# Engineering of Polymers and Chemical Complexity

## Volume 2 New Approaches, Limitations, and Control

Editors
Walter W. Focke, PhD
Hans-Joachim Radusch, PhD

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# ENGINEERING OF POLYMERS AND CHEMICAL COMPLEXITY

## Volume II: New Approaches, Limitations, and Control

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**Gennady E. Zaikov, DSc, and A. K. Haghi, PhD**

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## CHAPTER 15

# HYALURONAN–A HARBINGER OF THE STATUS AND FUNCTIONALITY OF THE JOINT

LADISLAV ŠOLTÉS and GRIGORIJ KOGAN

## CONTENTS

## 15.1 INTRODUCTION

Women often live longer than men. This fact could be associated with their enhanced redox load during the reproductive phase of their life. The physiological bleeding (with a periodicity of ca. 4 weeks) is accompanied by changes in the concentration of iron ions. Pre-menopausal women are believed to have a lower risk of common diseases because amounts of iron in their body are unlikely to be excessive at this time [1].

Fe ions are regarded as one of the most important catalytical agents that contribute to the augmented generation of the reactive oxygen species (for example, $^{\bullet}OH$ radicals). However, such "radical training" of female organism lasting on average 40 yr (that is in a period between ca. 15 to 55 yr) can have a positive effect on females in the sense that their organism is better adjusted to the oxidative stress. In the "free radical theory of ageing" oxidative stress is considered to be a risk factor that is usually associated with such negative consequences as serious diseases or even premature death [2,3].

Life can be in a simplified way divided into three periods: childhood, maturity, and senescence. Maturity is the longest lasting part of human life. It lasts from the end of development and growth of a skeleton (around ca. 20 yr) till the old age, which start can be marked as at ca. 70–75 yr. Thus, maturity lasts about half a century. During this period, human skeleton can be considered invariable regarding the number of bones (206), their size, and mass.

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

## 15.2 THE STRUCTURE OF A SYNOVIAL JOINT

### *15.2.1 CARTILAGE*

In a healthy synovial joint, heads of the bones are encased in a smooth (hyaline) cartilage layer. These tough slippery layers – for example, 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.

Figure 1 illustrates a normal healthy synovial joint indicating its major parts

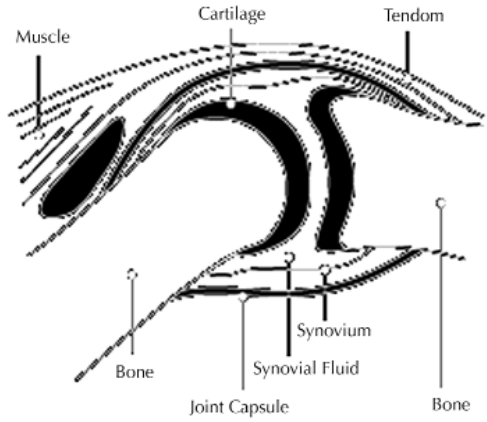



**FIGURE 1** Normal, healthy synovial joint [4].z.e The cartilage functions also as a shock absorber. This property is derived from. its high water-entrapping capacity, as well as from the structure and intermolecular interactions among polymeric components that constitute the cartilage tissue [5]. Figure 2 sketches a section of the cartilae– a chondrocyte cell that permanently restructures/rebuilds its extracellular matri.

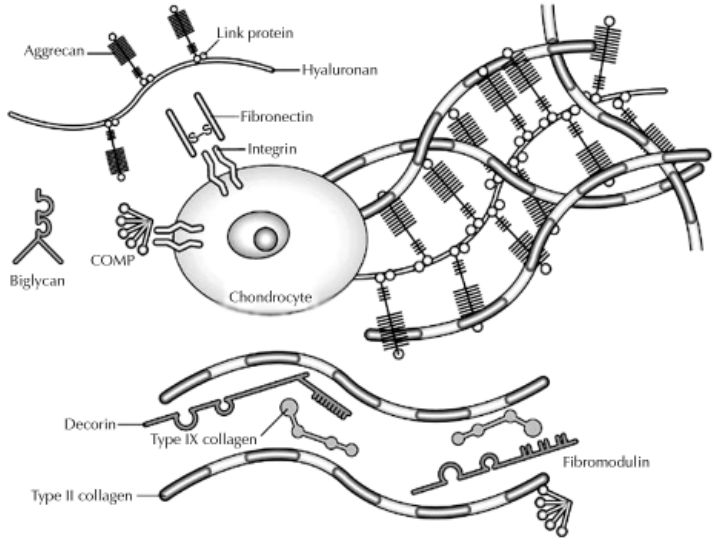



**FIGURE 2** Articular cartilage main components and structure [6].

Three classes of proteins exist in articular cartilage: collagens (mostly type II collagen); proteoglycans (primarily aggrecan); and other noncollagenous proteins (including link protein, fibronectin, COP– 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*.

### 15.2.2 SYNOVIUM/SYNOVIAL MEMBRANE

Each synovial joint is surrounded by a fibrous, highly vascular capsule/envelope called synovium, which 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 (SF).

### 15.2.3 SYNOVIAL FLUID

The synovial fluid, which consists of ateultra filtrate of blood plasma and glycoproteins, in normal/healthy joint contains HA macromolecules of molar mass ranging between 6–1nsmega Daltons [7]. 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 the motion [8].

The nutrients, including oxygen supply, upon crossing the synovial barrier, permeate through the viscous colloidal SF to the avascular articular cartilage, where they are utilized by the embedded chondrocytes. On the other hand, the chondrocyte catabolites (should) cross the viscous SF prior to being eliminated from the synovial joint [9]. It can thus be concluded that within SF, the process of "mixing" at the joint motion, significantly affects the equilibrium of influx and efflux of all low- and high-molar-mass solutes. It appears that the traffic of solutes is determined by molecular size, with small polar molecules being cleared by venular reabsorption, while high-molecular-sized solutes are removed by lymphatic drainage [10].

### 15.2.4 HYALURONAN

Figure 3 represents the structural formula of hyaluronan (also called hyaluronic acid, hyaluront) – regularly alternating disaccharide units composed from *N*-acetyl-D-glucosamine and D-glucuronic acid. HA is a polyelectrolyte component of SF; the concentration of HA in healthy human knee SF is 2.5 mg/ml on average [11]. While in the articular cartilage matrix HA is firmly associated via a link protein with proteoglycans (Figure 2), in SF the HA macromolecules are, if at all, only loosely interacting/bound to proteins.

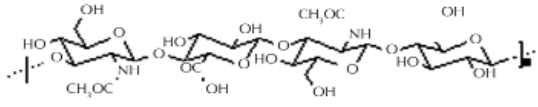



**FIGURE 3** Hyalurnn – the acid form.

The HA is a linear non-branched non-sulfated glycosaminoglycan (bio)polymer. In aqueous solutions, HA is represented by negatively charged hyaluronate macromolecules (p*K*a = 3.21 [12]) with extended conformations, which impart high viscosity/viscoelasticity, accompanied also by low compressibility – the characteristic property of SF [13].

### *15.2.5 REACTIVE OXYGEN SPECIES IN ARTICULAR CARTIALAR*

The articular cartilage is an avascular, acidic (pH 6.6–6.9) and hyperosmotic tissue dependent on diffusion of nutrients supplied mainly from SF (and perhaps partly from subchondral bone [14]) to provide for the metabolic requirements of chondrocytes. The oxygen levels in this tissue are low, ranging between 1 and 6% (cf. Figure 4). While reduction in O2 tension to 6% in all other tissues is already hypoxic, for chondrocytes such oxygen level is normoxic.

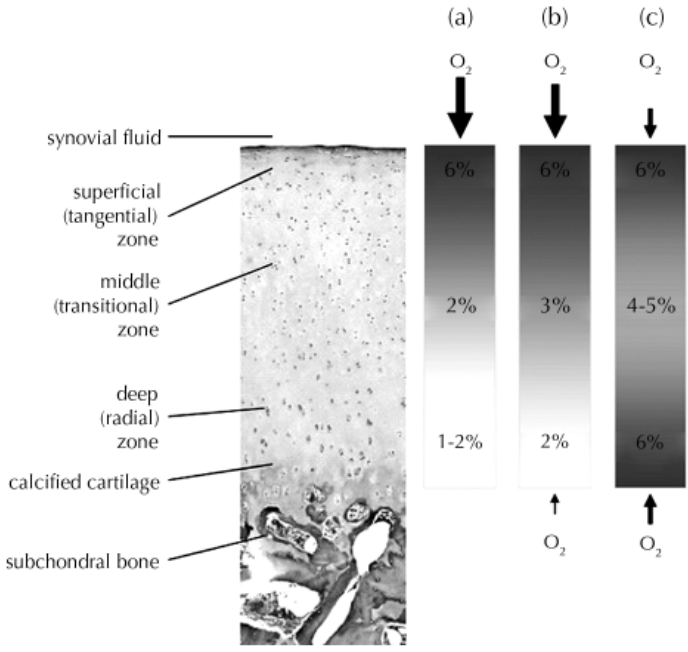



**FIGURE 4** The structure of articular cartilage and its oxygen supply (adapted from [14]). Estimated levels of $O_2$ within the cartilage tissue are shown for three scenarios: (a) Penetration of $O_2$ exclusively from SF; (b) $O_2$ supply mostly from SF with a small contribution from subchondral bone; and (c) Supply of $O_2$ in equivalent amounts from SF and subchondral bone.

