy humans exhibits no activity of hyaluronidase, it has been inferred that oxygen-derived free radicals are involved in a self-perpetuating process of HA catabolism within the joint (Grootveld *et al.*, 1991; Stankovska *et al.*, 2006; Rychly *et al.*, 2006). This radical-mediated process is considered to account for ca. twelve-hour half-life of native HA macromolecules in SF.

Acceleration of degradation of high-molecular-weight HA occurring under inflammation and/or oxidative stress is accompanied by impairment and loss of its viscoelastic properties (Parsons *et al.*, 2002; Soltes *et al.*, 2005; Stankovska *et al.*, 2005; Lath *et al.*, 2005; Hrabarova *et al.*, 2007; Valachova & Soltes, 2010; Valachova *et al.*, 2013a). Low-molecular weight HA was found to exert different biological activities compared to the native high-molecular-weight biopolymer. HA chains of 25–50 disaccharide units are inflammatory, immune-stimulatory, and highly angiogenic. HA fragments of this size appear to function as endogenous danger signals, reflecting tissues under stress (Noble, 2002; West *et al.*, 1985; Soltes *et al.*, 2007; Stern *et al.*, 2007; Soltes & Kogan, 2009). Figure 5 describes the fragmentation mechanism of HA under free radical stress.

- Initiation phase: the intact hyaluronan macromolecule entering the reaction with the HO• radical formed via the Fenton-like reaction:

$$\text{Cu}^+ + \text{H}_2\text{O}_2 \rightarrow \text{Cu}^{2+} + \text{HO}^\bullet + \text{OH}^-$$

$$\text{H}_2\text{O}_2$$
 has its origin due to the oxidative action of the Weissberger system (see Figure 6)
- Formation of an alkyl radical (C-centered hyaluronan macroradical) initiated by the HO• radical attack.
- Propagation phase: formation of a peroxy-type C-macroradical of hyaluronan in a process of oxygenation after entrapping a molecule of O₂.
- Formation of a hyaluronan-derived hydroperoxide via the reaction with another hyaluronan macromolecule.
- Formation of highly unstable alkoxy-type C-macroradical of hyaluronan on undergoing a redox reaction with a transition metal ion in a reduced state.

- Termination phase: quick formation of alkoxy-type C-fragments and the fragments with a terminal C=O group due to the glycosidic bond scission of hyaluronan. Alkoxy-type C fragments may continue the propagation phase of the free-radical hyaluronan degradation reaction. Both fragments are represented by reduced molar masses (Kogan, 2011; Rychly *et al.*, 2006; Hrabarova *et al.*, 2012; Surovcikova *et al.*, 2012; Valachova *et al.*, 2013b; Banasova *et al.*, 2012).

Several thiol compounds have attracted much attention from pharmacologists because of their reactivity toward endobiotics such as hydroxyl radical-derived species. Thiols play an important role as biological reductants (antioxidants) preserving the redox status of cells and protecting tissues against damage caused by the elevated reactive oxygen/nitrogen species (ROS/RNS) levels, by which oxidative stress might be indicated.

Soltes and his coworkers examined the effect of several thiol compounds on inhibition of the degradation kinetics of a high-molecular-weight HA *in vitro*. High molecular weight hyaluronan samples were exposed to free-radical chain degradation reactions induced by ascorbate in the presence of Cu(II) ions, the so called

Figure 5. Schematic degradation of HA under free radical stress (Hrabarova *et al.*, 2012).

Weissberger's oxidative system. The concentrations of both reactants [ascorbate, Cu(II)] were comparable to those that may occur during an early stage of the acute phase of joint inflammation (see Figure 6) (Banasova *et al.*, 2011; Valachova *et al.*, 2011; Soltes *et al.*, 2006a; Soltes *et al.*, 2006b; Stankovska *et al.*, 2004; Soltes *et al.*, 2006c; Soltes *et al.*, 2007; Valachova *et al.*, 2008; 2009; 2010; 2011; 2013; Hrabarova *et al.*, 2009, 2011; Rapta *et al.*, 2009; 2010; Surovcikova-Machova *et al.*, 2012; Banasova *et al.*, 2011; Drafi *et al.*, 2010; Fisher & Naughton, 2005).

Figure 7 illustrates the dynamic viscosity of hyaluronan solution in the presence and absence of bucillamine, D-penicillamine and L-cysteine as inhibitors for free radical degradation of HA. The study showed that bucillamine to be both a preventive and chain-breaking antioxidant. On the other hand, D-penicillamine and L-cysteine dose dependently act as scavenger of $\cdot\text{OH}$ radicals within the first 60 min. Then, however, the inhibition activity is lost and degradation of hyaluronan takes place (Valachova *et al.*, 2011; Valachova *et al.*, 2009; 2010; Hrabarova *et al.*, 2009).

L-Glutathione (GSH; L- γ -glutamyl-L-cysteinyl-glycine; a ubiquitous endogenous thiol, maintains the intracellular reduction-oxidation (redox) balance and regulates signaling pathways during oxidative stress/conditions. GSH is mainly cytosolic in the concentration range of ca. 1–10 mM; however, in the plasma as well as in SF, the range is only 1–3 μM (Haddad & Harb, 2005). This unique thiol plays a crucial role in antioxidant defense, nutrient metabolism, and in regulation of pathways essential for the whole body homeostasis. Depletion of GSH results in an increased vulnerability of the cells to oxidative stress (Hultberg & Hultberg, 2006).

It was found that L-glutathione exhibited the most significant protective and chain-breaking antioxidative effect against hyaluronan degradation. Thiol antioxidative activity, in general, can be influenced by many factors such as various molecule geometry, type of functional groups, radical attack accessibility, redox potential, thiol concentration and pK_a , pH, ionic strength of solution, as well as different ability to interact with transition metals (Hrabarova *et al.*, 2012).

Figure 8 shows the dynamic viscosity versus time profiles of HA solution stressed to degradation with Weissberger's oxidative system. As evident, addition of different concentrations of GSH resulted in a marked protection of the HA macromolecules against degradation. The greater the GSH concentration used, the longer was the observed stationary interval in the sample viscosity values. At the lowest GSH concentration used, *i.e.* 1.0 μM (Figure 8), the time-dependent course of the HA degradation was more rapid than that of the reference experiment with the zero thiol concentration. Thus, one could classify GSH traces as functioning as a pro-oxidant.

The effectiveness of antioxidant activity of 1,4-dithioerythritol expressed as the radical scavenging capacity was studied by a rotational viscometry method (Hrabarova *et al.*, 2010). 1,4-dithioerythritol, widely accepted and used as an effective antioxidant in the field of enzyme and protein oxidation, is a new potential antioxidant standard exhibiting very good solubility in a variety of solvents. Figure 9 describes the effect of 1,4-dithioerythritol on

degradation of HA solution under free radical stress (Hrabarova *et al.*, 2010).

N-Acetyl-L-cysteine (NAC), another significant precursor of the GSH biosynthesis, has broadly been used as effective antioxidant in a form of nutritional supplement (Soloveva *et al.*, 2007; Thibodeau *et al.*, 2001). At low concentrations, it is a powerful protector of α_1 -antiproteinase against the enzyme inactivation by HOCl . NAC reacts with $\text{HO}^\bullet$ radicals and slowly with H_2O_2 ; however, no reaction of this endobiotic with superoxide anion radical was detected (Aruoma *et al.*, 1989).

