Hyaluronan and synovial joint: function, distribution and healing

Tamer Mahmoud TAMER 1,2
1 Polymer Materials Research Department, Advanced Technologies and New Materials Research Institute (ATNMRI), City of Scientific Research and Technological Applications (SRTA-City), New Borg El-Arab City, Alexandria, Egypt
2 Laboratory of Bioorganic Chemistry of Drugs, Institute of Experimental Pharmacology & Toxicology, Slovak Academy of Sciences, Bratislava, Slovak Republic

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 cartilage.

Correspondence address:
Dr. Tamer Mahmoud Tamer
Polymer Materials Research Department, Advanced Technologies and New Materials Research Institute (ATNMRI), City of Scientific Research and Technological Applications (SRTA-City), New Borg El-Arab City 21934, Alexandria, Egypt.
E-MAIL: ttamer85@gmail.com
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; Ritig 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$_2^{-}$, H$_2$O$_2$, •OH) are generated in abundance by synovial neutrophils from RA patients, as compared with synovial neutrophils of osteo-arthritis (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 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.
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 (Chabrecke 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 (–c³) (Morris et al., 1980) and a solution of 10 g/l may have a viscosity at low shear of ~10⁶ times the viscosity of the solvent. At high shear the viscosity may drop as much as ~10³ 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 10g/l hyaluronan solution is of the same order as an 10g/l albumin solution. The exponential relationship makes hyaluronan and other polysaccharides excellent osmotic buffering substances – moderate changes in concentration lead
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 restrict the movements and not the viscosity of the solution. The excluded volume phenomenon will also affect the solubility of other macro-molecules 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.
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., 2001).

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; Rapta, 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.

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

\[ \text{Cu}^+ + \text{H}_2\text{O}_2 \rightarrow \text{Cu}^{2+} + \text{HO}^+ + \text{OH}^- \]

\( \text{H}_2\text{O}_2 \) has its origin due to the oxidative action of the Weissberger system (see Figure 6)

b. Formation of an alkyl radical (C-centered hyaluronan macroradical) initiated by the HO• radical attack.

c. Propagation phase: formation of a peroxy-type C-macroradical of hyaluronan in a process of oxygenation after entrapping a molecule of \( \text{O}_2 \).

d. Formation of a hyaluronan-derived hydroperoxide via the reaction with another hyaluronan macromolecule.

e. Formation of highly unstable alkoxy-type C-macroradical of hyaluronan on undergoing a redox reaction with a transition metal ion in a reduced state.

f. 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...
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 •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 pKₐ, 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
N-Acetyl-l-cysteine (NAC), another significant pre-cursor 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 α1-antiproteinase against the enzyme inactivation by HOCl. NAC reacts with HO• radicals and slowly with H2O2; 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 O2 to give O2•− (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).
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 peroxo radicals generated during the peroxo-degradation of HA (Hrabarova et al., 2012).

Acknowledgements
The author would like to thank the Institute of Experimental Pharmacology & Toxicology for having invited him and oriented him in the field of medical research. He would also like to thank Slovak Academic Information Agency (SAIA) for funding him during his work in the Institute.

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

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).

Adams ME. (1993). Viseosupplementation: 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.

AruomaOI,HalliwellB,HoeyBM,ButlerJ.(1989). The antioxidant action of N-acetylcysteine: Its reaction with hydrogen peroxide, hydroxyl radical, superoxide, and hypochlorous acid. Free Radic Biol Med 6: 593.

Ashhurst DE, BlandYS,LevickJR.(1991). An immunohistochemical study of the collagens of rabbit synovial interstitium. J Rheumatol 18: 1669–1672.

AthenasouNA,QuinnJ.(1991). Immunochemical analysis of human synovial lining cells: phenotypic relation to other marrow derived cells. Ann Rheum Dis 50: 311–315.

BalanizEA, DenlingerJL.(1989). Clinical uses of hyaluronan. Ciba Found Symp 143: 265–280.

BalanizEA, LaurentTC, JeanlozRW.(1986). Nomenclature of hyaluronic acid. Biochemical Journal 235: 903.