In the mitochondria of the eukaryotic cells, not all $O_2$ is fully reduced to water. A small fraction of oxygen is reduced incompletely yielding reactive oxygen species (ROS), which are assigned to the defense of the organism against viral/bacterial invaders

[15]. It has been established that while ROS content within the articular cartilage tissue remains normal at 6% $O_2$, it decreased at 1% $O_2$ [14].

Since hydrogen peroxide generated within the mitochondria of chondrocytes can freely permeate through the chondrocyte cell wall, one should admit the presence of $H_2O_2$ in all (deep, middle, and superficial) zones of the articular cartilage (Figure 4). The higher the $O_2$ tension, the greater is the content of $H_2O_2$ and vice-versa.

The ROS within the cartilage tissue could serve both as intra- and inter-cellular signaling devices and a reactant participating in the so-called Fenton reaction

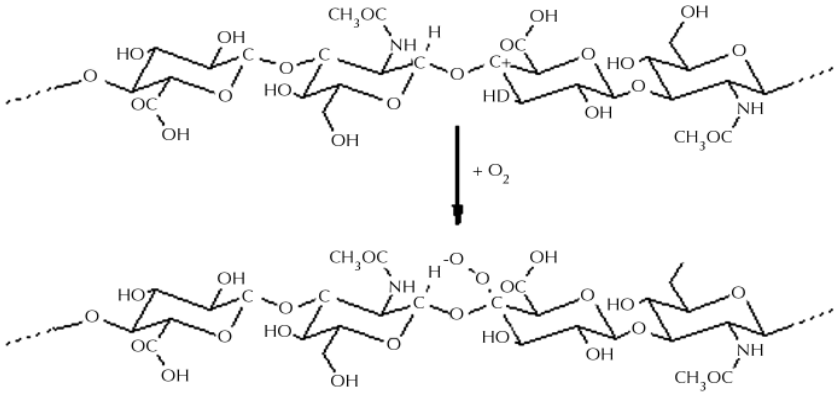
$$H_2O_2 + Me^{n+} \rightarrow {}^{\bullet}OH + Me^{(n+1)+} + {}^{-} \quad (1)$$

where, $Me^{n+}$ and $Me^{(n+1)+}$ represent a (biogenic) transition metal ion in reduced and oxidized state. Among these metals, primarily iron and copper are usually ranked, however, several further trace/biogenic metals can be taken into account as well [1,16].

### 15.2.6 ROS IN SF AND THEIR FUNCTION THEREOF

The capillaries within synovium continuously provide a plasma filtrate supplying in this way nutrients to the joint tissues (the arterial blood $O_2$ tension is 13% [17]). This is particularly important for homeostasis of the avascular articular cartilage [10]. As recently stated [16], taking into consideration that articular cartilage does not contain any teleneurons, chondrocytes should perform their autonomic (metabolic) regulation most plausibly using a chemical process, in which both $O_2$ and ROS play significant roles [17]. To understand this tenet, one should take into consideration that in the joint relaxed state – for example, at night – chondrocytes experience a decreased oxygen supply (a status termed "hypoxia"). However, when the status changes to an enhanced mobility in the morning, joint SF receives elevated supply of $O_2$ (a situation termed "re-oxygenaion"). Such increased content of oxygen can be, however, deleterious for the homeostasis of the chondrocytes – the cells that in adults lack mitotic activity.

Let us assume that $Me^{n+}$ ions in a given concentration are "entrapped" by (highly) negatively charged cartilage glycosaminoglycans (GAGs) within the superficial (tangential) zone of the articular cartilage (Figure 4). During the utilization of $O_2$ – respirain – by chondrocytes, a limited amount of $H_2O_2$ liberated from their mitochondria can react with the entrapped transition metal ions generating hydroxyl ($^{\bullet}OH$) radicals. Due to extremely short half-life of these species (picoseconds), they react *in situ nascendi* with GAGs – chondroitin sulfate (CS) and/or keratan sulfate (KS). The C-type radicals of CS or KS can, however, instantly undergo a reaction of hydrogen radical transfer onto the neighboring HA macromolecules within the SF. In such a way, free C-(mcals)radicals of hyaluronan appear nearby the superficial zone of the articular cartilage. And it is this very C-(macro)radical (denoted later as $A^{\bullet}$), which reacts and in this way reduces the (free "hyperoxic") $O_2$ tension within and nearby the superficial zone of the articular cartilage – according to the reaction presented in the following scheme:



**SCHEME 1** Entrapment of oxygen by the hyaluronan C-(macro)radical (A$^{\bullet}$) yielding a peroxyl (macro)radical (A–O-O$^{\bullet}$).

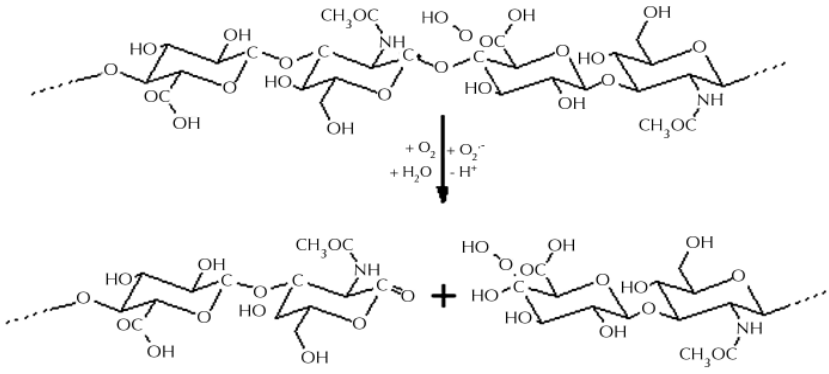
or briefly

$$A\text{–}H + {}^{\bullet}OH \rightarrow A^{\bullet} + H_2 \quad (2)$$

$$A^{\bullet} + O_2 \rightarrow A\text{–}O\text{-}O \quad (3)$$

where, A–H represents the intact hyaluronan macromolecule (Fig.Figure 3 and Scheme 1).

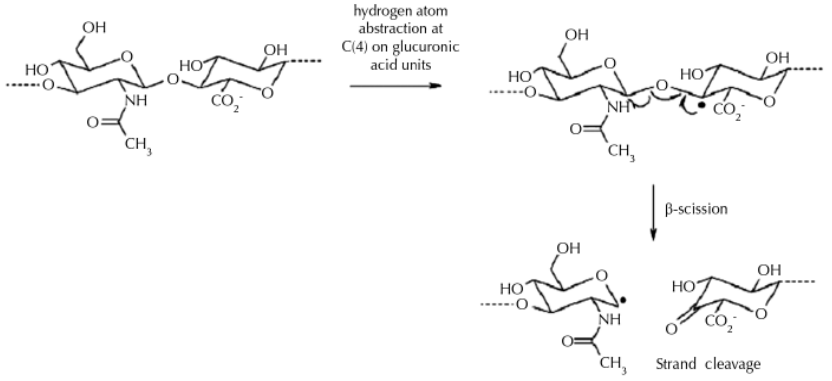
Subsequently, this A–O-O$^{\bullet}$ peroxyl (macro)radical can transform simply by an intramolecular 1,5-hydrogen shift to another C-(macro)radical – A$^{\bullet}$ (cf. Scheme 2). By participation of another $O_2$ molecule, this A$^{\bullet}$ radical can yield two fragments of the HA biopolymer: (i) the fragment, which possesses an aldehyde terminus, and (ii) the fragment bearing a hydroperoxide functional group. It is naturally evident that both fragments differ in their chemical structure from the initial HA macromolecule, not only due to the included novel substituents (–C=O; –O-OH) but above all by a reduced molar mass of both polymer fragments compared to that of the parent biopolymer.



**SCHEME 2** Strand scission of the C-(macro)radical (A•) yielding two fragments.

Since the intermolecular reaction between the CS and KS radicals and the native HA macromolecule could yield various A• radicals – formed for example at C(4) of the D-glucuronate/D-glucuronic acid (GlcA) unit (cf. Scheme 1) or at C(1) of GlcA unit, as well as at C(1) or C(3) of *N*-acetyl-D-glucosamine (GlcNAc) [18] – various biopolymer fragments are produced.