Investigation of the antioxidative effect of *N*-Acetyl-L-cysteine. Unlike L-glutathione, *N*-acetyl-L-cysteine was found to have preferential tendency to reduce Cu(II) ions to Cu(I) , forming *N*-acetyl-L-cysteinyl radical that may subsequently react with molecular O_2 to give $\text{O}_2^{\bullet-}$ (Soloveva *et al.*, 2007; Thibodeau *et al.*, 2001). Contrary to L-cysteine, NAC (25 and 50 μM), when added at the beginning of the reaction, exhibited a clear antioxidative effect within ca. 60 and 80 min, respectively (Figure 10A). Subsequently, NAC exerted a modest pro-oxidative effect, more profound at 25- μM than at 100- μM concentration (Figure 10A).

Figure 11. Evaluation of antioxidative effects of cysteamine against high-molar-mass hyaluronan degradation *in vitro* induced by Weissberger's oxidative system. Reference sample (black): 1 mM CuII ions plus 100 μM ascorbic acid; nil thiol concentration. Cysteamine addition at the onset of the reaction (a) and after 1 h (b) (25, 50, 100 μM). (Hrabarova *et al.*, 2012).

Application of NAC 1 h after the onset of the reaction (Figure 10B) revealed its partial inhibitory effect against formation of the peroxy-type radicals, independently from the concentration applied (Hrabarova *et al.*, 2012).

An endogenous amine, cysteamine (CAM) is a cystine-depleting compound with antioxidative and anti-inflammatory properties; it is used for treatment of cystinosis – a metabolic disorder caused by deficiency of the lysosomal cystine carrier. CAM is widely distributed in organisms and considered to be a key regulator of essential metabolic pathways (Kessler *et al.*, 2008).

Investigation of the antioxidative effect of cysteamine. Cysteamine (100 μM), when added before the onset of the reaction, exhibited an antioxidative effect very similar to that of GSH (Figure 8A and Figure 11A). Moreover, the same may be concluded when applied 1 h after the onset of the reaction (Figure 11B) at the two concentrations (50 and 100 μM), suggesting that CAM may be an excellent scavenger of peroxy radicals generated during the peroxidative degradation of HA (Hrabarova *et al.*, 2012).

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REFERENCES

Abeydeera LR. (2002). *In vitro* production of embryos in swine. *Theriogenology* **57**: 257–273.

- Adams ME. (1993). Viscosupplementation: A treatment for osteoarthritis. *J Rheumatol* **20**: Suppl. 39: 1–24.
- Altman RD. (2000). Intra-articular sodium hyaluronate in osteoarthritis of the knee. *Semin Arthritis Rheum* **30**: 11–18.
- Aruoma OI, Halliwell B, Hoey BM, Butler J. (1989). The antioxidant action of N-acetylcysteine: its reaction with hydrogen peroxide, hydroxyl radical, superoxide, and hypochlorous acid. *Free Radic Biol Med* **6**: 593.
- Ashurst DE, Bland YS, Levick JR. (1991). An immunohistochemical study of the collagens of rabbit synovial interstitium. *J Rheumatol* **18**: 1669–1672.
- Athanasou NA, Quinn J. (1991). Immunocytochemical analysis of human synovial lining cells: phenotypic relation to other marrow derived cells. *Ann Rheum Dis* **50**: 311–315.
- Balazs EA, Denlinger JL. (1989). Clinical uses of hyaluronan. *Ciba Found Symp* **143**: 265–280.
- Balazs EA, Laurent TC, Jeanloz RW. (1986). Nomenclature of hyaluronic acid. *Biochemical Journal* **235**: 903.
- Balazs EA. (2003). Analgesic effect of elastoviscous hyaluronan solutions and the treatment of arthritic pain. *Cells Tissues Organs* **174**: 49–62.
- Balazs EA, Denlinger JL. (1993). Viscosupplementation: a new concept in the treatment of osteoarthritis. *J Rheumatol* **20**: 3–9.
- Banasova M, Valachova K, Juranek I, Soltes L. (2012). Effect of thiol compounds on oxidative degradation of high molar hyaluronan *in vitro*. *Interdiscip Toxicol* **5**(Suppl. 1): 25–26.
- Banasova M, Valachova K, Juranek I, Soltes L. (2013b). Aloe vera and methylsulfonylmethane as dietary supplements: Their potential benefits for arthritic patients with diabetic complications. *Journal of Information Intelligence and Knowledge* **5**: 51–68.
- Banasova M, Valachova K, Rychly J, Priesolova E, Nagy M, Juranek I, Soltes L. (2011). Scavenging and chain breaking activity of buccillamine on free-radical mediated degradation of high molar mass hyaluronan. *ChemZi* **7**: 205–206.
- Baňasová M, Valachová K, Hrabárová E, Priesolová E, Nagy M, Juránek I, Šoltés L. (2011). Early stage of the acute phase of joint inflammation. *In vitro* testing of buccillamine and its oxidized metabolite SA981 in the function of antioxidants. 16th Interdisciplinary Czech-Slovak Toxicological Conference in Prague. *Interdiscip Toxicol* **4**(2): 22.
- Barrett J P, Siviero P. (2002). Retrospective study of outcomes in Hyalgan(R)-treated patients with osteoarthritis of the knee. *Clin Drug Invest* **22**: 87–97.
- Bergeret-Galley C, Latouche X, Illouz Y G. (2001). The value of a new filler material in corrective and cosmetic surgery: DermaLive and DermaDeep. *Aesthetic Plast Surg* **25**: 249–255.