BalanizEA.(2003). Analgesic effect of elastoviscous hyaluronan solutions and the treatment of arthritic pain. Cells Tissues Organs 174: 49–62.

BalanizEA, DenlingerJL.(1993). Vicosupplementation: a new concept in the treatment of osteoarthritis. J Rheumatol 20: 3–9.

Banasovam, ValachovaK, JuranekI, SoltesL.(2012). Effect of thiol compounds on oxidative degradation of high molar hyaluronan in vitro. Intercitiscop Toxicol 5(Suppl. 1): 25–26.

Banasovam, Valachovak, JuranekI, SoltesL.(2013b). Aloevera and methylsulfonylethane as dietary supplements: Their potential benefits for arthritic patients with diabetic complications. Journal of Information Intelligence and Knowledge 5: 51–68.

Banasovam, Valachovak, RychlyJ, PriesiolovaE, Nagym, JuranekI, SoltesL. (2011). Scavenging and chain breaking activity of buccilamine on free-radical mediated degradation of high molar mass hyaluronan. ChemZi 7: 205–206.

BaňasováM, ValachováK, HrabárováE, PriesišlováE, NagyM, JuránekI, ŠoltésL.(2011). Early stage of the acute phase of joint inflammation. In vitro testing of buccilamine and its oxidized metabolite S981 in the function of antioxidants. 16th Interdisciplinary Czech-Slovak Toxicological Conference in Prague. Interdiscip Toxicol 4(2): 22.

BarrettJP,SivieroP.(2002). Retrospective study of outcomes in Hyalgan(R)-treated patients with osteoarthritis of the knee. Clin Drug Invest 22: 87–97.

Bergeret-GalleyC, LatoucheX, IllouzYG,(2001). The value of a new filler material in corrective and cosmetic surgery: DermaLive and DermaDeep. Aesthetic Plast Surg 25: 249–255.
Hrabarova E, Valachova K, Ratpa P, Soltes L. (2010). An alternative standard for trolox-equivalent antioxidant-capacity estimation based on thiol antioxidants. Comparative. 2.2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) decolorization and rotational viscosimetry study regarding hyaluronan degradation. *Chemistry & Biodiversity* 7(9): 2191–2200.

Hrabarova E, Valachova K, Rychly J, Ratpa 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 antioxidative 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 oxidative degradation action on biological systems regarding biomolecular injury and inflammatory processes. *Chem Pap* 61: 417–437.

Hrabarova E, Valachova K, Juranek I, Soltes L. (2011). Free-radical degradation of high-molar-mass hyaluronan induced by ascorbate plus cupric ions. Antioxidative properties of the Paula-bions liquid water from heating peeland maturation pool. In: “Kinetics, Catalysis and Mechanism of Chemical Reactions” G. E. Zaikov (eds), Nova Science Publishers, New York, pp. 29–36.

Hrabarova E, Valachova K, Rychly J, Ratpa P, Sasinkova V, Gemeiner P, Soltes L. (2009). High-molar-mass hyaluronan degradation by the Weissberger’s system: pro- and antioxidative effects of some thiol compounds. *Polymer Degradation and Stability* 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-α-trypsin inhibitor, and proteinase inhibitor to synovial fluid hyaluronan and the influence of these proteins on its degradation byxyogen derived free radicals. *Ann Rheum Dis* 47: 377–85.

Inoue M, Katakami C. (1993). The effect of hyaluronic acid on corneal epithelial-cell cell proliferation. *Invest Ophthalmol Vis Sci* 34: 2313–2315.

Itano N, Kimata K. (2002). Mammalian hyaluronan synthases. *JUBMB Life* 54: 195–199.

Jaakma U, Zhang R B, Larsson B. (1997). Effects of sperm treatments on the in vitro development of bovine oocytes in semen 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 Del Rev* 6: 3–19.

Jay GD, Brett DE, Cha DJ. (2000). Lubricin is a product of megakaryocyte stimulation factor gene expression by human synovial fibroblasts. *J Rheumatol* 27: 599–605.

Joly T, Nibart M, Thibier M. (1992). Hyaluronic acid as a substitute for proteins in the preimplantation development of embryos derived from deep-freezing of embryos from mice and sheep – an in vitro study. *Chem Pap* 45: 377–382.

