A systematic review on correlation between biochemical and mechanical process of lubricant film formation in joint replacement of the last 10 years

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KEY WORDS

synovial fluid, lubricating film, Raman spectroscopy, bio-tribology, tribo-chemistry.

ABSTRACT

This study provides a systematic review of the synovial and simulated body fluid research for the last ten years (2006 to 2016). In particular, biochemical and mechanical properties of synovial fluid after joint replacement are focused, namely the chemical composition of the formed lubricating film and structural changes of the associated proteins under mechanical loading. In summary, the formation of the film depends on pH, viscosity and concentration of the solution, static, sliding and rolling conditions and other factors related to the joint replacement. However, chemical changes of the synovial fluid proteins after the joint replacement are rarely addressed and require further attention. To this end, we provide a preliminary study of selected proteins within the synovial fluid using Raman spectroscopy. We conclude that chemical analysis together with the analysis of mechanical and biological properties of the synovial fluid after total joint replacement will help in comprehension of the process.

1 INTRODUCTION

Synovial fluid (SF) of an articulating joint is a complex biological composite fluid enriched with protein, that derived from the blood plasma and cells within the joint tissues (such as synovium, cartilage, ligament, and meniscus) are contained in SF of an articulating joint [1]. This highly efficient water-based tribological system is optimized to provide low friction and wear protection at both low pressures and high contact pressures. The protection is maintained through the entire life of a human across various sliding velocities including during the resting periods [2]. Concerning mobility of the skeletal system, synovial joints act as bearings and SF is responsible for the lubrication of these bearings, adhering to various mechanisms [3]. The molecules participating in

joint lubrication are engaged mainly in providing an effective surface lubricant to reduce the friction and minimizing wear damage to the rubbing or shearing surfaces [2].
The naturally occurring components of SF can vary in concentration. In addition, differences in SF composition were observed for normal knee joint, patients without replacement of joint, patients with primary arthroplasties and patients with revisions of total hip and knee arthroplasties [4]. In particular, hyaluronic acid, proteins and phospholipid concentrations varied widely in patients undergoing the joint replacement [5, 6]. The thickness of lubricant film within hip prostheses is hypothesized to influence particular proteins [7]. It has also been stated that concentrations of bovine serum albumin and γ-globulin manipulate the frictional behaviour of the CoCr femoral head [8]. The optimum tribological performance of joint replacement would also be subject to the combination of composition, microstructural condition and manufacturing process of the used alloy [9].
Pursuing biochemical reactions underlying the formation of the lubricating film, vibrational fingerprint provided by Raman spectroscopy can be used to identify the components present and analyse the chemical bonding among them [10]. In addition, the correlation between the level of phospholipids and proteins exists and can be deduced from Raman spectra [11]. The technique has also been previously used to distinguish between healthy SF and SF changes in osteoarthritis patients as well as to osteoarthritis subchondral bone matrix change detection [10, 12].
The objectives of this study are to review and clarify the chemical reactions of SF components during the formation of the lubricating film on the joint replacement along with the focus on mechanical properties of the film. The selection of the review literature is described in Section 2, the result obtained is described in Section 3, while Section 4 contains the review itself. In Section 5, the discussion is provided including the preliminary Raman study on the behaviour of the selected SF proteins when subject to tribological experiment.

2 METHODOLOGY

2.1 Search strategy and eligibility criteria

Peer review articles published from 2006 to 2016 were searched for the review. To address both chemical and mechanical properties of the film that forms within artificial joints, articles subject to ‘synovial fluid film formation of artificial joints’ or ‘protein film chemistry of synovial fluid’ topics were considered. However, articles focused on bio-tribology in dental and optical science or protein film formation chemistry of outside synovial joint areas and/or mathematical modeling were excluded from the search.
The search explored online citation indexing services such as Web of Science and Scopus and the data range was limited to 2006 to 2016, considering this era most effective for research in this field. The search criteria were ‘film formation’ And ‘artificial joint’, ‘synovial fluid’ And ‘lubrication’, and ‘synovial fluid’ And ‘protein’ And ‘chemistry’.