- Bernatchez SF, Tabatabay C, Gurny R. (1993). Sodium hyaluronate 0.25-percent used as a vehicle increases the bioavailability of topically administered gentamicin. *Graefes Arch Clin Exp Ophthalmol* **231**: 157–161.
- Betts WH, Cleland LG. (1982). Effect of metal chelators and antiinflammatory drugs on the degradation of hyaluronic acid. *Arthritis Rheum* **25**: 1469–1476.
- Blake DR, Hall ND, Treby DA. (1981). Protection against superoxide and hydrogen peroxide in synovial fluid from rheumatoid patients. *Clin Sci* **61**: 483–486.
- Blewis ME, Nugent-Derfus GE, Schmidt TA, Schumacher BL, Sah RL. (2007). A model of synovial fluid lubricant composition in normal and injured. *European cells and materials* **13**: 26–39.
- Bothner H, Wik O. (1987). Rheology of hyaluronate. *Acta Otolaryngol Suppl* **442**: 25–30.
- Brandt K. (1970). Modification of chemotaxis by synovial fluid hyaluronate. *Arthritis Rheum* **13**: 308–309.
- Brown MB, Jones SA. (2005). Hyaluronic acid: a unique topical vehicle for the localized delivery of drugs to the skin. *J Eur Acad Dermatol Venereol* **19**: 308–318.
- Brown TJ, Laurent UBG, Fraser JRE. (1991). Turnover of hyaluronan in synovial joints: elimination of labelled hyaluronan from the knee joints of the rabbit. *Exp Physiol* **76**: 125–34.
- Bucolo C, Mangiafico P. (1999). Pharmacological profile of a new topical pilocarpine formulation. *J Ocul Pharmacol Ther* **15**: 567–573.
- Bucolo C, Spadaro A, Mangiafico S. (1998). Pharmacological evaluation of a new timolol/pilocarpine formulation. *Ophthalmic Res* **30**: 101–106.
- Camber O, Edman P, Gurny R. (1987). Influence of sodium hyaluronate on the meiotic effect of pilocarpine in rabbits. *Curr Eye Res* **6**: 779–784.
- Camber O, Edman P. (1989). Sodium hyaluronate as an ophthalmic vehicle – some factors governing its effect on the ocular absorption of pilocarpine. *Curr Eye Res* **8**: 563–567.
- Cantor JO, Cerreta JM, Armand G, Turino GM. (1998). Aerosolized hyaluronic acid decreases alveolar injury induced by human neutrophil elastase. *Proc Soc Exp Biol Med* **217**: 471–475.
- Chabreck P, Soltes L, Kallay Z, Fugedi A. (1990). Isolation and characterization of high molecular weight (3H) hyaluronic acid. *J Label Compd Radiopharm* **28**: 1121–1125.
- Chabreck P, Soltes L, Kallay Z, Novak I. (1990). Gel permeation chromatographic characterization of sodium hyaluronate and its reactions prepared by ultrasonic degradation. *Chromatographia* **30**: 201–204.
- Chen FH, Rousche KT, Tuan RS. (2006). Technology Insight: adult stem cells in cartilage regeneration and tissue engineering. *Nat Clin Pract Rheumatol* **2**(7): 373–82.
- Coleman P, Kavanagh E, Mason RM, Levick JR, Ashhurst DE. (1998). The proteoglycans and glycosaminoglycan chains of rabbit synovium. *Histochem J* **30**: 519–524.
- Comper WD, Laurent TC. (1978). Physiological function of connective tissue polysaccharides. *Physiol Rev* **58**: 255–315.
- Cowman MK, Matsuoka S. (2005). Experimental approaches to hyaluronan structure. *Carbohydrate Research* **340**: 791–809.
- Dahl LB, Dahl IM, Engstrom-Laurent A, Granath K. (1985). Concentration and molecular weight of sodium hyaluronate in synovial fluid from patients with rheumatoid arthritis and other arthropathies. *Ann Rheum Dis* **44**: 817–822.
- Dobbie JW, Hind C, Meijers P, Bodart C, Tasiaux N, Perret J, Anderson JD. (1995). Lamellar body secretion: ultrastructural analysis of an unexplored function of synoviocytes. *Br J Rheumatol* **34**: 13–23.
- Dougados M. (2000). Sodium hyaluronate therapy in osteoarthritis: arguments for a potential beneficial structural effect. *Semin Arthritis Rheum* **30**: 19–25.
- Dráfi F, Valachová K, Hrabárová E, Juránek I, Bauerová K, Šoltés L. (2010). Study of methotrexate and β -alanine-L-histidine in comparison with L-glutathione on high-molar-mass hyaluronan degradation induced by ascorbate plus Cu (II) ions via rotational viscometry. 60th Pharmacological Days in Hradec Králové. *Acta Medica* **53**(3): 170.
- Drobnik J. (1991). Hyaluronan in drug delivery. *Adv Drug Dev Rev* **7**: 295–308.
- Duranti F, Salti G, Bovani B, Calandra M, Rosati ML. (1998). Injectable hyaluronic acid gel for soft tissue augmentation – a clinical and histological study. *Dermatol Surg* **24**: 1317–1325.
- Edwards JCW, Wilkinson LS, Jones HM. (1994). The formation of human synovial cavities: a possible role for hyaluronan and CD44 in altered interzone cohesion. *J Anat* **185**: 355–67.
- Edwards JCW (1995). Consensus statement. Second international meeting on synovium. Cell biology, physiology and pathology. *Ann Rheum Dis* **54**: 389–91.
- Eliaz RE, Szoka FC. (2001). Liposome-encapsulated doxorubicin targeted to CD44: a strategy to kill CD44-overexpressing tumor cells. *Cancer Res* **61**: 2592–2601.
- Figueiredo F, Jones GM, Thouas GA, Trounson AO. (2002). The effect of extracellular matrix molecules on mouse preimplantation embryo development *in vitro*. *Reprod Fertil Dev* **14**: 443–451.
- Fisher AE, Naughton ODP. (2005). Therapeutic chelators for the twenty first century: new treatments for iron and copper mediated inflammatory and neurological disorders. *Curr Drug Delivery* **2**: 261–268.
- Fraser JRE, Foo WK, Maritz JS. (1972). Viscous interactions of hyaluronic acid with some proteins and neutral saccharides. *Ann Rheum Dis* **31**: 513–20.
- Fraser JRE, Kimpton WG, Pierscionek BK, Cahill RNP. (1993). The kinetics of hyaluronan in normal and acutely inflamed synovial joints – observations with experimental arthritis in sheep. *Semin Arthritis Rheum* **22**: 9–17.
- Furness CC, deMatos DG, Martinez AG. (1998). Effect of hyaluronic acid on development of *in vitro* produced bovine embryos. *Theriogenology* **49**: 1489–99.
- Gandolfi SA, Massari A, Orsoni JG. (1992). Low-molecular-weight sodium hyaluronate in the treatment of bacterial corneal ulcers. *Graefes Arch Clin Exp Ophthalmol* **230**: 20–23.
- Gardner DK, Lane M, Stevens J, Schoolcraft WB. (2003). Changing the start temperature and cooling rate in a slow-freezing protocol increases human blastocyst viability. *Fertil Steril* **79**: 407–410.
- Gardner DK, Rodriegez-Martinez H, Lane M. (1999). Fetal development after transfer is increased by replacing protein with the glycosaminoglycan hyaluronan for mouse embryo culture and transfer. *Hum Reprod* **14**: 2575–2580.
- Ghosh P, Guidolin D. (2002). Potential mechanism of action of intraarticular hyaluronan therapy in osteoarthritis: are the effects molecular weight dependent? *Semin Arthritis Rheum* **32**: 10–37.
- Ghosh S, Jassal M. (2002). Use of polysaccharide fibres for modern wound dressings. *Indian J Fibre Textile Res* **27**: 434–450.
- Gibbs DA, Merrill EW, Smith KA, Balazs EA. (1968). Rheology of hyaluronic acid. *Biopolymers* **6**: 777–91.
- Glogau RG. (2000). The risk of progression to invasive disease. *J Am Acad Dermatol* **42**: S23–S24.
- Grootveld M, Henderson EB, Farrell A, Blake DR, Parkes HG, Haycock P. (1991). Oxidative damage to hyaluronate and glucose in synovial fluid during exercise of the inflamed rheumatoid joint. Detection of abnormal low-molecular-mass metabolites by proton-N.M.R. spectroscopy. *Biochem J* **273**: 459–467.
- Guidolin DD, Ronchetti IP, Lini E. (2001). Morphological analysis of articular cartilage biopsies from a randomized, clinical study comparing the effects of 500–730 kDa sodium hyaluronate Hyalgan(R) and methylprednisolone acetate on primary osteoarthritis of the knee. *Osteoarthritis Cartilage* **9**: 371–381.
- Gurny R, Ibrahim H, Aebi A. (1987). Design and evaluation of controlled release systems for the eye. *J Control Release* **6**: 367–373.
- Haddad JJ, Harb HL. (2005). L-gamma-Glutamyl-L-cysteinyl-glycine (glutathione; GSH) and GSH-related enzymes in the regulation of pro- and anti-inflammatory cytokines: a signaling transcriptional scenario for redox(y) immunologic sensor(s). *Mol Immunol* **42**: 987–1014.
- Hamburger MI, Lakhanpal S, Moar PA, Oster D. (2003). Intra-articular hyaluronans: a review of product-specific safety profiles. *Semin Arthritis Rheum* **32**: 296–309.
- Haubeck HD, Kock R, Fischer DC, van de Leur E, Hoffmeister K, Greiling H. (1995). Transforming growth factor β 1, a major stimulator of hyaluronan synthesis in human synovial lining cells. *Arthritis Rheum* **38**: 669–677.
- Herrero-Vanrell R, Fernandez-Carballido A, Frutos G, Cadorniga R. (2000). Enhancement of the mydriatic response to tropicamide by bioadhesive polymers. *J Ocul Pharmacol Ther* **16**: 419–428.
- Hills BA, Crawford RW. (2003) Normal and prosthetic synovial joints are lubricated by surface-active phospholipid: a hypothesis. *J Arthroplasty* **18**: 499–505.
- Hlavacek M. (1993). The role of synovial fluid filtration by cartilage in lubrication of synovial joints. *J Biomech* **26**(10): 1145–50.
- Hochberg MC. (2000). Role of intra-articular hyaluronic acid preparations in medical management of osteoarthritis of the knee. *Semin Arthritis Rheum* **30**: 2–10.