Very recently Kennett and Davies [19] reported the data obtained with both the C(1)- and the C(2)- $^{13}$C-labeled *N*-acetyl-D-glucosamine, and the apparent highly selective generation of radicals at the C(2) position of the isopropyl group of the β-isopropyl glycoside, which allow the authors to rationalize the specific banding pattern observed on oxidation of hyaluronan: The lack of reactivity at C(1)/C(2) of the *N*-acetyl-D-glucosamine monomers and the specific formation of radicals on the isopropyl group, which models the C(4) glycosidic linkage site of the glucuronic acid, implicate attack at C(4) of the glucuronic acid subunits and subsequent β-scission of this radical as a major route to cleavage of the hyaluronan backbone (Scheme 3). A contribution from reaction at C(1) of the glucuronic acid and subsequent cleavage of the alternative glycosidic linkage cannot be discounted; however, it is clear that an alternative route involving C(3) on the *N*-acetyl-D-glucosamine monomer is less favored, as only low levels of initial hydrogen atom abstraction seem to occur at this position as judged by the low yield of radicals that did not have additional $^{13}$C couplings observed with the two labeled *N*-acetyl-D-glucosamine species. It should be pointed, however, that the products of the hyaluronan strand cleavage depicted in Scheme 3 do not take into account that the ubiquitous oxygen participate within the strand scission reaction and thus, analogously to Scheme 2, the involved $O_2$ molecule with the A• radical yields two fragments of the HA biopolymer: (i) the fragment bearing a hydroperoxide functional group, and (ii) the fragment, which possesses an aldehyde terminus. As stated above, both fragments naturally differ in their chemical structure due to the included –C=O or –O-OH substituent and, above all, by the reduced molar mass of both polymer fragments compared to that of the parent HA biopolymer.




**SCHEME 3** Potential mechanism of hyaluronan strand cleavage as a result of hydrogen abstraction and radical formation on C(4) of the glucuronic acid unit (adapted from [19]).

Along with the fragmentation reactions shown in Schemes 2 and 3, the radical attack on the GlcA and GlcNAc moieties can also lead to the ring opening without breaking the polymer chain [11,18,20,21].

There exists, however, a remarkable phenomenon of *in vivo* free-radical oxidative degradation of hyaluronan: Under physiological conditions, the SF viscosity does not undergo any changes since the content of "naive" hyaluronan remains constant due to permanent *de novo* production of mega Dalton HA macromolecules by (stimulated) type B synoviocytes. Thus, the self-perpetuating oxidative (non-enzymatic)HA "catabolism" in SF represents a rather delicate and properly balanced mechanism that presumably plays significant role in regulating the physiological – normoxygen – homeostasis for chondrocytes. At the same time, the produced polymer fragments, which are probably cleared from the joint by drainage pathways, serve most likely as chemical messengers/feedback molecules. These play role in the adjustment of the optimum mode of functioning of the synovial membrane and of the HA-producing cells, B synoviocytes, localized within. In other words, during physiologic joint functioning, the hyaluronan in SF plays the role of a "scavenger antioxidant", whereas the produced polymer fragments can subsequently serve as messengers mediating information on the changes occurring in the homeostasis of the joint [16].

High "protective/scavenging efficiency" of hyaluronan against the *in vitro* action of $^{\bullet}OH$ radicals has been earlier pointed out by some authors [22,23]. Presti and Scott [23] described that high-molar-mass hyaluronan (megaDalton HA) was much more effective than the lower-molar-mass HAs (hundreds of kiloDaltons HAs) in scavenging $^{\bullet}OH$ radicals generated by a Fenton-type system comprising glucose and glucose oxidase *plus* $Fe^{2+}$-EDTA chelate.

### *15.2.7 HYPOXIA AND RE-OXYGENATION OF THE JOINT*

As SF of healthy human exhibits no activity of the hyaluronidase enzyme, it has been inferred that oxygen-derived free radicals are involved in a self-perpetuating process

of HA catabolism within the joint [24]. This radical-mediated process is considered to account for ca. twelve-hour half-life of native HA macromolecules in F.

To understand how to maintain a radical reaction active/self-perpetuating, its propagation stage should first be analyzed. If a peroxyl-type (macro)radical (A–O-O$^{\bullet}$) exists within SF, due to the relatively high reactivity of the unpaired electron on oxygen, the following intermolecular reaction can be assumed

$$\text{A–O-O}^{\bullet} + \text{A–H} \rightarrow \text{A–O-OH} + \qquad (4)$$

In the case when A$^{\bullet}$ is a C-type (macro)radical, it is this very reactant that traps the dioxygen molecule, dissolved in SF, according to the reaction

$$\text{A}^{\bullet} + \text{O}_2 \rightarrow \text{A–O-O}^{\bullet} \qquad (5)$$

Hence, by combining the reactions 4 and 5, the net reacion,

$$\text{A–H} + \text{O}_2 \rightarrow \text{A–O-} \qquad \text{(net reaction)}$$

corroborates the statement that one particular function of (a high-molar-mass) HA is to trap the oxygen excess during the phase of joint re-oxygenation [16].

## 15.3 PHYSIOLOGIC OXIDATIVE CATABOLISM OF HYALURONAN: PARTICIPATION OF BIOGENIC TRANSITION METALIONS

As stated in Scheme 2 and reaction 4, A–O-OH hydroperoxides are generated during the self-perpetuating – propagation – stage of the hyaluronan oxidative catabolism. The fate of A–O-OH type hydroperoxides, however, is significantly dependent on the presence or absence of the transition metal ions within SF. In the former case, the following reactions could be suggested for decomposition of the generated A–O-OH hydroperoxides

$$\text{A–O-OH} + \text{Me}^{n+} \rightarrow \text{A–O}^{\bullet} + \text{HO}^{-} + \text{Me}^{(n+1)} \qquad (6)$$

$$\text{A–O-OH} + \text{Me}^{(n+1)+} \rightarrow \text{A–O-O}^{\bullet} + \text{Me}^{n+} + {}^{+} \qquad (7)$$

As can be seen, while the “propagator” that participates in reaction 4 is (re)generated by reaction 7, reaction 6 produces an alkoxyl type (macro)radical A–O$^{\bullet}$. The ratio of the A–O-O$^{\bullet}$ to A–O$^{\bullet}$ radicals is, however, governed by the present transition metal ions, or, more precisely, by the ratio of $\text{Me}^{(n+1)+}$ to $\text{Me}^{n+}$. To answer the question, which transition metals may be present in SF and cells or tissues of healthy human beings, one should take into account the data presented in Tables 1 and 2.

**TABLE 1** Contents of transition metals in blood serum of healthy human volunteers and in *post mortem* collected SF from subjects without evidence of connective tissue disease

| Element | Mean concentration in blood serum [μg/100 mL][a] | Mean concentration in synovial fluid [μg/100 g][a] |
|---|---|---|
| Iron | 131.7 (23.6)[b] | 29.0 (5.19)[b] |
| Copper | 97.0 (15.3) | 27.5 (4.33) |
| Zinc | 115.4 (17.7) | 17.6 (2.69) |
| Manganese | 2.4 (0.44) | 2.4 (0.44) |
| Nickel | 4.1 (0.70) | 1.2 (0.20) |
| Molybdenum | 3.4 (0.35) | 1.0 (0.10) |

[a]Reported by Niedermeier and Griggs [25].
[b]Data in parentheses are the values in μM calculated in assumption that 100 g of SF has a volume of 100 mL.

**TABLE 2** Average relative abundance of some biogenic transition metals in the mammalian blood plasma and cells/tissues

| Element | Blood plasma [μM][a] | Cell/Tissue [μM][a] |
|---|---|---|
| Iron | 22 | ≈ 68 |
| Copper | 8-24 | 0.001-10 |
| Zinc | 17 | 180 |
| Manganese | 0.1 | 180 |
| Nickel | 0.04 | 2 |
| Molybdenum | - | 0.005 |

[a]Adapted from [26].

Based on the data listed in Table 1, iron and copper are the two prevailing redox active transition metals in SF. It should be, however, pointed out that the respective concentrations of ca. 5.2 μM of iron ions and 4.3 μM of copper ones do not represent those, which are (freely) disposable to catalyze the oxidative catabolism of hyaluronan within SF. As has been reported, the availability of iron to stimulate *in vivo* generation of ·OH radicals is very limited, since concentrations of "free" iron, are seldom larger than 3 μM in human samples [27].

Let us now deal with the oxidation states of iron within SF of a healthy human. By accepting that the concentration of ascorbate in SF of healthy subjects reaches the values close to those established in blood serum, that is 40–140 μM [28], it must be admitted that the transition metal ions in SF of a healthy human being are in the reduced oxidation state, that is $Me^{n+}$. Thus, in the case of the ascorbate level, which many times exceeds the concentration of transition metal ions, the actual concentration of ferrous ions should

exceed that of ferric ones, and thus A–O$^{\cdot}$ radicals should prevail. These radicals could, similarly to the A–O-O$^{\cdot}$ ones, propagate the radical chain reaction as follows

$$A–O^{\cdot} + A–H \rightarrow A–OH + A^{\cdot} \quad (8)$$

Yet, due to the redox potential of the pair RO$^{\cdot}$,H$^{+}$/ROH = + 1.6 V, which surpasses significantly that of ROO$^{\cdot}$,H$^{+}$/ROOH = + 1.0 V, the actual content of A–O$^{\cdot}$ in SF is practically nil; the half-life of the A–O$^{\cdot}$ radicals is much shorter than that of A–O-O$^{\cdot}$ ones – microseconds *vs*. seconds.

### *15.3.1 OXIDATIVE/NITROSATIVE STRESS*

Oxidative and/or nitrosative stress are terms used to describe situations, in which the organism's production of oxidants exceeds the capacity to neutralize them. The excess of oxidative species can cause "fatal" damage to lipids within the cell membranes, cellular proteins and nucleic acids, as well as to the constituents of the extracellular matrix, such as collagens, proteoglycans, and so on. [29].