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: 978-0-16668-923-0, 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. Petrick 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 bio- polymer with a broad range of biomedical and industrial applications. *Bio- technol Lett* 29: 17–25.

Krell G. (1995). Hyaluronidas 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 recombiant albumin and hyaluronan. *Mol Reprod Dev* 64: 70–78.

Langer K, Mutschler A, Lambrecht G. (1997). Methylmethacrylate sulfopropyly methacrylate copolymer nanoparticles for drug delivery – Part III. Evaluation as drug delivery system for ophthalmic applications. *Int J Pharm* 158: 219–232.

Lau D, Csomorova K, Kollarikova G, Stankovska M, Soltes L. (2005). Molar mass-intrinsic viscosity relationship of high-molar-mass hyaluronans: 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 Symposi- um. 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, Piuszkielewicz 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.

Levdan J, Narins RS, Brandt F. (2003). A randomized, double-blind, multi- center comparison of the efficacy and tolerability of Restylane versus Zyplast 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* 219: 73–82.

Luo Y, Prestwich GD. (1999). Synthesis and selective cytotoxicity of a hyaluron- ionic acid-antimurin biocongjugate. *Bioconjug Chem* 10: 755–763.

Luo Y, Ziebell MR, Prestwich GD. (2000). A hyaluronic acid-taxol antimurin biocongjugate targeted to cancer cells. *Biomacromolecules* 1: 208–218.

Mahué E, Ayral X, Dougados M. (2002). A hyaluronan preparation (500– 730 kDa) in the treatment of osteoarthritis: a review of clinical trials with HylanG-R. *Int J Clin Pract* 56: 804–813.

Manuskiati W, Mailach HI. (1996). Hyaluronic acid and skin: wound healing and aging. *Int J Dermatol* 35: 535–544.

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 pres- sure-flow relation across synovium in anesthetized rabbits. *J Physiol* 485(Pt 1): 179–93.

Mendichi R, Soltes L. (2002). Hyaluronan molecular weight and polydispers- ity in some commercial intra-articular injectable preparations and in syn- ovial fluid *Inflamm Res* 51: 115–126.

Meyer K, Palmer JW. (1934). The polysaccharide of the vitreous humor. *Ann Rheum Dis* 33: 177–199.

Meyers R, Picker F, Strobel F, Stavropoulos A. (1998). Synthesis and antiscru- mulating factor gene expression by human synovial fibroblasts. *Immunology and Cell Biology* 76: 177–80.

Minkowitz RS, Pickard DL. (1982). Liposomes in the treatment of synovitis. *Adv Drug Dev Rev* 5: 231–235.
Morimoto K, 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 ac- tion of hyaluronic sodium, Japan Pharmacological Conference. Tokyo, April 4, 1983.

Miyazaki T, Miyaschi 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.

Moberger TS, Levick JR, Mason RM. (2005). Hyaluronan secretion by synoviocytes is mechanosensitive. Matrix Biol 24: 510–519.

Moreira CA, Armstrong DK, Jelliffe RW. (1991). Sodium hyaluronate as a car- rier 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 intracocular gentamicin. Acta Ophthalmol (Copenh) 69: 50–56.

Morimoto K, Mtsugi 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, Ishikawa Y. (1997). Effects of viscous hyaluronate- sodium solutions on the nasal absorption of vasopressin and an analog. Pharmacol Res 8: 471–474.

Morris EE, 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 pulse radiolysis studies. Biochim Biophys Acta 925: 194–202.

Necas J, Bartosikova L, Brauner P, Kolar J. (2008). Hyaluronic acid (hyaluronan): a review. Veterinarni Medicina 53(8): 397–411.

Niwa Y, Sakane T, Shingu M, Yokoyama MM. (1983). Effect of stimulated neutrophils on the growth of rabbit cornea epithelial cells in vitro. J Ocul Pharmacol 9: 25–29.

Niwa Y, Sakane T, Shingu M, Yokoyama MM. (1983). Effect of stimulated neutrophils on the growth of rabbit cornea epithelial cells in vitro. J Ocul Pharmacol 9: 25–29.