- Hrabarova E, Valachova K, Rapta P, Soltes L. (2010). An alternative standard for trolox-equivalent antioxidant-capacity estimation based on thiol antioxidants. Comparative 2,2'-azinobis[3-ethylbenzothiazoline-6-sulfonic acid] decolorization and rotational viscometry study regarding hyaluronan degradation. *Chemistry & Biodiversity* **7**(9): 2191–2200.
- Hrabarova E, Valachova K, Rychly J, Rapta P, Sasinkova V, Malikova M, Soltes L. (2009). High-molar-mass hyaluronan degradation by Weissberger's system: Pro- and anti-oxidative effects of some thiol compounds. *Polymer Degradation and Stability* **94**: 1867–1875.
- Hrabarova E, Valachova K, Juranek I, Soltes L. (2012). Free-radical degradation of high-molar-mass hyaluronan induced by ascorbate plus cupric ions: evaluation of antioxidant effect of cysteine-derived compounds. *Chemistry & Biodiversity* **9**: 309–317.
- Hrabarova E, Gemeiner P, Soltes L. (2007). Peroxynitrite: *In vivo* and *in vitro* synthesis and oxidant degradative action on biological systems regarding biomolecular injury and inflammatory processes. *Chem Pap* **61**: 417–437.
- Hrabárová E, Valachová K, Juránek I, Šoltés L. (2011). *Free-radical degradation of high-molar-mass hyaluronan induced by ascorbate plus cupric ions. Antioxidative properties of the Piešťany-spa curative waters from healing peloid and maturation pool*. In: "Kinetics, Catalysis and Mechanism of Chemical Reactions" G. E. Zaikov (eds), Nova Science Publishers, New York, pp. 29–36.
- Hrabárová E, Valachová K, Rychlý J, Rapta P, Sasinková V, Gemeiner P, Šoltés L. (2009). High-molar-mass hyaluronan degradation by the Weissberger's system: pro- and antioxidative effects of some thiol compounds. *Polym Degrad Stab* **94**: 1867–1875.
- Hultberg M, Hultberg B. (2006). The effect of different antioxidants on glutathione turnover in human cell lines and their interaction with hydrogen peroxide. *Chem Biol Interact* **163**(3): 192–198.
- Hutadilok N, Ghosh P, Brooks PM. (1988). Binding of haptoglobin, inter-alpha-trypsin inhibitor, and I proteinase inhibitor to synovial fluid hyaluronate and the influence of these proteins on its degradation by oxygen derived free radicals. *Ann Rheum Dis* **47**: 377–85.
- Inoue M, Katakami C. (1993). The effect of hyaluronic-acid on corneal epithelial-cell proliferation. *Invest Ophthalmol Vis Sci* **34**: 2313–2315.
- Itano N, Kimata K. (2002). Mammalian hyaluronan synthases. *IUBMB Life* **54**: 195–199.
- Jaakma U, Zhang B R, Larsson B. (1997). Effects of sperm treatments on the *in vitro* development of bovine oocytes in semidefined and defined media. *Theriogenology* **48**: 711–720.
- Jang G, Lee BC, Kang SK, Hwang WS. (2003). Effect of glycosaminoglycans on the preimplantation development of embryos derived from *in vitro* fertilization and somatic cell nuclear transfer. *Reprod Fertil Dev* **15**: 179–185.
- Jarvinen K, Jarvinen T, Urtti A. (1995). Ocular absorption following topical delivery. *Adv Drug Dev Rev* **16**: 3–19.
- Jay GD, Britt DE, Cha DJ. (2000). Lubricin is a product of megakaryocyte stimulating factor gene expression by human synovial fibroblasts. *J Rheumatol* **27**: 594–600.
- Joly T, Nibart M, Thibier M. (1992). Hyaluronic-acid as a substitute for proteins in the deep-freezing of embryos from mice and sheep – an *in vitro* investigation. *Theriogenology* **37**: 473–480.
- Juranek I, Soltes L. (2012). *Reactive oxygen species in joint physiology: Possible mechanism of maintaining hypoxia to protect chondrocytes from oxygen excess via synovial fluid hyaluronan peroxidation*. In: "Kinetics, Catalysis and Mechanism of Chemical Reactions: From Pure to Applied Science. Volume 2 – Tomorrow and Perspectives" R.M. Islamova, S.V. Kolesov, G.E. Zaikov (eds), Nova Science Publishers, New York pp. 1–10
- Kano K, Miyano T, Kato S. (1998). Effects of glycosaminoglycans on the development of *in vitro* matured and fertilized porcine oocytes to the blastocyst stage *in vitro*. *Biol Reprod* **58**: 1226–1232.
- Kelly MA, Goldberg VM, Healy WL. (2003). Osteoarthritis and beyond: a consensus on the past, present, and future of hyaluronans in orthopedics. *Orthopedics* **26**: 1064–1079.
- Kemmann E. (1998). Creutzfeldt-Jakob disease (CJD) and assisted reproductive technology (ART) – quantification of risks as part of informed consent. *Hum Reprod* **13**: 1777.
- Kessler A, Biasibetti M, da Silva Melo DA, Wajner M, Dutra-Filho CS, de Souza Wyse AT, Wannmacher CMD. (2008). Antioxidant effect of cysteamine in brain cortex of young rats. *Neurochem Res* **33**: 737–44.
- Kim A, Checkla DM, Dehazya P, Chen WL. (2003). Characterization of DNA-hyaluronan matrix for sustained gene transfer. *J Control Release* **90**: 81–95.
- Kirwan J. (2001). Is there a place for intra-articular hyaluronate in osteoarthritis of the knee? *Knee* **8**: 93–101.
- Knight AD, Levick JR. (1984). Morphometry of the ultrastructure of the blood-joint barrier in the rabbit knee. *Q J Exp Physiol* **69**: 271–288.
- Kogan G. (2010). *Hyaluronan – A High Molar mass messenger reporting on the status of synovial joints: part 1. Physiological status* In: New Steps in Chemical and Biochemical Physics. ISBN: 97 8-1-61668-923 -0. pp. 121–133.
- Kogan G, Soltes L, Stern R, Mendichi R. (2007a). *Hyaluronic acid: A biopolymer with versatile physico-chemical and biological properties. Chapter 31* – in: Handbook of Polymer Research: Monomers, Oligomers, Polymers and Composites. Pethrick R. A, Ballada A, Zaikov G. E. (eds.), Nova Science Publishers, New York, pp. 393–439.
- Kogan G, Soltes L, Stern R, Gemeiner P. (2007). Hyaluronic acid: A natural biopolymer with a broad range of biomedical and industrial applications. *Bio-technol Lett* **29**: 17–25.
- Kreil G. (1995). Hyaluronidases-A group of neglected enzymes. *Protein Sciences* **4**: 1666–1669.
- Lane M, Maybach JM, Hooper K. (2003). Cryo-survival and development of bovine blastocysts are enhanced by culture with recombinant albumin and hyaluronan. *Mol Reprod Dev* **64**: 70–78.
- Langer K, Mutschler E, Lambrecht G. (1997). Methylmethacrylate sulfopropyl-methacrylate copolymer nanoparticles for drug delivery – Part III. Evaluation as drug delivery system for ophthalmic applications. *Int J Pharm* **158**: 219–231.
- Lath D, Csomorova K, Kollarikova G, Stankovska M, Soltes L. (2005). Molar mass-intrinsic viscosity relationship of high-molar-mass yaluronans: Involvement of shear rate. *Chem Pap* **59**: 291–293.
- Laurent TC, Laurent UBG, Fraser JRE. (1996). The structure and function of hyaluronan: An over view. *Immunology and Cell Biology* **74**: A1–A7.
- Laurent TC. (1989). *The biology of hyaluronan*. In: Ciba Foundation Symposium. John Wiley and Sons, New York. **143**: 1–298.
- Laurent TC, Fraser JRE. (1992). Hyaluronan. *FASEB J* **6**: 2397–2404.
- Laurent TC, Laurent UBG, Fraser JRE. (1995). Functions of hyaluronan. *Ann Rheum Dis* **54**: 429–32.
- Laurent TC, Ryan M, Pictruskiewicz A. (1960). Fractionation of hyaluronic acid. The polydispersity of hyaluronic acid from the vitreous body. *Biochim Biophys Acta* **42**: 476–85.
- Levick JR. (1994). An analysis of the interaction between interstitial plasma protein, interstitial flow, and fenestral filtration and its application to synovium. *Microvasc Res* **47**: 90–125.
- Leyden J, Narins RS, Brandt F. (2003). A randomized, double-blind, multicenter comparison of the efficacy and tolerability of Restylane versus Z-plast for the correction of nasolabial folds. *Dermatol Surg* **29**: 588–595.
- Lim ST, Forbes B, Berry DJ, Martin GP, Brown MB. (2002). *In vivo* evaluation of novel hyaluronan/chitosan microparticulate delivery systems for the nasal delivery of gentamicin in rabbits. *Int J Pharm* **231**: 73–82.
- Luo Y, Prestwich GD. (1999). Synthesis and selective cytotoxicity of a hyaluronic acid-antitumor bioconjugate. *Bioconjug Chem* **10**: 755–763.
- Luo Y, Ziebell MR, Prestwich GD. (2000). A hyaluronic acid-taxol antitumor bioconjugate targeted to cancer cells. *Biomacromolecules* **1**: 208–218.
- Maheu E, Ayral X, Dougados M. (2002). A hyaluronan preparation (500–730 kDa) in the treatment of osteoarthritis: a review of clinical trials with Hyalgan(R). *Int J Clin Pract* **56**: 804–813.
- Manuskiatti W, Maibach HI. (1996). Hyaluronic acid and skin: wound healing and aging. *Int J Dermatol* **35**: 539–544.
- Mazzucco D, Scott R, Spector M. (2004). Composition of joint fluid in patients undergoing total knee replacement and revision arthroplasty: correlation with flow properties. *Biomaterials* **25**: 4433–4445.
- McCord JM. (1974). Free radicals and inflammation: protection of synovial fluid by superoxide dismutase. *Science* **185**: 529–531.
- McDonald JN, Levick JR. (1988). Morphology of surface synoviocytes in situ at normal and raised joint pressure, studied by scanning electron microscopy. *Ann Rheum Dis* **47**: 232–240.
- McDonald JN, Levick JR. (1995). Effect of intra-articular hyaluronan on pressure-flow relation across synovium in anaesthetized rabbits. *J Physiol* **485**(Pt.1): 179–93.
- Mendichi R, Soltes L. (2002). Hyaluronan molecular weight and polydispersity in some commercial intra-articular injectable preparations and in synovial fluid *Inflamm Res* **51**: 115–116.
- Meyer K, Palmer JW. (1934). The polysaccharide of the vitreous humor. *Journal of Biology and Chemistry* **107**: 629–634.

