Chapter 4
Biochemical Composition of Synovial Fluid in Health and Disease

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Lubricant Molecules

A primary function of SF is to lubricate the joint reducing friction and mechanical stress on the articular cartilage. Large molecules of SF such as hyaluronan (HA), products of the proteoglycan 4 (PRG4) gene including lubricin and superficial zone protein work synergistically to decrease friction and mechanical damage [1]. Hyaluronan is a non-sulfated glycosaminoglycan composed of repeating disaccharide units of D-glucuronic acid and D-N-acetylglucosamine. It was discovered in 1934 by Myer and Palmer, deriving its name from the Greek-derived *haylos* combined with uronic acid [2]. Produced by fibroblast-derived type B synovial cells, HA has the viscosity of an egg white, and is the most abundant large molecule of SF with concentrations ranging from 1–4 mg/ml [3]. It is a biopolymer molecule several million daltons in size. HA can take the form of hyaluronic acid, or at physiological pH can take the form of a sodium salt [2].

Hyaluronan is a rheological marvel that is extremely lubricious and hydrophilic. In solution, the hyaluronan polymer chain takes on the form of an expanded coil allowing it to hold 1000 times its weight in water [2]. The chains entangle with each other at very low concentrations, and at higher concentrations have an extremely high shear-dependent viscosity separating tissue surfaces that slide along each other. These characteristics give SF the empirical "string sign" that can be demonstrated by letting the thick normal fluid drip out of a syringe one drop at time or by pinching a drop between the thumb and forefinger [4]. A fluid "string sign" of 3 cm or more before breaking is considered normal, while <2.5 cm (more similar to water) is considered abnormal and indicative of inflammatory fluid with degraded HA.

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B. F. Mandell (ed.), *Synovial Fluid Analysis and The Evaluation of Patients With Arthritis*, https://doi.org/10.1007/978-3-030-99612-3_4

Hyaluronan has other important biological functions. The constant secretion, then degradation has led to the suggestion that it acts as a scavenger for cellular debris [5]. Since the 1940s it has been known that HA is degraded by oxidizing systems via chain cleavage induced by hydroxy radicals, and through this reaction molecular "debris" can be caught in the network of HA and removed at the same rate as the polysaccharide [6, 7]. HA also has a concentration and molecular weight-dependent effect on angiogenesis [2, 8]. High molecular weight and concentrations of HA inhibit capillary formation which may contribute to the avascularity of the joint capsule and cartilage. Interestingly, under certain conditions HA binds to cell surface proteins. Synovial cells can change their expression of HA-binding receptors in disease states, thus HA can influence immunological reactions and inflammatory cell traffic in and out of the synovial fluid [9]. Finally, HA may interact directly or indirectly with pain receptors within the joint, perhaps explaining transient analgesic benefit experienced by some patients following intra-articular HA injections [2, 10].

In states of inflammation and oxidative stress there is accelerated (beyond physiologic) degradation of HA resulting in impairment and loss of viscosity. Degraded HA of low-molecular-weight has different biological activities compared to the high-molecular-weight HA in the healthy joint [2]. Smaller HA chains can serve as endogenous immunostimulating danger signals contributing to inflammation and angiogenesis [2, 11, 12].

There are many commercially available products for HA "viscosupplementation" in OA which may decrease pain and improve function in some patients with mild to moderate disease [13]. An intent with intra-articular injection of exogenous HA is to temporarily improve intra-articular viscosity, stimulate endogenous HA production, stimulate chondrocytes and synthesis of cartilage matrix components, and inhibit enzymatic degradation of chondrocytes and inflammatory processes [14].

Lubricin is a surface-active mucin-like glycoprotein, encoded by the PRG4 gene, which is produced by synovial fibroblasts and chondrocytes along the surface of articular cartilage [15]. The role of lubricin is to retain a protective layer of water molecules, lubricate the joint, and prevent cell and protein adhesion. Along with hyaluronan, it provides protection of the joint by reducing friction. Patients with inflammatory arthritis, joint trauma, or genetic lubricin deficiencies have insufficient amounts to protect the cartilage. There are recombinant forms of lubricin in clinical development for the treatment of OA, autoimmune arthritis, as well as dry eyes and other applications [16].

Proteins

There are similarities between blood plasma and SF protein composition, with the synovial membrane selectively blocking very large plasma proteins from entering into the joint space under normal physiological conditions [17]. The average joint SF contains approximately one-third of the protein concentration found in plasma, or 19–28 mg/ml [17, 18]. The major protein found in SF is albumin (approximately

12 mg/ml or 37% of the plasma concentration); transferrin and globulins make up most of the rest. In contrast, large molecular weight plasma proteins such as fibrinogen are at very low concentrations.

In joint inflammation the concentration and quantity of proteins increase. Patients with synovial inflammation including osteoarthritis (OA), rheumatoid arthritis (RA), gout, systemic lupus erythematosus (SLE), and traumatic arthritis have increased protein concentrations [17]. The inflammation of the synovium compromises the ability of the synovium to selectively filter and retain proteins [2, 17]. For example, active RA patients will have high levels of large plasma proteins such as fibrinogen, β2 macroglobulin, β1 lipoprotein, α2 glycoprotein, and α2 macroglobulin [17]. Small proteins such as C-reactive protein, calprotectin, and defensins also more readily influx into SF during joint inflammation, and may have clinical significance and some diagnostic value. But measurement of the SF protein level, unlike the situation with other body fluids, is not diagnostically useful.