2.2 Study selection and data extraction

The selection of articles for the review was independently performed by two researchers. First, duplicate results coming from different keywords or different citation database searches were merged. In the next step, the title and abstract of the obtained citations were screened to exclude

work with low relevance to the topic. In particular, exclusion criteria were chosen to exempt articles on dental and optical tribology, on film formation other than on joint replacement and on mathematical modelling of joint replacement. In result, articles dealing with mechanical or biochemical properties of film formation within the synovial joint, in particular dealing with lubrication mechanism, protein concentration and adsorption, film composition, usage of spectroscopy in this field, or pH and buffer of protein films, were accepted as relevant for the review.

3 RESULT

In the below flowchart the journal selection process has been demonstrated, using inclusion and exclusion method. Initially, 1138 articles were found in online citation databases, 676 in Scopus and 462 in Web of Science. Duplicate items were merged by using Endnote and, reviewing title and abstract, the article extraction continued via the inclusion and exclusion criteria. Finally, two more articles were added explicitly. In result, 14 articles were selected for review in this section.

Table 1: Inclusion and exclusion process of article selection for the systematic review.

Table 2: Summary of the systematic review, with characteristics of the eligible studies

4 BIOCHEMICAL AND MECHANICAL PROPERTIES OF SYNOVIAL FLUID AND JOINT REPLACEMENTS

4.1 Properties of synovial fluid

The SF constitutes a complex mixture of large and surface-active molecules, such as proteins, phospholipids, hyaluronic acid, cholesterol and glycoproteins, [19, 21]. However, the proteome composition of healthy SF and cellular origins of its components are yet to be understood completely [1]. The prominent functions of SF within the joint lies in its biological lubrication but SF also acts as a biochemical pool for nutrients and regulatory cytokines outpour. Of course, the tribological performance of cartilage cannot be featured with a single mechanism. Rather, multiple functions of lubrication take place mutually. When lubrication is optimum, normal loads, shear stresses, and rates change are provided at articulating cartilage surfaces, yielding low-friction and low-wear properties [2]. Erosion of articulating cartilage surfaces may occur due to arthritis if there is a lack of proper SF lubricating system or any disruption of chemical environment within synovial joint takes place. In damaged joint SF normal electrostatic interactions are not workable, probably due to chemical-changes-induced secondary structure modification of the proteins [12, 23].
Within the synovial space, SF is accumulated and separated by the semipermeable synovial lining. The chemical regulation is assumed to be controlled by lubricant concentration, as secreted by

chondrocytes and synoviocytes [23]. Therefore, SF also plays a transporting role of the boundary lubricant to its site of adsorption [5].

At the beginning of bio-tribology research, Dowson and Walker et.al. [22], had provided the idea that the cartilage surfaces are prevented from contacting each other primarily because of the formation of trapped pools of lubricant. Due to compression and concentration of the fluid between the cartilage surfaces an acid-protein complex is formed as a protective gel. While proteoglycan 4 (PRG4), hyaluronic acid (HA) and surface active phospholipids (SAPL) contribute to the total arrangement of the system [23]. Walker, Dowson et.al. [22], termed this combination of trapped pool and fluid concentration as "boosted lubrication". The proteins are produced from synovium, cartilage, ligament and meniscus [1], and their structural and functional properties are directly connected with the phospholipids [11]. On the other hand, HA contributes more as a potential boundary lubricant for cartilage and shows a small amount of lubrication activity [2].

In a model described by Blewis et al. [23], SF is compared to an ultra-filtrate of plasma, which has the ability of filtration through the synovial membrane with chondrocytes in articular cartilage and synoviocytes in synovium secrete lubricants.