Oxidative and/or nitrosative stress has been implicated in various pathological conditions involving several diseases, which fall into two groups:

1. Diseases characterized by "inflammatory oxidative conditions" and enhanced activity of either NAD(P)H oxidase (leading to atherosclerosis and chronic inflammation) or xanthine oxidase-induced formation of oxidants (implicated in ischemia and reperfusion injury),
2. Diseases characterized by the implication of pro-oxidants that shift the thiol/disulphide redox equilibrium and cause impairment of glucose tolerance - the so-called "mitochondrial oxidative stress" conditions (leading to cancer and diabetes mellitus) [3].

### *15.3.2 OXIDANTS*

In a broader sense, oxidation concerns the reaction of any substance with molecules of oxygen, the primary oxidant. In chemistry, however, the term "oxidant" is used for all species able to render one or more (unpaired) electrons.

**TABLE 3** Main ROS and RNS

| Radical | | **Non-radical** | |
|---|---|---|---|
| hydroxyl | $^{\cdot}OH$ | peroxynitrite anion | $ONOO^{-}$ |
| superoxide anion radical | $O_2^{\cdot-}$ | hypochloric acid | HOCl |
| nitric oxide | $^{\cdot}NO$ | hydrogene peroxide | $H_2O_2$ |
| thyil | $–RS^{\cdot}$ | singlet oxygen | $^1\Delta_g$ ($^{-1}O_2$) |
| alkoxyl | $RO^{\cdot}$ | ozone | $O_3$ |
| peroxyl | $ROO^{\cdot}$ | nitrosyl cation | $NO^{+}$ |
| | | nitroxyl anion | $NO^{-}$ |
| | | nitryl chloride | $NO_2Cl$ |

In a simplified way, oxidants can be classified as free-radical and non-radical species (cf. Table 3; adapted from [30]). They are often classified as reactive oxygen species (ROS) and reactive nitrogen species (RNS). Although the latter, similarly to ROS, contain oxygen atom(s) – for example, $NO^+$, $NO^-$, and $NO_2Cl$ – the RNS usually participate at nitrosylation reactions.

### 15.3.3 OXYGEN METABOLISM–SOURCE OF ENERGY

Several oxidant species are produced at the processes occurring in animal cells, including human ones, during metabolism of oxygen, when these cells generate energy. Although the substrate ($O_2$) is – by a cascade of enzymatically driven reactions – reduced within subcellular organelles, mitochondria, to a completely harmless substance, the waste product – water, a fraction of generated ROS may escape from the enzymatically controlled processes:

$$O_2 + 1e^- \rightarrow O_2^{\bullet -} \tag{9}$$

$$O_2^{\bullet -} + 1e^- + 2H^+ \rightarrow H_2O_2 \tag{10}$$

$$H_2O_2 + 1e^- + H^+ \rightarrow {}^{\bullet}OH + H_2O \tag{11}$$

$$^{\bullet}OH + 1e^- + H^+ \rightarrow H_2O \tag{12}$$

net reaction

$$O_2 + 4e^- + 4H^+ \rightarrow 2H_2O \tag{13}$$

As indicated by the reaction steps (9), (10), and (11), oxidants, namely $O_2^{\bullet -}$, $H_2O_2$, and $^{\bullet}OH$ are intermediate products of the enzymatically controlled cascade. Their reactivity and presumable site of action can be assessed by physico-chemical parameters, such as standard reduction potential ($E^{\theta}$) and half-life ($t_{1/2}$) of the given species (cf. Table 4).

**TABLE 4** Standard reduction potential (and half-life) for some dioxygen species in water, pH 7, 25°C[a]

| Species | (reaction) | $E^{\theta}$ [V] | $t_{1/2}$ [s] |
|---|---|---|---|
| $O_2$ | (9) | - 0.33[b] | reactive |
| $O_2^{\bullet -}$ | (10) | + 0.89 | $10^{-6}$ |
| $H_2O_2$ | (11) | + 0.38 | long living |
| $^{\bullet}OH$ | (12) | + 2.31 | $10^{-9}$ |

[a]Adapted from [31].
[b]The greater the positive $E^{\theta}$ value, the greater is generally the species reactivity, that is the ability to catch an electron [cf. reactions (9)–(12)].

With regard to the high (positive) value of $E^0$ and to the short half-life values, escape of $^{\bullet}OH$ and $O_2^{\bullet-}$ from the sphere immediately surrounding mitochondrion can be virtually excluded. Yet the neutral molecule $H_2O_2$ is considered to be movable one, which can escape as from the "body" of the mitochondrion as well as from the cell body itself. It is comprehensible that in some tissues the actual $H_2O_2$ concentrations may reach 100 μM or more as for example, in human and other animal aqueous and vitreous humors. The hydroperoxide levels at or below 20–50 μM seem, however, to have limited cytotoxicity to many cell types [32].

## 15.4 OXYGEN METABOLISM–A DEFENSE MECHANISM AGAINST VIRAL/ BACTERIAL INVADERS

Along with the above four-electron reaction (13), several specialized cells – or more precisely their specific (sub)cellular structures – are able to reduce $O_2$ molecules producing the superoxide anion radical, which in aqueous (acidic) milieu can form the reactive perhydroxyl radical ($^{\bullet}O_2H$).

Nitric oxide, called also nitrogen monoxide ($^{\bullet}NO$), a (bioactive) free radical, is produced in various cells/tissues by NO-synthase (NOS) enzymes. The three distinct NOS isoforms are $P_{450}$-related hemoproteins that during L-arginine oxidation to L-citrulline produce $^{\bullet}NO$. Two of the permanently present enzymes that participate in the regulation of the blood vessel tonus are termed constitutive NOS (cNOS), while the third one is called an inducible NOS (iNOS). The level of $^{\bullet}NO$ produced by iNOS increases markedly during inflammation, a process accompanied with abundant production of the superoxide anion radical.

The two radical intermediates – $O_2^{\bullet-}/^{\bullet}O_2H$ and $^{\bullet}NO$ – serve as precursors of various ROS and RNS, including hydrogen peroxide, peroxynitrite/peroxynitrous acid, hypochlorous acid, and so on. On respiring air, human beings by utilizing one mole of $O_2$ ingest $6.023 \times 10^{23}$ molecules of oxygen, of which approximately 1–3% is assigned to the generation of ROS/RNS that defend the organism against viral/bacterial invaders [15].

It has been noted that certain organ systems are predisposed to greater levels of oxidative stress and/or nitrosative stress. Those organ systems most susceptible to damage are the pulmonary system (exposed to high levels of oxygen), brain (exhibits intense metabolic activity), eye (constantly exposed to damaging UV light), circulatory system (victim to fluctuating oxygen and nitric oxide levels) and the reproductive systems (at risk from the intense metabolic activity of sperm cells) [30]. In some cases, however, the intermediate and/or the "final" reactive oxidative species may also damage cells/tissues of the human host. Imbalance between the extent of damage and self-repair of the functionally essential structures may result in a broader host tissue injury, eventually leading to a specific disease.

Because of the highly reactive nature of ROS/RNS, it is difficult to directly demonstrate their presence *in vivo*. It is considerably more practical to measure the "footprints" of ROS and RNS, such as their effects on various lipids, proteins, and nucleic acids [29].

### 15.4.1 INDIRECT ROS/RNS EVIDENCE

Most ROS/RNS have very short half-live times thus they cannot be directly detected in the organisms. That is why, as reported also by Valko *et al*. [3], convincing evidence for the association of oxidative/nitrosative stress and acute and chronic diseases lies on validated biomarkers of these stresses. Table 5 summarizes most representative biomarkers of oxidative damage associated with several human diseases.

**TABLE 5** Biomarkers of oxidative damage associated with several chronic human diseases (adapted from [3])

| **Disease** *Biomarker*[a] | **Alzheimer's disease** | **Atherosclerosis** | **Cancer** | **Cardiovascular disease** | **Diabetes mellitus** | **Parkinson's disease** | **Rheumatoid arthritis** |
|---|---|---|---|---|---|---|---|
| *8-OH-dG* | | | + | | | | |
| Acrolein | | + | | + | | | |
| *AGE* | + | | | | + | | |
| Carbonylated proteins | | | | | | + | |
| *$F_2$-isoprostanes* | + | + | | + | + | | + |
| *GSH/GSSG* | + | | + | + | + | + | + |
| *HNE* | + | + | | + | | + | |
| Iron level | | | | | | + | |
| *MDA* | + | + | + | | + | | |
| *$NO_2$-Tyr* | + | + | + | + | + | | |
| S-glutathiolated proteins | | | | | + | | |

[a]Abbreviations: *8-OH-dG, 8-hydroxy-20-deoxyguanosine; AGE, advanced glycation end products; GSH/GSSG, ratio of glutathione/oxidized glutathione; HNE, 4-hydroxy-2-nonenal; MDA, malondialdehyde; $NO_2$-Tyr, 3-nitro-tyrosine*

There are numerous further diseases whose pathology involves reactive oxidative/oxygen-derived species that is ROS and/or RNS, at the onset and/or at later stages of the disease [33]. The magnitude and duration of the change in the concentrations of these species appear to belong among the main regulatory events (cf. Figure 5).