- Miltner O, Schneider U, Siebert CH. (2002). Efficacy of intraarticular hyaluronic acid in patients with osteoarthritis—a prospective clinical trial. *Osteoarthritis Cartilage* **10**: 680–686.
- Miyano T, Hirooka RE, Kano K. (1994). Effects of hyaluronic-acid on the development of 1-cell and 2-cell porcine embryos to the blastocyst stage in vitro. *Theriogenology* **41**: 1299–1305.
- Miyazaki M, Sato S, Yamaguchi T. (1983). *Analgesic and antiinflammatory action of hyaluronic sodium*, Japan Pharmacological Conference. Tokyo, April 4, 1983.
- Miyazaki T, Miyauchi S, Nakamura T. (1996). The effect of sodium hyaluronate on the growth of rabbit cornea epithelial cells in vitro. *J Ocul Pharmacol Ther* **12**: 409–415.
- Momberger TS, Levick JR, Mason RM. (2005). Hyaluronan secretion by synovocytes is mechanosensitive. *Matrix Biol* **24**: 510–519.
- Moreira CA, Armstrong DK, Jelliffe RW. (1991). Sodium hyaluronate as a carrier for intravitreal gentamicin – an experimental study. *Acta Ophthalmol (Copenh)* **69**: 45–49.
- Moreira CA, Moreira AT, Armstrong DK. (1991). In vitro and in vivo studies with sodium hyaluronate as a carrier for intraocular gentamicin. *Acta Ophthalmol (Copenh)* **69**: 50–56.
- Morimoto K, Metsugi K, Katsumata H. (2001). Effects of lowviscosity sodium hyaluronate preparation on the pulmonary absorption of rh-insulin in rats. *Drug Dev Ind Pharm* **27**: 365–371.
- Morimoto K, Yamaguchi H, Iwakura Y. (1991). Effects of viscous hyaluronate-sodium solutions on the nasal absorption of vasopressin and an analog. *Pharmacol Res* **8**: 471–474.
- Morris ER, Rees DA, Welsh EJ. (1980). Conformation and dynamic interactions in hyaluronate solutions. *J Mol Biol* **138**: 383–400.
- Myint P. (1987). The reactivity of various free radicals with hyaluronic acid steady-state and pulseradiolysis studies. *Biochim Biophys Acta* **925**: 194–202.
- Necas J, Bartosikova L, Brauner P, Kolar J. (2008). Hyaluronic acid (hyaluronan): a review. *Veterinari Medicina* **53**(8): 397–411.
- Niwa Y, Sakane T, Shingu M, Yokoyama MM. (1983). Effect of stimulated neutrophils from the synovial fluid of patients with rheumatoid arthritis on lymphocytes: a possible role of increased oxygen radicals generated by the neutrophils. *J Clin Immunol* **3**: 228–240.
- Noble PW. (2002). Hyaluronan and its catabolic products in tissue injury and repair. *Matrix Biol* **21**: 25–29.
- Oates KMN, Krause WE, Colby RH. (2002). Using rheology to probe the mechanism of joint lubrication: polyelectrolyte/protein interactions in synovial fluid. *Mat Res Soc Symp Proc* **711**: 53–58.
- Ogston AG, Stanier JE. (1953). The physiological function of hyaluronic acid in synovial fluid viscous, elastic and lubricant properties. *J Physiol* **199**: 244–52.
- Ortonne JP. (1996). A controlled study of the activity of hyaluronic acid in the treatment of venous leg ulcers. *J Dermatol Treatment* **7**: 75–81.
- Orvisky E, Soltes L, Chabreck P, Novak I, Kery V, Stancikova M, Vins I. (1992). The determination of hyaluronan molecular weight distribution by means of high performance size exclusion chromatography. *J Liq Chromatogr* **15**: 3203–3218.
- Parsons BJ, Al-Assaf S, Navaratnam S, Phillips GO. (2002). *Comparison of the reactivity of different oxidative species (ROS) towards hyaluronan*, in: Kennedy JF, Phillips GO, Williams PA, Hascall VC (Eds.), *Hyaluronan: Chemical, Biochemical and Biological Aspects*, Woodhead, Publishing Ltd, Cambridge, MA, pp. 141–150.
- Peer D, Florentin A, Margalit R. (2003). Hyaluronan is a key component in cryoprotection and formulation of targeted unilamellar liposomes. *Biochim Biophys Acta-Biomembranes* **1612**: 76–82.
- Peer D, Margalit R. (2000). Physicochemical evaluation of a stability-driven approach to drug entrapment in regular and in surface-modified liposomes. *Arch Biochem Biophys* **383**: 185–190.
- Poli A, Mason RM, Levick JR. (2004). Effects of Arg- Gly-Asp sequence peptide and hyperosmolarity on the permeability of interstitial matrix and fenestrated endothelium in joints. *Microcirculation* **11**: 463–476.
- Praest BM, Greiling H, Kock R. (1997). Effects of oxygen-derived free radicals on the molecular weight and the polydispersity of hyaluronan solutions. *Carbohydr Res* **303**: 153–157.
- Price FM, Levick JR, Mason RM. (1996). Glycosaminoglycan concentration in synovium and other tissues of rabbit knee in relation to synovial hydraulic resistance. *J Physiol (Lond)* **495**: 803–820.
- Prisel PT, Camber O, Hiselius J, Norstedt G. (1992). Evaluation of hyaluronan as a vehicle for peptide growth factors. *Int J Pharm* **85**: 51–56.
- Radin EL, Swann DA, Weisser PA. (1970). Separation of a hyaluronate-frec lubricating fraction from synovial fluid. *Nature* **228**: 377–8.
- Rapta P, Valachova K, Gemeiner P, Soltes L. (2009). High-molar-mass hyaluronan behavior during testing its radical scavenging capacity in organic and aqueous media: Effects of the presence of Manganese (II) ions. *Chem Biodivers* **6**: 162–169.
- Rapta P, Valachová K, Gemeiner P, Šoltés L. (2009). High-molar-mass hyaluronan behavior during testing its antioxidant properties in organic and aqueous media: effects of the presence of Mn(II) ions. *Chem Biodivers* **6**: 162–169.
- Rapta P, Valachová K, Zalibera M, Šnirc V, Šoltés L. (2010). *Hyaluronan degradation by reactive oxygen species: scavenging effect of the hexapyridindole stobadine and two of its derivatives*. In Monomers, Oligomers, Polymers, Composites, and Nanocomposites, Ed: R. A. Pethrick P. Petkov, A. Zlatarov G. E. Zaikov, S. K. Rakovsky, Nova Science Publishers, N.Y. Chapter 7, pp. 113–126.
- Rees MD, Kennett EC, Whitelock JM, Davies MJ. (2008). Oxidative damage to extracellular matrix and its role in human pathologies. *Free Radical Biol. Med* **44**: 1973–2001.
- Revell PA. (1989). Synovial lining cells. *Rheumatol Int* **9**: 49–51.
- Risberg B. (1997). Adhesions: preventive strategies. *Eur J Surg* **163**: 32–39.
- Rittig M, Tittor F, Lutjen-Drecoll E, Mollenhauer J, Rauterberg J. (1992). Immunohistochemical study of extracellular material in the aged human synovial membrane. *Mech Ageing Dev* **64**: 219–234.
- Rychly J, Soltes L, Stankovska M, Janigova I, Csomorova K, Sasinkova V, Kogan G, Gemeiner P. (2006). Unexplored capabilities of chemiluminescence and thermoanalytical methods in characterization of intact and degraded hyaluronans. *Polym Degrad Stab* **91**(12): 3174–3184.
- Saettone MF, Giannaccini B, Chetoni P, et al. (1991). Evaluation of highmolecular-weight and low-molecular-weight fractions of sodium hyaluronate and an ionic complex as adjuvants for topical ophthalmic vehicles containing pilocarpine. *Int J Pharm* **72**: 131–139.
- Saettone MF, Monti D, Torracca MT, Chetoni P. (1994). Mucoadhesive ophthalmic vehicles – evaluation polymeric low-viscosity formulations. *J Ocul Pharmacol* **10**: 83–92.
- Sakurai K, Miyazaki K, Koda Y. (1997). Anti-inflammatory activity of superoxide dismutase conjugated with sodium hyaluronate. *Glycoconj J* **14**: 723–728.
- Sasaki H, Yamamura K, Nishida K. (1996). Delivery of drugs to the eye by topical application. *Prog Retinal Eye Res* **15**: 583–620.
- Sattar A, Kumar S, West DC. (1992). Does hyaluronan have a role in endothelial cell proliferation of the synovium. *Semin. Arthritis Rheum* **22**: 37–43.
- Schartz RA. (1997). The actinic keratoses. A perspective and update. *Dermatol Surg* **23**: 1009–1019.
- Schiller J, Volpi N, Hrabarova E, Soltes L. (2011). *Hyaluronic acid: a natural biopolymer* In: “Handbook of Biopolymers and Their Applications” S. Kalia and L. Averous (eds), Wiley & Scrivener Publishing, USA pp. 3–34.
- Schmid T, Lindley K, Su J, Soloveychik V, Block J, Kuettner K, Schumacher B. (2001a). Superficial zone protein (SZP) is an abundant glycoprotein in human synovial fluid and serum. *Trans Orthop Res Soc* **26**: 82.
- Schmid T, Soloveychik V, Kuettner K, Schumacher B. (2001b). Superficial zone protein (SZP) from human cartilage has lubrication activity. *Trans Orthop Res Soc* **26**: 178.
- Schumacher BL, Block JA, Schmid TM, Aydelotte MB, Kuettner KE. (1994). A novel proteoglycan synthesized and secreted by chondrocytes of the superficial zone of articular cartilage. *Arch Biochem Biophys* **311**: 144–152.
- Schumacher BL, Hughes CE, Kuettner KE, Caterson B, Aydelotte MB. (1999). Immunodetection and partial c DNA sequence of the proteoglycan, superficial zone protein, synthesized by cells lining synovial joints. *J Orthop Res* **17**: 110–120.
- Schumacher BL, Schmidt TA, Voegtline MS, Chen AC, Sah RL. (2005). Proteoglycan 4 (PRG4) synthesis and immunolocalization in bovine meniscus. *J Orthop Res* **23**: 562–568.
- Schwarz IM, Hills BA. (1996). Synovial surfactant: lamellar bodies in type B synovocytes and proteolipid in synovial fluid and the articular lining. *Br J Rheumatol* **35**: 821–827.
- Schwarz IM, Hills BA. (1998). Surface-active phospholipids as the lubricating component of lubricin. *Br J Rheumatol* **37**: 21–26.
- Scott DL, Shipley M, Dawson A, Edwards S, Symmons DP, Woolf AD. (1998). The clinical management of rheumatoid arthritis and osteoarthritis: strategies for improving clinical effectiveness. *Br J Rheumatol* **37**: 546–554.