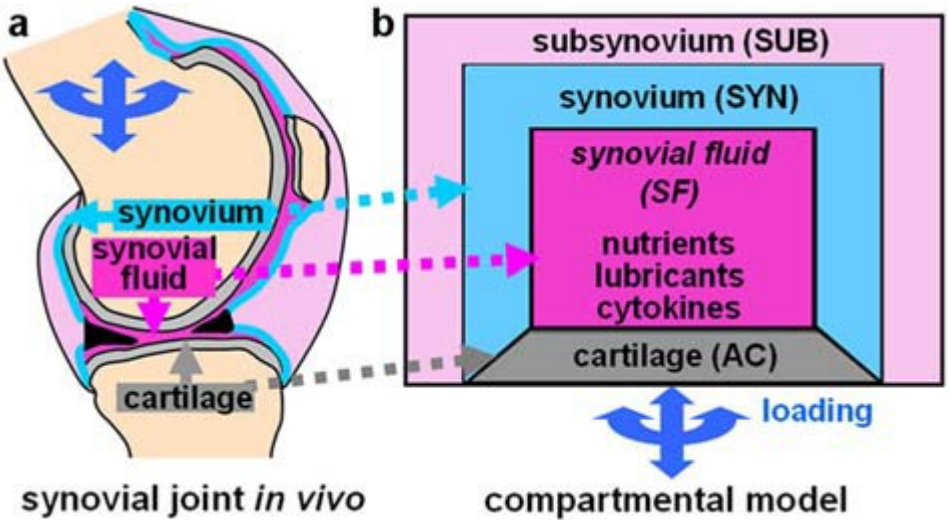

Fig.1 (a) Synovial joints composed of cartilage, synovium, and synovial fluid; (b) communicating compartments are present in synovial fluid, while lubricant secretion is regulated by chemical and mechanical factors [23].

4.2 Biochemical composition of synovial fluid

The principal element of SF is phospholipids, amounting to about 61% in the normal joint constitution. Being the main phospholipid component, phosphatidylcholine is extremely surface-active due to large saturation of joint phospholipids. Another element abundant in SF is the SAPL. These small molecules function as binders of amino acid groups [5].

Albumin, the most abundant protein of SF, shows affinity to hydrophobic surfaces such as OH-terminated surfaces. Adherence of albumin to artificial surfaces may thus be regulated by altering the pH value. Therefore, for lubrication of articular surfaces and interaction with other components albumin plays an important role.

On the other hand, globulin activity is more focused on boundary lubrication. At low speeds globulin plays the role of pH-independent boundary lubricant. When speed is higher, boundary lubrication by globulin depends on pH, which in turn helps in producing hydrodynamic or mixed lubrication [15].
Being another essential element of SF, HA produces a cross-linked network by complexing with the glycoprotein lubricin, whose solutions are slippery to the touch. This network provides wear prevention mechanism of joints and is also (chemically) responsible for boundary lubrication [2, 5]. Lubricin is formed by a long, heavily glycosylated, mucinous domain, separating two somatomedin domains at the N-terminus and a haemopexin region at the C-terminus. The total amount of lubricin in SF is small, but assists in a substantial amount in the boundary lubrication as well as lubrication properties, transporting, anchoring of phospholipids to the cartilage surface and the friction coefficient of the different area of articular cartilage. In addition, lubricin contributes essentially to chondroprotective properties of articular cartilage; by interaction with HA via dissipation of shear-induced energy, this activity is further increased. Thus, effective biolubrication is provided by lubricin in the presence of other major components in SF [5, 15].
In result, the chemical behaviour, friction and wear properties of the joints are controlled by prime constituents of human SF, which are proteins such as serum albumin and γ-globulin, phospholipids, and HA [4, 16].

4.3 Synovial liquid content correlated to joint replacement

The levels of different components within SF may differ with the person, age and they also depend on the presence of disease. The total volume of SF, protein concentration, pH value, viscosity and other properties are known to vary between healthy persons and patients with Osteoarthritis (OA) at different stages [4, 15].
The concentration of proteins and other components differs for diseased and periprosthetic SF and the condition includes decreased effective viscosity, increased protein content and increased pH [4, 19, 21]. In case of inflammation, stimulation of tissue protective and regenerative mechanisms takes place to prevent collateral damage to periprosthetic tissues.
The tissues architecture is balanced by various hormones such as cytokines, chemokines and specific cell populations, including macrophages, dendritic and stem cells to minimize inflammation. As a result of the failure of local tissue homeostatic mechanism, periprosthetic osteolysis may arise [24].
The bulk properties of the SF may control the film lubrication as proposed by Mazzuccoa et al. [6] some connections between the composition of the joint fluid and the tribology of joint replacement prostheses in vivo. Determination of protein, phospholipid and hyaluronic acid contents of joint fluid samples obtained from patients undergoing total knee arthroplasty(TKA) and revision TKA reveals that despite normal total protein content, the individual protein concentrations deflect in the diseased joints from that in healthy joints. To the implant wear and failure of joint replacement, patient SF chemistry plays an important role [19].