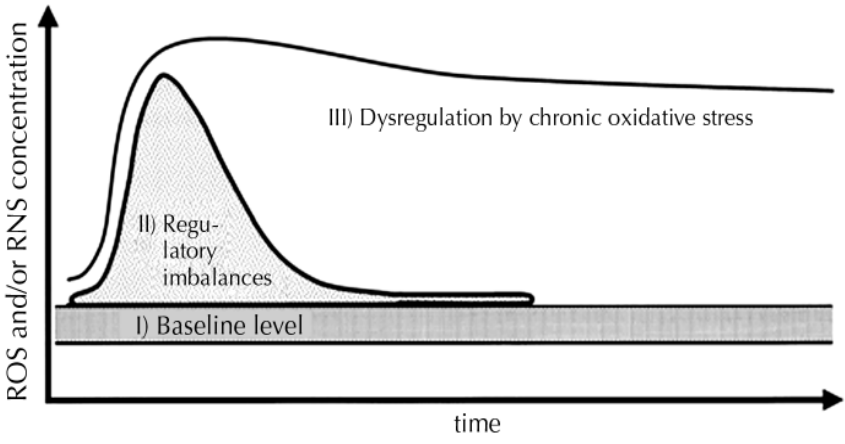



**FIGURE 5** Regulatory events and their dysregulation depend on the magnitude and duration of the change in ROS and/or RNS concentration(s) (adapted from [34]).

Today it is a widely accepted fact that ROS and RNS normally occur in living tissues at relatively low steady-state levels (cf. Figure 5, stage I "Baseline level"). The regulated increase in the production of superoxide anion radical or nitric oxide leads to a temporary imbalance, which forms the basis of redox regulation (stage II in Figure 5, "Regulatory imbalances"). The persistent production of abnormally large amounts of ROS or RNS, however, may lead to persistent changes in signal transduction and gene expression, which, in turn, may give rise to pathological conditions (as seen in Figure 5, stage III "Dysregulation by chronic oxidative stress") [**34**]. One of the classes of such diseases includes arthritic conditions – inflammatory diseases of joints. A substantial amount of evidence exists for an increased generation of oxidants in patients suffering from acute and chronic inflammatory joint diseases [36,37] – see Table 6.

**TABLE 6** Some characteristics registered within SF during inflammatory joint diseases[a]

| | | Blood characteristics | | | |
|---|---|---|---|---|---|
| **Diagnosis** | **SF viscosity** | **White cells/ µL** | **% of PMNLs** | **PMNLs/ µL** | **$H_2O_2$ flux [µM/ min]** |
| Healthy | normal | <200 | 7 | <14 | <0.003 |
| OA | decreased | 600 | 13 | 48 | 0.017 |
| RA | decreased | 1900 | 66 | 1254 | 0.276 |

[a]Adapted from [37].

### 15.4.2 REGULATORY IMBALANCES WITHIN A SYNOVIAL JOINT

As schematically reported by Dröge [34], under physiological status, "Baseline level" (cf. Figure 5) of ROS and/or RNS concentration play an important role as regulatory mediators in signaling processes. In case of the composition of SF of healthy organisms, one may state two border concentrations of ROS (and RNS as well), which are primarily determined by the $O_2$ level within SF, or more precisely by the $H_2O_2$ level escaped from mitochondria of chondrocytes and from those of cells of the synovial membrane. A lower one exists at rest regimen of the joint and a higher $H_2O_2$ level at reoxygenation of the joint tissues during movement of the subject. The high-molar-mass HA however keeps most probably the joint ROS/RNS homeostasis between the two concentration values inside the "Baseline level" (see Figure 5, stage I).

On accepting the tenet that concentrations of $H_2O_2$ ranging around 50 μM (sometimes even up to 100 μM) are not toxic to any cells [32], the highest limit (cf. stage I, Figure 5) of the hydrogen peroxide level in SF, and thus in contact with both chondrocytes and synovial-membrane cells, is close to this concentration (<100 μM). The flux of $H_2O_2$ in the amount of less than 0.003 μM per minute does not change SF viscosity (cf. Table 6). In light of this observation one can propose that the ROS action, that is $H_2O_2$-degradative action on the high-molar-mass HA, is fully compensated by the *de novo* synthesis of megaDalton hyaluronans by the synoviocytes embedded within the synovial membrane of healthy human beings. Our detailed studies focusing on the $H_2O_2$-degradative action to HA macromolecules also showed that hydrogen peroxide up to hundreds of micromolar concentrations led to practically no cleavage/decay of high-molar-mass hyaluronan samples when the reaction system was "free" of any transition metal ions, namely those of iron and/or copper [M. Stankovská *et al*., not published].

Let us now admit the situation of occurrence of temporary "Regulatory imbalances" (stage II in Figure 5), or more precisely the situation at which an acute inflammation is initiated within the synovial joint. On taking into account the data given in Table 6, the increase in ROS concentration, or more precisely the increase in $H_2O_2$ flux, appears to be functionally related to the rising number of PMNLs in the SF, presenting in the initial phase as Regulatory inbalance. This increase is however associated with the following events: i) infiltration of the increased number of white cells (PMNLS and/or macrophages) from the blood circulation into the SF, and ii) activation of these cells in the SF. Yet concerning the event given in ii), it has to be emphasized that at the time of infiltration movement of the white blood cells is impeded in the SF, due to its viscosity, which can be characterized as "normal" (cf. Table 6; see Figure 6) or high caused by the presence of high-molar-mass HA macromolecules. Moreover, it is a well-known fact that especially high-molar-mass hyaluronans exert antiimflammatory action or more precisely, the long-sized HA chains quench the PMNLs and macrophages.

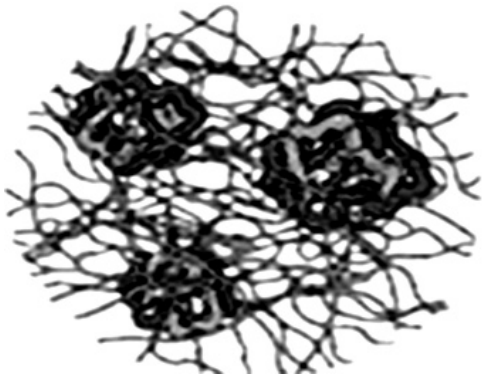


**FIGURE 6** The movement of the white blood cells in the normal/highly viscous SF. The long-sized HA chains are sketched as blue strands.

Thus one may admit that infiltration of an increased number of white cells into a millieu such as that of SF of healthy human beings need not immediately result in a rise of the ROS concentration or the $H_2O_2$ level enhancement, respectively. The demand of rapid/acute growth of ROS/RNS level within the joint during the stage II (cf. Figure 5, "Regulatory imbalances") could not be met in this way. Resulting from our experimental findings, we may hereby offer/recommend our hypothesis/speculation in point of process sequencing which can very quickly, owing to their physiological status, bring about – for a temporary time period – the status possibly be defined as accute inflammation, or – by taking into account the Dröge scheme (cf. Figure 5 [34]) – the "Regulatory inbalances".

## *INFLAMMATION*

Inflammation generally means a complex biological response of tissues to harmful stimuli, such as infective pathogens, damaged cells, toxins, physical and/or chemical irritants. It is a protective attempt by the organism to remove injurious stimuli and to initiate the healing process for the tissue. Yet inflammation that runs unchecked can lead to various diseases (cf. Table 5), including those connected to synovial joints. Normally, however, inflammation is critically controlled and closely regulated by the body.

Inflammation can be classified as acute or chronic (Table 7). Acute inflammation is the initial response of the body to harmful stimuli and is achieved by the increased movement of PMNLs from the blood into the injured tissues. Then a cascade of biochemical events propagates and matures the (local) inflammatory response. Chronic inflammation usually leads to a progressive shift in the type of immune cells which are present at the site of inflammation and is characterized by destruction and often by (partial) healing of damaged tissues.

**TABLE 7** Comparison between acute and chronic inflammation (from [38])

| Inflammation | Acute | Chronic |
|---|---|---|
| *Causative agent* | Pathogens, injured tissues | Persistent acute inflammation due to non-degradable pathogens, persistent foreign bodies, or autoimmune reactions |
| *Major cells involved* | Neutrophils, mononuclear cells (monocytes, macrophages) | Mononuclear cells (monocytes, macrophages, lymphocytes, plasma cells), fibroblasts |
| *Primary mediators* | Vasoactive amines, eicosanoids | IFN-γ and other cytokines, growth factors, reactive oxygen species, hydrolytic enzymes |
| onset | immediate | delayed |
| duration | few days | up to many months or yr |
| outcomes | resolution, abscess formation, chronic inflammation | tissue destruction, fibrosis |

Acute inflammation – a short-term process appearing in a few minutes or hours – is usually characterized by five cardinal signs: rubor, calor, tumor, dolor, and *functio laesa*. However, the acute inflammation of an internal organ may not be manifested by the full set of signs.

Inflammation, and especially the acute one, is associated with elevated systemic levels of acute-phase proteins. These proteins prove beneficial in acute inflammation.