Noble PW. (2002). Hyaluronan and its catabolic products in tissue injury and repair. Matrix Biol 21: 25–29.

Oates KM, Krause WE, Colley RH. (2002). Using rheology to probe the mecha- nism 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: 53–58.

Ortonne JP. (1996). A controlled study of the activity of hyaluronic acid in the treatment of venous leg ulcers. J Dermatol Treatment 7: 25–30.

Peer D, Florentin A, Margalit R. (2003). Hyaluronan is a key component in cryoprotection and formulation of targeted unilamellar liposomes. Biochim Biophys Acta-Biомembranes 1612: 76–82.

Peer D, Florentin A, Margalit R. (2003). Hyaluronan is a key component in cryoprotection and formulation of targeted unilamellar liposomes. Biochim Biophys Acta 1612: 76–82.

Peer D, Margalit R. (2000). Physicochemical evaluation of a stability-driven approach to drug entrapment in regular and in surface-modified lipo- somes. Arch Biochem Biophys 383: 185–190.

Poli A, Mason RM, Levick JR. (2004). Effects of Arg-Gly-Asp sequence peptide and hyperosmolality on the permeability of intestinal matrix and fenes- trated 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.
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.

Simkovíc I, Hricovinová 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 hyaluronic acid 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, Mendić F, 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, Gmeiner P. (2006a). Degradation of high-molecular-weight hyaluronan by hydrogen peroxide in the presence of cupric ions. Carbohydr Res 341: 639–644.

Soltes L, Mendić F, Kogan G, Schiller J, Stankovska M, Arnold K. (2006b). Degradative action of reactive oxygen species on hyaluronan. Biomacroolecules 7: 659–668.

Soltes L, Stankovska M, Brezova V, Stankovska M, Kogan G, Gmeiner P. (2006c). Hyaluronan degradation by copper (II) chloride and ascorbate. Carbohydrate Polym 71: 1234–1245.

Soltes L, Valachová K, Mendić F, Kogan G, Arnold K, Gmeiner P. (2007). Solution properties of high-molar-mass hyaluronans: the bipolymer 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 Chemistry 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, Arnold F, Rychly J, Spaltenthal P. (2007). In vitro screening of the action of non-steroidal anti-inflammatory drugs on hyochylophilic acid-induced hyaluronan degradation. Polym Degrad Stabil 92: 644–652.

Stankovska M, Soltes L, Vikartovska A, Mendić F, Lath D, Molnarova M, Gmeiner P. (2004). Study of hyaluronan degradation by means of rotational Viscometry: Contribution of the material of viscometer. Chem Pap 58: 348–352.

Stankovska M, Harabarova E, Valachová K, Molnarova M, Gmeiner P, Soltes L. (2006). The degradative action of pepsin on high-molecular-weight hyaluronan. Neuroendocrinol Lett 27(Suppl. 2): 31–34.

Stankovska M, Soltes L, Vikartovska A, Gmeiner P, Soltes G, Bakos D. (2005). Degradation of high-molecular-weight hyaluronan: a rotational visco- meter study. Biologii 60(Suppl. 17): 149–152.

Sterz R, Kogan G, Jedezejs WJ, Soltes L. (2007). The many ways to cleave hyaluronan. Biotechnol Adv 25: 537–557.

Stiebel-Kahl H, Gatton DD, Weinberger D. (1998). A comparison of the effect of hyaluronic acid versus gentamicin on corneal epithelial healing. Eye 12: 829–833.

Suchaneck 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, Hodges ECM. (2003). Sustained release of insulin from sodium hyaluronate based dry powder formulations after pul- monary delivery to beagle dogs. J Control Release 91: 385–394.

Suri N, Akyaama H, Morishita M. (2003). Polysaccharide effect of chitosan and so- dium hyaluronate as an implant device for insulin delivery. STP Pharm Sci 13: 265–268.

Suticnic L, Valachová K, Banasova M, Snivc V, Priesolova E, Nagy M, Juránek J, Soltes L. (2012). Free-radical degradation of high-molar-mass hyaluronan induced by ascorbate plus cupric ions: Testing of stabodine and its two derivatives in function as antioxidants. General Physiol Biophys 31: 57–64.