From the experiment of Galandáková et al. [4], four types of sample were collected as below:

Group I	Patients with aseptic loosening of total joint replacement
Group II	Patients with total joint replacement, but without any sign of aseptic loosening
Group III	Patients without total joint replacement and the end stage of osteoarthritis
Group IV	Healthy SF

In the experiment, γ-globulin concentration was found significantly higher in patients with the revision of total joint replacement compared to patients without total joint replacement. On the other hand, the concentration of phospholipid was found to be significantly lower: on average 0.312 mg/ml for healthy SF and 0.154 mg/ml in the patient with aseptic loosening of total joint replacement. no significant difference was found among the groups in HA concentration and viscosity.

The average concentrations of different components of the joint fluid in the four distinct groups are summarized in Fig. 2.

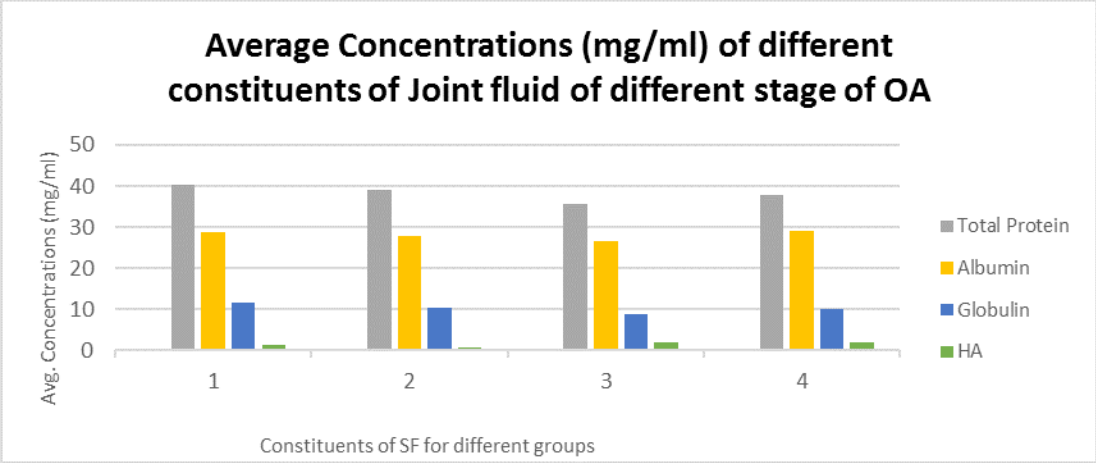

Fig. 2 The average concentrations of the constituents of the joint fluid from the different stages of osteoarthritis. Average total concentration of protein found 40.3-35.5 mg/ml, average concentration of albumin 29.1-26.7 mg/ml, average concentration of globulin 11.5-8.7 mg/ml and HA 0.8-2.0 mg/ml for different groups of patients as described above [4].

Galandáková et al. [4] concluded that lubricant film is predominantly formed because of bovine serum proteins. Cong et.al. [8] stated that the concentration of bovine serum albumin (BSA) and γ-globulin regulates the frictional behaviour of the CoCr femoral head.

The protein concentration dependent lubrication system could be the result of the difference in the adsorption strength of BSA and γ-globulin. The α-helix structure of albumin causes low adsorption strength of this protein and the bearing surface while the β-sheet structure of γ-globulin rather plays a role in its strong adsorption on the rubbing surfaces [8] (and the references cited therein [8]), which leads to the formation of large cohesive and/or adhesive forces on the bearing surface within a mixed or boundary lubrication regime. At high concentrations, BSA seems to act as an effective boundary lubricant but in the physiological concentration range of the human SF, the lubricating ability of γ-globulin for boundary lubrication is most effective [8].

4.4 SF lubricant film formation on artificial joint replacement

The SAPL provides a thin hydrophobic outermost lining to the normal particular surface. Through SAPL, the boundary lubricant reduces friction to significantly lower levels [5].
Optimal concentrations of HA and phospholipids, such as dipalmitoylphosphatidylcholine (DPPC), for effective lubrication are equivalent to those observed in normal human SF. These concentrations regulate the microscale frictional response of the retrieved CoCr femoral head [16]. In case of arthritis, injury, and artificial joint failure, the friction between the articulating surfaces is enhanced through concomitant erosion of the load-bearing elements [23, 28]. On the other hand, Parks et al. [18] stated that under static conditions, the lubrication properties of proteins are only partially governed by their adsorption properties.