## *ACUTE-PHASE PROTEINS*

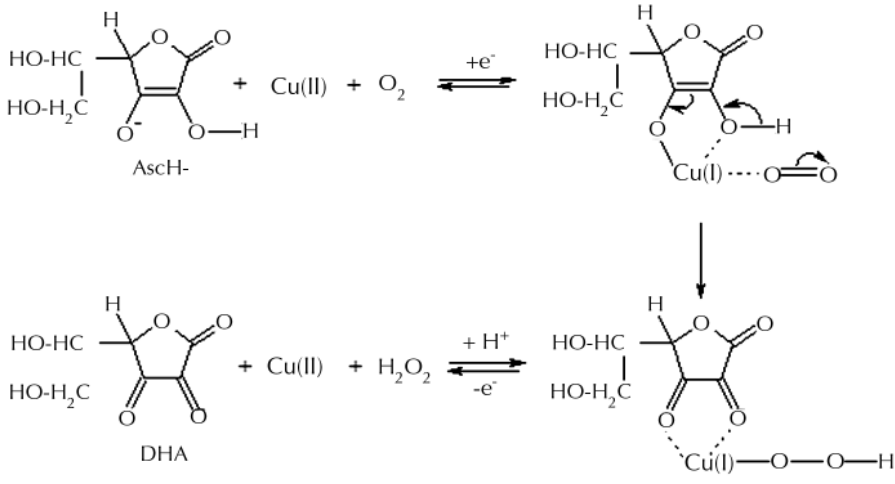
The acute-phase proteins are a class of proteins whose plasma concentrations increase (positive acute-phase proteins) or decrease (negative acute-phase proteins) in response to inflammation. This response is called the acute-phase reaction or acute-phase response. The acute-phase reactants are produced by the liver in response to specific stimulations. The following positive acute-phase proteins belong to the physiologically most prominent ones: C-reactive protein, $\alpha_1$-antitrypsin and $\alpha_1$-antichymotrypsin, fibrinogen, prothrombin, complement factors, ferritin, serum amyloid A, $\alpha_1$-acid glycoprotein, ceruloplasmin, and haptoglobin. Others – negative acute-phase proteins such as albumin, transferrin – give negative feedback on the inflammatory response.

## *CERULOPLASMIN*

The concentration of ceruloplasmin, whose molar mass (≈ 134 kDa) exceeds nearly twice that of albumin, increases markedly under certain circumstances – including those of acute inflammation. Since each ceruloplasmin macromolecule complexes/binds up to eight Cu(II)/Cu(I) ions of which two can liberate relatively easily [39], at the early stage of acute inflammation the actual copper level increases markedly. The consequence of higher ceruloplasmin concentration in blood plasma – accompanied with a rise in the concentration of copper ions – would mean a larger amount of this biogenic trace element that might cross the synovial membrane [16]. Yet, due to the gel-like consistency of SF, the copper ions entering into this specific environment start their redox action in the vicinity of the synovial membrane.

### *15.4.3 WEISSBERGER'S OXIDATIVE SYSTE*

The concentration of ascorbate in SF of healthy subjects reaches the values close to those established in blood seru,.that is 40–140 μM [28]. Ascorbate, an "actor of physiologic HA catabolism in SF" with copper liberated from ceruloplasmin, creates easily the so-called Weissberger's oxidative system [40,41] – ascorbate-Cu(I)-oxygen – generating $H_2O_2$ (cf. Scheme 4) [42-44]. Moreover, due to the simultaneous decomposition of hydrogen peroxide by the redox active copper ions, a large flux of hydroxyl radicals may occur [45].




**SCHEME 4** Generation of $H_2O_2$ by Weissberger's system from ascorbate and Cu(II) under aerobic conditions (adapted from Fisher and Naughton [44]).

As evident from the data listed in Table 1, iron and copper are the two prevailing redox active transition metals in SF. Although just only a minor fraction of their re-

spective total levels equaling 5.2 μM and 4.3 μM is disposable for Weissberger's and/or Fenton-type reactions, it are the copper ions that better fulfill the requirement of acute (rapid) generation of ROS – particularly of •OH radicals (cf. Figure 7).

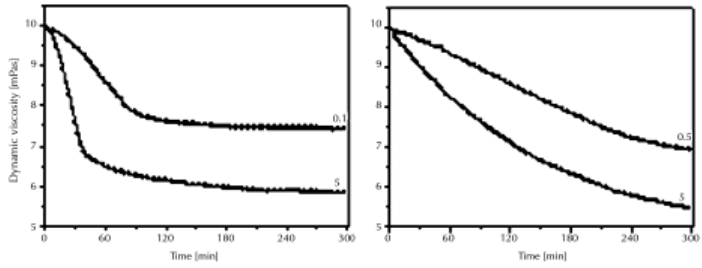



**FIGURE 7** Time dependences of dynamic viscosity of solutions of a high-molar-mass HA sample.

- *Left panel*: Solutions of the HA sample with addition of 100 μM ascorbic acid immediately followed by admixing 0.1 or 5 μM of $CuCl_2$.
- *Right panel*: Solutions of the HA sample with addition of 100 μM ascorbic acid immediately followed by admixing 0.5 or 5 μM of $FeCl_2$.

Figure 7 illustrates the degradative action of ROS by monitoring the viscosity-time profiles of a HA solution into which – along with 100 μM ascorbate – a single transition metal was added [46]. As evident, a significant reduction of the solution dynamic viscosity (η), corresponding to the degradation of the high-molar-mass HA sample, clearly indicates a concentration-dependent manner for each metal (cf. left and right panels ig.Figure 7). While the character of the time dependence of η value upon the addition of $FeCl_2$ (5.0 μM) can be described as a gradual monotonous decline, the addition of $CuCl_2$ (5.0 μM) resulted in a literally "dramatic" drop of η value within a short time interval (30 min). A similar drop of η value and two-phase reaction kinetics are identifiable upon the addition of even a minute (0.1 μM) amount of $CuCl_2$ (seg. Figure 7, left panel). A possible explanation of this dissimilarity lies most probably in different reaction kinetics of the processes leading to generation of oxygen-derived reactive species in the system ascorbate *plus* $CuCl_2$ and in that comprising ascorbate *plus* $FeCl_2$.

As seen in Figure 7, the transition metal – either iron or copper – can play an active role in oxidative HA catabolism. However, the increase in Cu(II) concentration within the joint (and particularly in SF) could lead to an extremely rapid degradation of the native HA macromolecules. How efficiently the chemically generated •OH radicals are "scavenged" within this microenvironment by the locally disposable albumin as well as by the HA polymer fragments of lower molecular size, remains questionable. The oxidative process may escape the control mechanisms and damage/disrupt the synovial membrane. Moreover, the intermediate-sized HA-polymer fragments generated within this microenvironment could participate in the activation of "defender" cells. They may further intensify the inflammation state of the injured tissue(s) as the HA-

polymer fragments can in turn augment the inflammatory responses. As reported by Jiang *et al.*, the HA fragments in the, for example, $2 \times 10^5$ Da range induce the expression of a number of inflammatory mediators in macrophages, including chemokines, cytokines, growth factors, proteases, and nitric oxide [47]. In this way, the oxidants generated by activated defender cells may enlarge the damage within the involved joint tissues such as the synovial membrane (cf. Figure 8). Such an increase in unmediated reactive radicals, generally termed oxidative stress, is an active area of research in a variety of diseases where copper may play an insidious role.

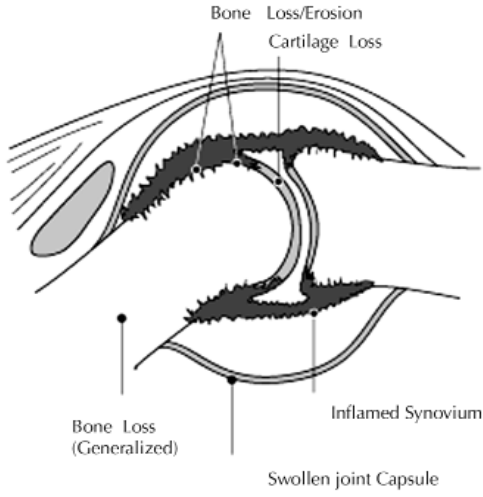



**FIGURE 8** Damages within the inflamed joint tissues.

Moreover, reactive oxygen species appear to disrupt copper binding to ceruloplasmin, thereby releasing "free" copper ions, which in turn may promote oxidative pathology [39]. The damage can be manifested by visually localizable cardinal signs of inflammation – that is rubor, calor, tumor, dolor, and *functio laesa*, yet less distinct, repeated (micro-acute) inflammatory injures may lead to a disastrous outcomeg.for example, an autoimmune disease such as rheumatoid arthritis.