- Scott JE, Cummings C, Brass A, Chen Y. (1991). Secondary and tertiary structures of hyaluronan in aqueous solution, investigated by rotary shadowing-electron microscopy and computer simulation. *Biochem J* **274**: 600–705.
- Servaty R, Schiller J, Binder H, Arnold K. (2000). Hydration of polymeric components of the cartilage – An infrared spectroscopic study on hyaluronic acid and chondroitin sulfate. *Int J Biol Macromol* **28**: 123–129.
- Simkovic I, Hricovini M, Soltes L, Mendichi R, Cosentino C. (2000). Preparation of water soluble/insoluble derivatives of Hyaluronic acid by cross linking with epichlorohydrin in aqueous NaOH/NH₄OH solution. *Carbohydr Polym* **41**: 9–14.
- Simon A, Safran A, Revel A. (2003). Hyaluronic acid can successfully replace albumin as the sole macromolecule in a human embryo transfer medium. *Fertil Steril* **79**: 1434–1438.
- Soldati D, Rahm F, Pasche P. (1999). Mucosal wound healing after nasal surgery. A controlled clinical trial on the efficacy of hyaluronic acid containing cream. *Drugs Exp Clin Res* **25**: 253–261.
- Soloveva ME, Solovev VV, Faskhutdinova AA, Kudryavtsev AA, Akatov VS. (2007). Prooxidant and cytotoxic action of N-acetylcysteine and glutathione in combinations with vitamin B12b. *Cell Tissue Biol* **1**: 40–49.
- Soltes L, Kogan G. (2009). *Impact of transition metals in the free-radical degradation of hyaluronan biopolymer* In: "Kinetics & Thermodynamics for Chemistry & Biochemistry: Vol. 2" E. M. Pearce, G. E. Zaikov, G. Kirshenbaum (eds), Nova Science Publishers, New York (181–199).
- Soltes L, Mendichi R, Kogan G, Mach M. (2004). Associating Hyaluronan Derivatives: A Novel Horizon in Viscosupplementation of Osteoarthritic Joints. *Chem Biodivers* **1**: 468–472.
- Soltes L, Brezova V, Stankovska M, Kogan G, Gemeiner P. (2006a). Degradation of high-molecular-weight hyaluronan by hydrogen peroxide in the presence of cupric ions. *Carbohydr Res* **341**: 639–644.
- Soltes L, Mendichi R, Kogan G, Schiller J, Stankovska M, Arnhold J. (2006b). Degradative action of reactive oxygen species on hyaluronan. *Biomacromolecules* **7**: 659–668.
- Soltes L, Stankovska M, Brezova V, Schiller J, Arnhold J, Kogan G, Gemeiner P. (2006c). Hyaluronan degradation by copper (II) chloride and ascorbate: rotational viscometric, EPR spin-trapping, and MALDI-TOF mass spectrometric investigations *Carbohydr Res* **341**: 2826–2834.
- Soltes L, Stankovska M, Kogan G, Gemeiner P, Stern R. (2005). Contribution of oxidative reductive reactions to high molecular weight hyaluronan catabolism. *Chem Biodivers* **2**: 1242–1245.
- Soltes L, Valachova K, Mendichi R, Kogan G, Arnhold J, Gemeiner P. (2007). Solution properties of high-molar-mass hyaluronans: the biopolymer degradation by ascorbate. *Carbohydr Res* **342**: 1071–1077.
- Soltes L. (2010). Hyaluronan – A High-Molar-Mass Messenger Reporting on the Status of Synovial Joints: Part II. *Pathophysiological Status* In: "New Steps in Chemical and Biochemical Physics. Pure and Applied Science" E. M. Pearce, G. Kirshenbaum, G. E. Zaikov (eds), Nova Science Publishers, New York pp. 137–152.
- Stankovska M, Arnhold J, Rychly J, Spalteholz H, Gemeiner P, Soltes L. (2007). *In vitro* screening of the action of non-steroidal anti-inflammatory drugs on hypochlorous acid-induced hyaluronan degradation. *Polym Degrad Stabil* **92**: 644–652.
- Stankovska M, Soltes L, Vikartovska A, Mendichi R, Lath D, Molnarova M, Gemeiner P. (2004). Study of hyaluronan degradation by means of rotational Viscometry: Contribution of the material of viscometer. *Chem Pap* **58**: 348–352.
- Stankovska M, Hrabarova E, Valachova K, Molnarova M, Gemeiner P, Soltes L. (2006). The degradative action of peroxynitrite on high-molecular-weight hyaluronan. *Neuroendocrinol Lett* **27**(Suppl. 2): 31–34.
- Stankovska M, Soltes L, Vikartovska A, Gemeiner P, Kogan G, Bakos D. (2005). Degradation of high-molecular-weight hyaluronan: a rotational viscometry study. *Biologia* **60**(Suppl. 17): 149–152.
- Stern R, Kogan G, Jedrzejak M. J, Soltes L. (2007). The many ways to cleave hyaluronan. *Biotechnol Adv* **25**: 537–557.
- Stiebel-Kalish H, Gatton DD, Weinberger D. (1998). A comparison of the effect of hyaluronic acid versus gentamicin on corneal epithelial healing. *Eye* **12**: 829–833.
- Suchanek E, Simunic V, Juretic D, Grizelj V. (1994). Follicular-fluid contents of hyaluronic-acid, follicle-stimulating-hormone and steroids relative to the success of *in-vitro* fertilization of human oocytes. *Fertil Steril* **62**: 347–352.
- Surendrakumar K, Martyn GP, Hodgson ECM. (2003). Sustained release of insulin from sodium hyaluronate based dry powder formulations after pulmonary delivery to beagle dogs. *J Control Release* **91**: 385–394.