Fibrinogen levels are relatively low in the healthy joint SF, but during inflammatory states such as RA and gout, levels can increase 3–5-fold [19]. Studies have shown influx of fibrinogen and other coagulation proteins into synovial fluid that is not paralleled by increased fibrinolytic activity. While normal joint fluid does not clot, the increased fibrinogen in inflamed synovial fluid can cause clotting when transported in a test tube without EDTA or sodium heparin [20, 21]. In the RA patient, forms of citrullinated fibrinogen may bind to anti-citrullinated protein antibodies (anti-CCP ab) within the synovial fluid and joint [22].

Similar to serum CRP, synovial CRP increases in states of inflammation, particularly infection. Serum CRP is commonly used as a screening test for acute infection, but synovial fluid has been found to have a sensitivity and specificity of 92% and 90%, respectively, in periprosthetic joint infection (PJI). High concentrations of CRP are also found in the synovial fluid of inflammatory arthropathies such as RA [23]. Although thought to be exclusively produced in the liver, there is evidence that fibroblast-like synoviocytes may produce CRP, releasing it into the SF [24] and perhaps into the blood.

There are several small proteins and antimicrobial peptides (AMPs) released by neutrophils and macrophages as part of the innate immune response that can be found in SF during states of infection or sterile inflammation. Cathelicidin LL-37, α-defensin, and calprotectin in SF have potential clinical utility when used with other markers such as CRP, white blood count (WBC) with differential and cultures when a septic joint is suspected. Commercial point-of-care testing is available to detect calprotectin and α-defensin in SF [25, 26], but their clinical utility to differentiate between septic and aseptic inflammatory arthropathies is not fully understood.

Cathelicidin LL-37 is a 37 amino acid peptide produced by neutrophils that has been implicated in the pathogenesis of several inflammatory diseases including SLE, RA, psoriasis, and atherosclerosis [27]. It has been found in SF of RA patients and found to associate with inflammation and to increase apoptosis of osteoblasts [27]. Defensins are small (29–35 amino acids) proteins produced by circulating white blood cells and tissue cells [26]. Defensins are classified into alpha and beta families with α-defensin found in and released by neutrophils, macrophages, and

Paneth cells of the intestine. In the presence of pathogens in SF, neutrophils release α-defensin inducing the depolarization of the bacterial cell membrane promoting lysis and death [26]. In addition to direct antimicrobial activity, α-defensin also contributes to chemotaxis, cytokine induction, and phagocytosis [28]. Consistently high levels of α-defensin observed in the SF of infected joints have led to it being established as a biomarker and part of the diagnostic criteria for PJI [26, 29]. Interestingly, α-defensin is not influenced by prior antibiotic administration, comorbid conditions of the patient (with the possible exception of an autoimmune disease), or type of infectious organism [30]. Elevated levels can be found in inflammatory arthropathies including crystalline (gout and calcium pyrophosphate deposition disease (CPPD)), psoriatic arthritis, and rheumatoid arthritis [29, 31]. Similar to α-defensin levels, increased numbers of neutrophils within the SF contribute to the levels of lactate dehydrogenase (LD), while the serum LD levels remain normal [4].

Calprotectin is another AMP secreted by neutrophils and monocytes that has chemotactic properties. It has been shown to be more sensitive and specific for the diagnosis of PJI than erythrocyte sedimentation rate, CRP, and SF WBC [32, 33]. Unlike cathelicidin LL-37 and α-defensin, calprotectin has been shown to have potential clinical utility as a biomarker to differentiate septic arthritis from other inflammatory arthropathies including CPPD and RA [34].

Small Molecules

Small molecules in SF include glucose, urate, and lactate. Unlike other body fluids where active transport dictates the movement of many molecules, in SF smaller molecule concentrations typically parallel serum levels under normal conditions. For example, glucose levels in SF are typically ~10 mg/dL less than serum levels. In inflammatory states there are often significantly larger decreases in SF glucose levels in a septic joint compared to serum glucose levels [4], but there is too much overlap with other inflammatory joint conditions for this to be a reliable diagnostic test for infection.

Uric acid in the plasma and SF circulates as urate, the mono-deprotonated ionic form of uric acid under normal physiologic conditions [35]. Elevated serum urate levels will elevate SF levels with subsequent monosodium urate monohydrate (MSU) deposition (MSU being the most common crystallized urate) which may ultimately result in gouty arthritis (see Chap. 10). The various factors that cause crystallization of uric acid have not been fully illuminated, and data on how HA and its degradation products influence urate solubility have been conflicting [35].

Measurement of SF protein, glucose, and even the presence of crystals will not aid in differentiating between septic and aseptic arthritis. While WBC with differential, gram stain and culture remain the most important tests to obtain, lactate may have diagnostic potential to distinguish septic arthritis from other inflammatory states including gout [36–38]. SF lactate levels above 10 mmol/L suggest septic arthritis, and levels lower than 4.3 mmol/L are more likely to be aseptic [36].

Another potential biomarker to distinguish septic arthritis from crystalline and aseptic inflammatory arthropathies is the SF lactate/glucose ratio [39]. A large European study evaluating 233 SF specimens found elevated lactate/glucose ratio had a greater area under curve, sensitivity, specificity, and likelihood ratio in septic arthritis, outperforming individual SF lactate and glucose levels.

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