## 15.5 RELEVANCY AND FUNCTION OF WEISSBERGER'S OXIDATIVE SYSTEM AT ACUTE INFLAMMATION OF THE JOINT

As demonstrated by the results depicted in Figure 7 (left panel) Weissberger's oxidative system is really a prompt/ultimate generator of hydrogen peroxide leading immediately to dramatic flux of $^{\bullet}OH$ radicals. Subsequently these radicals initiate a significant degradation of long-chain HA macromolecules, the process which diminishes markedly the dynamic viscosity of the hyaluronan solution. A similar HA degradative process can be anticipated in SF at the early stage of acute (synovial) joint inflammation. The lower SF viscosity may markedly promote the transition of defender cells

from blood through the synovial membrane and further enhance the movement of these cells to the target synovial and periarticular tissues. These cells may simultaneously undergo activation in contact with/binding to biopolymer fragments resulted from (•OH) radical degradation of native high-molar-mass hyaluronans present in SF. The infiltrated defender cells thus may start their more or less specific action inside the intraarticular space.

### 15.5.1 CHRONIC INFLAMMATION

In acute inflammation, if the injurious agent persists, chronic inflammation will ensue. This process marked by inflammation lasting many days, months or eversyears, may lead to the formation of a chronic wound. Chronic inflammation is characterized by the dominating presence of macrophages in the injured tissue. These cells are powerful defensive agents of the body, but the "toxins" they release – including ROS and/or RNS – are injurious to the organism's own tissues. Consequently, chronic inflammation is almost always accompanied by tissue destruction. Destructed tissues are recognized by the immunity system and, when "classified" by the body as foreign ones, a cascade of autoimmune reactions could start. Such reactions are well established in diseases such as rheumatoid arthritis, where – along with the (synovial) joints – several further tissues/organs e.g. for example, lungs, heart, and blood vessels, are permanently atacked e.g. that is misrecognized as foreign ones.

### 15.5.2 MEDICATIONS USED TO TREAT INFLAMMATORY JOINT DISEASES

There are many medications available to decrease joint pain, swelling, inflammation and to prevent or minimize the progression of the inflammatory disease. These medications include:

- Non-steroidal anti-inflammatory drugs (NSAIDs – such as acetylsalicylic acid/aspirin, ibuprofen or naproxen).
- Corticosteroids (such as prednisone).
- Anti-malarial medications (such as hydroxychloroquine).
- Other medications, including methotrexate, sulfasalazine, leflunomide, anti-TNF medications, cyclophosphamide, and mycophenolate.

As reported in the Section "Relevancy and function of Weissberger's oxidative system at acute inflammation of the joint", the early acute-phase of (synovial) joint inflammation should, most plausibly, be accompanied with generation of ROS (and RNS) – particularly with •OH radicals. These, however, due to their extrememly high electronegativity (-2.31 V) should – in contact with any hydrogen atom containing compounds – entrap a proton (•H). By that process the •OH radicals are partially or fully scavenged (cf. Figure 9). If the resulting radical generated from the given compound/medication is not able to initiate HA degradation, we speak of drug-scavenging, which could moderate the free radical process within the inflamed joint.

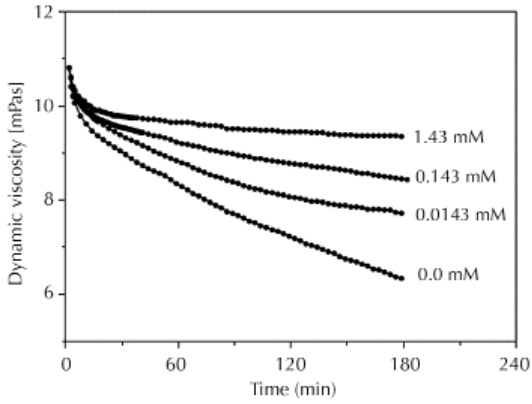



**FIGURE 9** Effect of acetylsalicylic acid on HA degradation in the system 0.1 μM $CuCl_2$ + 100 μM ascorbic acid + 2 mM NaOCl.

Concentration of acetylsalicylic acid added into the system before initiation of HA degradation in mM: 0.0, 0.0143, 0.143, and 1.43.

Figure 9 illustrates such an *in vitro* testing of the scavenging efficiency of acetylsalicylic acid/aspirin. As evident, this drug – based on its activity under aerobic conditions within the system HA-ascorbate-$Cu^{2+}$-NaOCl – can be classified as a potent scavenger of $^{\bullet}OH$ radicals [48].

## 15.6 CONCLUSIONS

With the current understanding that free radicals can act as cell signaling or "messenger" agents it is likely that they also play a role in normal cellular function as well as various disease etiologies. Researchers are now making rapid progress in understanding the role of oxidative stress and nitrosative stress in cardiovascular diseases such as atherosclerosis, ischemia/reperfusion injury, restenosis and hypertension; cancer; inflammatory diseases such as acute respiratory distress syndrome (ARDS), asthma, inflammatory bowel disease (IBD), dermal and ocular inflammation and arthritis; metabolic diseases such as diabetes; and diseases of the central nervous system (CNS) such as amyotrophic lateral sclerosis (ALS), Alzheimer's, Parkinson's, and stroke. The increased awareness of oxidative stress related to disease and the need to measure the delicate balance that exists between free radicals and the given systems in regulating them has given rise to a demand for new research tools.

**KEYWORDS**

- **Osteoarthritis (OA)**
- **Polymorphonuclear leukocyte (PMNL)**
- **Rheumatoid arthritis (RA)**

## ACKNOWLEDGMENT

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## REFERENCES

1. Yan, D., Gao, C., and Frey, H. "*Hyperbranched Polymers Synthesis, Properties, and Applications*, John Wiley & Sons, New Jersey (2011).
2. Frechet, J. M. J. and Tomalia, D. A. *Dendrimers and other dendritic polymers*, John Wiley & Sons, UK (2011).
3. Hasanzadeh, M., Moieni, T., and Hadavi Moghadam, B. *Adv. Polym. Tech.*, **32**, 792 (2013).
4. Mishra, M. K. and Kobayashi, S. *Star and Hyperbranched Polymers*, Marcel Dekker, New York (1999).
5. Gates, T. S. and Odegard, G. M., Frankland, S. J. V., Clancy, T. C. *Compos. Sci. Technol.*, **65**, 2416 (2005).
6. Jikei, M. and Kakimoto, M. *Prog. Polym. Sci.*, **26**, 1233 (2001).
7. Gao, C. and Yan, D. *Prog. Polym. Sci.*, **29**, 183 (2004).
8. Voit, B. I. and Lederer, A. *Chem. Rev.*, **109**, 5924 (2009).
9. Kumar, A. and Meijer, E. W. *Chem. Commun.*, **16**, 1629 (1998).
10. Grabchev, I., Petkov, C., Bojinov, V., *Dyes. Pigments.*, **62**, 229 (2004).
11. Qing-Hua, C., Rong-Guo, C., Li-Ren, X., Qing-Rong, Q., and Wen-Gong, Z. *Chinese J. Struct. Chem.*, **27**, 877 (2008).
12. Kou, Y., Wan, A., Tong, S., Wang, L., and Tang, J. *React. Funct. Polym.*, **67**, 955 (2007).
13. Schmaljohann, D., Pötschke, P., Hässler, R., Voit, B. I., Froehling, P. E., Mostert, B., and Loontjens, J. A. *Macromolecules*, **32**, 6333 (1999).
14. Kim, Y. H. *J. Polym. Sci. A*, **36**, 1685 (1998).
15. Liu, G. and Zhao, M. *Iranian Polym. J.*, **18**, 329 (2009).
16. Inoue, K. *Prog. Polym. Sci.*, **25**, 453 (2000).
17. Seiler, M. *Fluid Phase Equilibr.*, **241**, 155 (2006).
18. Yates, C. R. and Hayes, W. *Eur. Polym. J.*, **40**, 1257 (2004).
19. Voit, B. *J. Polym. Sci. A*, **38**, 2505 (2000).
20. Nasar, A. S., Jikei, M., and Kakimoto, M. *Eur. Polym. J.*, **39**, 1201 (2003).
21. Froehling, P. E. *Dyes. Pigments.*, **48**, 187 (2001).
22. Jikei, M., Fujii, K., and Kakimoto, M. *Macromol. Symp.*, **199**, 223 (2003).
23. Radke, W., Litvinenko, G., and Müller, A. H. E. *Macromolecules*, **31**, 239 (1998).
24. Maier, G., Zech, C., Voit, B., and Komber, H. *Macromol. Chem. Phys.*, **199**, 2655 (1998).
25. Voit, B., Beyerlein, D., Eichhorn, K., Grundke, K., Schmaljohann, D., and Loontjens, T. *Chem. Eng. Technol.*, **25**, 704 (2002).
26. Frey, H. and Hölter, D. *Acta. Polym.*, **50**, 67 (1999).
27. Hölter, D., Burgath, A., and Frey, H. *Acta. Polymerica.*, **48**, 30 (1997).
28. Le, T.C., Todd, B. D., Daivis, P. J., and Uhlherr, A. *J. Chem. Phys.*, **130**, 074901 (2009).
29. Haj-Abed, M. Master Degree Thesis, McMaster University (2008).
30. Stiriba, S. E., Kautz, H., and Frey, H. *J. Am. Chem.*, **124**, 9698 (2002).
31. Zou, J., Zhao, Y., Shi, W., Shen, X., and Nie, K. *Polym. Advan. Technol.*, **16**, 55 (2005).
32. Zhu, L., Shi, Y., Tu, C., Wang, R., Pang, Y., Qiu, F., Zhu, X., Yan, D., He, L., Jin, C., and Zhu, B. *Langmuir*, **26**, 8875 (2010).
33. Zhou, Y., Huang, W., Liu, J., Zhu, X., and Yan, D. *Adv. Mater.*, **22**, 4567 (2010).