- Surini S, Akiyama H, Morishita M. (2003). Polyion complex of chitosan and sodium hyaluronate as an implant device for insulin delivery. *STP Pharm Sci* **13**: 265–268.
- Surovcikova L, Valachova K, Banasova M, Snirc V, Priesolova E, Nagy M, Juranek I, Soltes L. (2012). Free-radical degradation of high-molar-mass hyaluronan induced by ascorbate plus cupric ions: Testing of stobadine and its two derivatives in function as antioxidants. *General Physiol Biophys* **31**: 57–64.
- Swann DA, Silver FH, Slayter HS, Stafford W, Shore E. (1985). The molecular structure and lubricating activity of lubricin isolated from bovine and human synovial fluids. *Biochem J* **225**: 195–201.
- Takayama K, Hirata M, Machida Y. (1990). Effect of interpolymer complex-formation on bioadhesive property and drug release phenomenon of compressed tablet consisting of chitosan and sodium hyaluronate. *Chem Pharmaceut Bull* **38**: 1993–1997.
- Tani E, Katakami C, Negi A. (2002). Effects of various eye drops on corneal wound healing after superficial keratectomy in rabbits. *Jpn J Ophthalmol* **46**: 488–495.
- Tascioglu F, Oner C. (2003). Efficacy of intra-articular sodium hyaluronate in the treatment of knee osteoarthritis. *Clin Rheumatol* **22**: 112–117.
- Thibodeau PA, Kocsis-Bedard S, Courteau J, Niyonsenga T, Paquette B. (2001). Thiols can either enhance or suppress DNA damage induction by catecholestrogens. *Free Radic Biol Med* **30**: 62–73.
- Turino GM, Cantor JO. (2003). Hyaluronan in respiratory injury and repair. *Am J Respir Crit Care Med* **167**: 1169–1175.
- Uthman I, Raynauld JP, Haraoui B. (2003). Intra-articular therapy in osteoarthritis. *Postgrad Med J* **79**: 449–453.
- Valachova K, Vargova A, Raptá P, Hrabarova E, Drafi F, Bauerova K, Juranek I, Soltes L. (2011). Aurothiomalate as preventive and chain-breaking antioxidant in radical degradation of high-molar-mass hyaluronan. *Chemistry & Biodiversity* **8**: 1274–1283.
- Valachova K, Banasova M, Machova L, Juranek I, Bezec S, Soltes L. (2013b). Antioxidant activity of various hexahydropyridindoles. *Journal of Information Intelligence and Knowledge* **5**: 15–32.
- Valachova K, Hrabarova E, Priesolova E, Nagy M, Banasova M, Juranek I, Soltes L. (2011). Free-radical degradation of high-molecular-weight hyaluronan induced by ascorbate plus cupric ions. Testing of buccillamine and its SA981-metabolite as antioxidants. *J Pharma & Biomedical Analysis* **56**: 664–670.
- Valachová K, Hrabárová E, Drafi F, Juránek I, Bauerová K, Priesolová E, Nagy M, Šoltés L. (2010a). Ascorbate and Cu(II) induced oxidative degradation of high-molar-mass hyaluronan. Pro- and antioxidative effects of some thiols. *Neuroendocrinol Lett* **31**(2): 101–104.
- Valachová K, Hrabárová E, Gemeiner P, Šoltés L. (2008). Study of pro- and anti-oxidative properties of d-penicillamine in a system comprising high-molar-mass hyaluronan, ascorbate, and cupric ions. *Neuroendocrinol Lett* **29**: 697–701.
- Valachová K, Hrabárová E, Juránek I, Šoltés L. (2011b). Radical degradation of high-molar-mass hyaluronan induced by Weissberger oxidative system. Testing of thiol compounds in the function of antioxidants. 16th Interdisciplinary Slovak-Czech Toxicological Conference in Prague. *Interdiscip Toxicol* **4**(2): 65.
- Valachová K, Kogan G, Gemeiner P, Šoltés L. (2008b). Hyaluronan degradation by ascorbate: Protective effects of manganese (II). *Cellulose Chem. Technol* **42**(9–10): 473–483.
- Valachova K, Kogan G, Gemeiner P, Šoltés L. (2009b). *Hyaluronan degradation by ascorbate: protective effects of manganese (II) chloride*. In: Progress in Chemistry and Biochemistry. Kinetics, Thermodynamics, Synthesis, Properties and Application, Nova Science Publishers, N.Y, Chapter 20, pp. 201–215.
- Valachová K, Mendichi R, Šoltés L. (2010c). *Effect of L-glutathione on high-molar-mass hyaluronan degradation by oxidative system Cu(II) plus ascorbate*. In: Monomers, Oligomers, Polymers, Composites, and Nanocomposites, Ed: R. A. Pethrick P. Petkov, A. Zlatarov G. E. Zaikov, S. K. Rakovsky, Nova Science Publishers, N.Y, Chapter 6, pp. 101–111.
- Valachová K, Raptá P, Kogan G, Hrabárová E, Gemeiner P, Šoltés L. (2009a). Degradation of high-molar-mass hyaluronan by ascorbate plus cupric ions: effects of d-penicillamine addition. *Chem Biodivers* **6**: 389–395.
- Valachová K, Raptá P, Slovák M, Priesolová E, Nagy M, Mislovičová D, Drafi F, Bauerová K, Šoltés L. (2013a). *Radical degradation of high-molar-mass hyaluronan induced by ascorbate plus cupric ions. Testing of arbutin in the function of antioxidant*. In: Advances in Kinetics and Mechanism of Chemical Reactions, G. E. Zaikov, A. J. M. Valente, A. L. Iordanskii (eds), Apple Academic Press, Waretown, NJ, USA, pp. 1–19.