Swain DA, Silver FH, Slatyer HS, Stafford W, Shore E. (1985). The molecular structure and lubricating activity of lubricin isolated from bovine and hu- man synovial fluids. Biochem J 225: 195–201.

Takayama K, Hirata M, Machida Y. (1990). Radial degradation of high-molar-mass hyaluronan. Pro- and antioxidative effects of some thiols. Neuroendocrinol Lett 11: 615–621.

Taylor GM, Cantor JD. (2003). Hyaluronan in respiratory injury and repair. Am J Respir Crit Care Med 167: 1169–1175.

Uthman I, Raynauld JP, Harauzi B. (2003). Intra-articular therapy in osteoarthritis. Postgrad Med J 79: 449–453.

Valachová K, Vargova A, Rapta P, Harabarova E, Dráfi F, Baurová K, Juránek J, Soltes L. (2011). Antioxidant activity of various polyhexamethylene biguanide derivatives. J Pharm Sci 100: 348–352.

Valachová K, Harabarova E, Priesolová E, Nagy M, Banasova M, Juránek I, Soltes L. (2011). Free-radical degradation of high-molecular-weight hyaluronan induced by ascorbate plus cupric ions. Testing of curcumin and its SA981-metabolite as antioxidants. J Pharma & Biomedical Analysis 56: 644–670.

Valachová K, Harabarová E, Dráfi F, Juránek J, Baurová K, Priesolová E, Nagy M, Soltes 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, Harabarová E, Gmeiner P, Soltes L. (2008). Study of pro- and anti-oxidative properties of diclenacin in a system comprising high- molar-mass hyaluronan, ascorbate, and cupric ions. Neuroendocrinol Lett 29: 697–701.

Valachová K, Harabarová E, Juránek J, Soltes L. (2011b). Radical degradation of high-molar-mass hyaluronan induced by Weisberger oxidative system. Testing of thiols compounds in the function of antioxidants. 16th Interdis- ciplinary Slovak-Czech Toxicological Conference in Prague. Interdiscip Toxicol 42(2): 65.

Valachová K, Gmeiner P, Soltes L. (2008b). Hyaluronan degrada- tion by ascorbate: Protective effects of manganese (II). Cellulose Chem Technol 42(9–10): 473–483.

Valachová K, Gmeiner P, Soltes L. (2009b). Hyaluronan degrada- tion by ascorbate: protective effects of manganese (II) chloride. In: Progress in Chemistry and Biochemistry. Kinetics, Thermodynamics, Synthesis, Proper- ties and Application, Nova Science Publishers, N.Y., Chapter 20, pp. 201–215.

Valachová K, Merchant R, Soltes L. (2010a). Effect of l-glutathione on high-mo- lar-mass hyaluronan degradation by oxidative system Cu(II) plus ascorbate. In: Monomers, Oligomers, Polymers, Composites, and Nanocomposites, Ed: R. A. Pethrick P, Petkow, A. Zlatarov G, E. Zaikov, S. K. Rakozy, Nova Science Publishers, N.Y., Chapter 6, pp. 101–111.

Valachová K, Rakta P, Gmeiner P, Harabarova E, Gmeiner P, Soltes 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, Rakta P, Slováková M, Priesolová E, Nagy M, Mislovicková D, Dráfi F, Baurová K, Soltes L. (2013a). Radical degradation of high-molar-mass hy- aluronan induced by ascorbate plus cupric ions. Testing of curcumin in the function of antioxidant. In: Advances in Kinetics and Mechanism of Chemical Re- actions, G. E. Zaikov, A. J. M. Valente, A. L. Iordanski (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. Zaitkov, 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 hyaluronan. 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 fertilization 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 fibroblast-like synovial intimal cell (synoviocyte). Arthritis Rheum 35: 1179–1184.

Worrall JG, Bayliss MT, Edwards JC. (1991). Morphological localization of hyaluronan 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 hyaluronic 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 liposomes as local drug depots: effects of cellular and fluid dynamics on liposome retention at target sites. Arch Biochem Biophys 349: 21–26.

Yun YH, Goetz DJ, Yellen P., Chen W. (2004). Hyaluronan microspheres for sustained 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.