34. Dodiuk-Kenig, H., Lizenboim, K., Eppelbaum, I., Zalsman, B., and Kenig, S. *J. Adhes. Sci. Technol.*, **18**, 1723 (2004).
35. Burkinshaw, S. M., Froehling, P. E., and Mignanelli, M. *Dyes. Pigments.*, **53**, 229 (2002).
36. Mulkern, T.J. and Tan, N. C. *Polymer*, **41**, 3193 (2000).
37. Boogh, L., Pettersson, B., and Månson, J. A. E. *Polymer*, **40**, 2249 (1999).
38. Zhang, F., Chen, Y., Lin, H., and Lu, Y. *Color. Technol.*, **123**, 351 (2007).
39. Zhang, F., Chen, Y., Lin, H., Wang, H., and Zhao, B. *Carbohyd. Polym.*, **74**, 250 (2008).
40. Zhang, F., Chen, Y.Y., Lin, H., and Zhang, D. S. *Fiber. Polym.*, **9**, 515 (2008).
41. Zhang, F., Chen, Y., Ling, H., and Zhang, D. *Fiber. Polym.*, **10**, 141 (2009).
42. Hasanzadeh, M., Moieni, T., and Hadavi Moghadam, B. *J. Polym. Eng.*, **20**, 2191 (2013).
43. Khatibzadeh, M., Mohseni, M., and Moradian, S. *Color. Technol.*, **126**, 269 (2010).
44. Mahmoodi, R., Dodel, T., Moieni, T., and Hasanzadeh, M. *Polym. Res. J.*, **7**, 20, (2013).
45. Hua, Y., Zhang, F., Lin, H., and Chen, Y. *Textile Auxiliaries*, **20** (2008).
46. Zhang, F., Zhang, D., Chen, Y., and Lin, H. *Cellulose*, **16**, 281 (2009).
47. Burkinshaw, S. M., Mignanelli, M., Froehling, P. E., and Bide, M. J. *Dyes. Pigments.*, **47**, 259 (2000).
48. Gao, C., Xu, Y., Yan, D., and Chen, W. *Biomacromolecules*, **4**, 704 (2003).
49. Wu, D., Liu, Y., Jiang, X., He, C., Goh, S. H., and Leong, K. W. *Biomacromolecules*, **7**, 1879 (2006).
50. Kima, T. H., Cooka, S. E., Arote, R. B., Cho, M. H., Nah, J. W., Choi, Y. J., and Cho, C. S. *Macromol. Biosci.*, **7**, 611 (2007).
51. Reul, R., Nguyen, J., and Kissel, T. *Biomaterials*, **30**, 5815 (2009).
52. Goswami, A. and Singh, A. K. *React. Funct. Polym.*, **61**, 255 (2004).
53. Fang, J., Kita, H., and Okamoto, K. *Macromolecules*, **33**, 4639 (2000).
54. Seiler, M., Köhler, D., and Arlt, W. *Sep. Purif. Technol.*, **30**, 179 (2003).
55. Itoh, T., Hirai, K., Tamura, M., Uno, T., Kubo, M. and Aihara, Y. *J. Solid. State. Electr.*, **14**, 2179 (2010).
56. Tang, W., Huang, Y., Meng, W., Qing, F. L. *Eur. Polym. J.*, **46**, 506 (2010).
57. Hong, X., Chen, Q., Zhang, Y., and Liu, G. *J. Appl. Polym. Sci.*, **77**, 1353 (2000).
58. Shokrieh, M. M. and Rafiee, R. *Mater. Design.*, **31**, 790 (2010).
59. Tserpes, K. I. and Papanikos, P. *Composites B*, **36**, 468 (2005).
60. Arani, A. G., Rahmani, R., and Arefmanesh, A. *Physica E*, **40**, 2390 (2008).
61. Ruoff, R. S., Qian, D., Liu, and W. K. *C. R. Physique*, **4**, 993 (2003).
62. Guo, X., Leung, A. Y. T., He, X. Q., Jiang, H., and Huang, Y. *Composites B*, **39**, 202 (2008).
63. Xiao, J. R., Gama, B. A., and Gillespie Jr, J. W. *Int. J. Solid. Struct.*, **42**, 3075 (2005).
64. Ansari, R. and Motevalli, B. *Commun. Nonlinear. Sci. Numer. Simulat.*, **14**, 4246 (2009).
65. Li, C. and Chou, T. W. *Mech. Mater.*, **36**, 1047 (2004).
66. Natsuki, T. and Endo, M. *Carbon*, **42**, 2147 (2004).
67. Ansari, R., Sadeghi, F., and Motevalli, B. *Commun. Nonlinear. Sci. Numer. Simulat.*, **18**, 769 (2013).
68. Alisafaei, F. and Ansari, R. *Comput. Mater. Sci.*, **50**, 1406 (2011).
69. Natsuki, T., Ni, Q.Q., and Endo M. *Appl. Phys. A*, **90**, 441 (2008).
70. Joshi, U. A., Sharma, S. C., and Harsha, S. P. *Proc. IMechE, Part N: J. Nanoeng. Nanosys.*, **225**, 23 (2011).
71. Shokrieh, M. M. and Rafiee, R. *Mech. Compos. Mater.*, **46**, 155 (2010).
72. Sadus, R. J. "*Molecular Simulation of Fluids: Algorithms and Object- Orientation*", Elsevier, Amsterdam, 1999.

73. Allen, M. P. and Tildesley, D. J. "*Computer simulation in chemical physics*", Dordrecht Kluwer Academic Publishers, Dordrecht (1993).
74. Haile, J. M. "*Molecular dynamics simulation: elementary methods*", Wiley, New York (1997).
75. Liu, W. K., Karpov, E. G., and Park, H. S. "*Nano Mechanics and Materials: Theory, Multiscale Methods and Applications*", John Wiley & Sons, New York (2006).
76. Rapaport, D. C. "*The Art of Molecular Dynamics Simulation*", Cambridge University Press, New York (1995).
77. Aerts, J. *Comput. Theor. Polym. Sci.*, **8**, 49 (1998).
78. Lescanec, R. L. and Muthukumar, M. *Macromolecules*, **23**, 2280 (1990).
79. Le, T.C., Todd, B. D., Daivis, P. J., and Uhlherr, A. *J. Chem. Phys.*, **130**, 074901 (2009).
80. Lyulin, A. V., Adolf, D. B., and Davies, G. R. *Macromolecules*, **34**, 3783 (2001).
81. Konkolewicz, D., Thorn-Seshold, O., and Gray-Weale, A. *J. Chem. Phys.*, **129**, 054901 (2008).
82. de Gennes, P. G. and Hervet, H. J. *J. Phys. Lett. (Paris)*, **44**, L351 (1983).
83. Maiti, P. K., Cagın, T., Wang, G. F., and Goddard, W. A. *Macromolecules*, **37**, 6236 (2004).
84. Cagin, T., Wang, G., Martin, R., Breen, N., and Goddard, W. A. *Nanotechnology*, **11**, 77 (2000).
85. Cagin, T., Wang, G., Martin, R., Zamanakos, G., Vaidehi, N., Mainz, D.T., and Goddard, W. A. *Comput. Theor. Polym. Sci.*, **11**, 345 (2001).
86. Lue, L. *Macromolecules*, **33**, 2266 (2000).
87. Le, T. C. Ph.D. Degree Thesis, Swinburne University of Technology (2010).
88. Zacharopoulos, N. and Economou, I. G. *Macromolecules*, **35**, 1814 (2002).
89. Lee, I., Athey, B. D., Wetzel, A. W., Meixner, W., and Baker, J. R. *Macromolecules*, **35**, 4510 (2002).
90. Hana, M., Chen, P., and Yang, X. *Polymer*, **46**, 3481 (2005).
91. Laferla, R. *J. Chem. Phys.*, **106**, 688 (1997).
92. Avila-Salas, F., Sandoval, C., Caballero, J., Guiñez-Molinos, S., Santos, L. S., Cachau, R. E., and González-Nilo, F. D. *J. Phys. Chem. B*, **116**, 2031 (2012).
93. He, X., Liang, H., and Pan, C. *Macromol. Theory Simul.*, **10**, 196 (2001).
94. Neelov, I. M. and Adolf, D. B. *J. Phys. Chem. B*, **108**, 7627 (2004).
95. Bosko, J. T. and Prakash, J. R. *J. Chem. Phys.*, **128**, 034902 (2008).
96. Dalakoglou, G. K., Karatasos, K., Lyulin, S. V., and Lyulin, A. V. *J. Chem. Phys.*, **129**, 034901 (2008).
97. Konkolewicz, D., Gilbert, G. R., and Gray-Weale, A. *Phys. Rev. Lett.*, **98**, 238301 (2007).
98. Sheridan, P. F., Adolf, D. B., Lyulin, A. V., Neelov, I., and Davies, G. R. *J. Chem. Phys.*, **117**, 7802 (2002).