- Valachová K, Šoltés L. (2010b). *Effects of biogenic transition metal ions Zn(II) and Mn(II) on hyaluronan degradation by action of ascorbate plus Cu(II) ions*. In: New Steps in Chemical and Biochemical Physics. Pure and Applied Science, Nova Science Publishers, Ed: E. M. Pearce, G. Kirshenbaum, G.E. Zai-kov, Nova Science Publishers, N.Y, Chapter 10, pp. 153–160.
- Valachová K, Vargová A, Rapta P, Hrabárová E, Dráfi F, Bauerová K, Juránek I, Šoltés L. (2011a). Aurothiomalate in function of preventive and chain-breaking antioxidant at radical degradation of high-molar-mass hyaluro-nan. *Chem Biodivers* **8**: 1274–1283.
- Vanos HC, Drogendijk AC, Fetter WPF. (1991). The influence of contamination of culture-medium with hepatitis-B virus on the outcome of *in vitro* fertil-ization pregnancies. *Am J Obstet Gynecol* **165**: 152–159.
- Vazquez JR, Short B, Findlow AH. (2003). Outcomes of hyaluronan therapy in diabetic foot wounds. *Diabetes Res Clin Pract* **59**: 123–127.
- Weigel PH, Hascall VC, Tammi M. (1997). Hyaluronan synthases. *J Biol Chem* **272**: 13997–14000.
- West DC, Hampson IN, Arnold F, Kumar S. (1985). Angiogenesis induced by degradation products of hyaluronic acid. *Science* **228**: 1324–1326.
- Wilkinson LS, Pitsillides AA, Worrall JG, Edwards JC. (1992). Light microscopic characterization of the fibroblastlike synovial intimal cell (synoviocyte). *Ar-thritis Rheum* **35**: 1179–1184.
- Worrall JG, Bayliss MT, Edwards JC. (1991). Morphological localization of hyal-uronan in normal and diseased synovium. *J Rheumatol* **18**: 1466–1472.
- Worrall JG, Wilkinson LS, Bayliss MT, Edwards JC. (1994). Zonal distribution of chondroitin-4-sulphate/ dermatan sulphate and chondroitin-6-sulphate in normal and diseased human synovium. *Ann Rheum Dis* **53**: 35–38.
- Yerushalmi N, Arad A, Margalit R. (1994). Molecular and cellular studies of hy-aluronic acid-modified liposomes as bioadhesive carriers for topical drug-delivery in wound-healing. *Arch Biochem Biophys* **313**: 267–273.
- Yerushalmi N, Margalit R. (1998). Hyaluronic acid-modified bioadhesive lipo-somes as local drug depots: effects of cellular and fluid dynamics on lipo-some retention at target sites. *Arch Biochem Biophys* **349**: 21–26.
- Yun YH, Goetz DJ, Yellen P, Chen W. (2004). Hyaluronan microspheres for sus-tained gene delivery and site-specific targeting. *Biomaterials* **25**: 147–157.
- Zhu YX, Granick S. (2003). Biolubrication: hyaluronic acid and the influence on its interfacial viscosity of an antiinflammatory drug. *Macromolecules* **36**: 973–976.