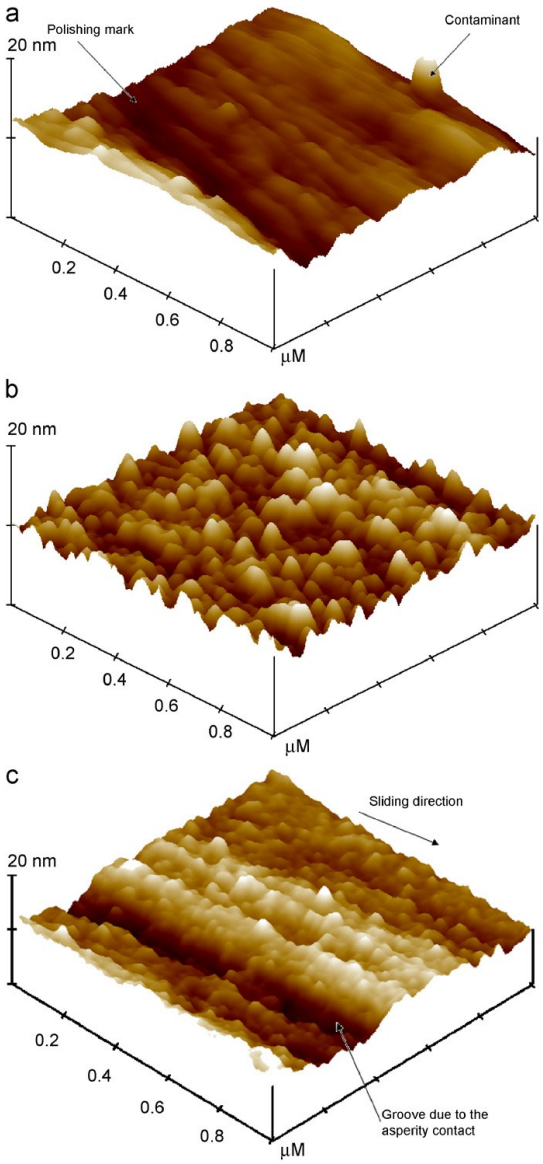

Fig.3 AFM study of the CoCrMo surface (a) as polished, (b) after immersion in 25% BS for 30 min at 37°C and (c) after Sc test in 25% BS at 37°C [27].

The corrosion-enhanced wear occurs mainly due to the presence of the proteins. More precisely [27, 29], the presence of the proteins increases wear component; probably, adsorption of proteins in the particle entrainment also enhance the rolling efficiency of the abrasives. The CoCrMo implanted into the human body (pH 7.4) within a tribological contact of a knee or a hip is known to be susceptible to corrosion. In particular, Co 2+ and Cr 2+ ions in acidic conditions and CoO and

CrO species at neutral pH are produced as a result of initial corrosion of CoCrMo alloy. Actually, the wear-corrosion behaviour of the cast CoCrMo alloy can be deflected noticeably with a minor alteration in test solution chemistry [27, 29].

4.5 pH dependency of protein films formation

Parkes et.al. stated [13, 18] that film formation within protein solutions is dependent both on the protein content and pH of the solution. When comparison of the film thickness during and after rolling tests shows that the deposited films formed by bovine γ-globulin (BGG) and mixed proteins were quite strongly bound.

The BSA films were, however, only weakly bound. Comparing BSA and BGG deposited layers, the BGG films were much thicker. In the case of mixed proteins, the ability of BGG to form thick layers was decreased. Therefore, the interaction between the proteins influences the process of film formation [13].

At physiological pH, the films are formed by large irregular deposits in solutions. However, both the protein adsorption kinetics and the formation of tribofilms is influenced by the solution pH, whether statically or under the rolling conditions.

At the lower pH, the adsorbed protein layers are initially rigid and relaxed over time to form viscoelastic layers while the rate of adsorption also increases with time. At higher pH, even the initially adsorbed films are found to be substantial viscoelastic [18]. However, for the solutions tested in different buffer chemistry, no significant changes to protein adsorption were observed. In particular, for all buffer chemistry, the rate of protein adsorption as well as the viscoelastic properties of the adsorbed layer are similar. In buffers with pH 7.4 or lower, thicker protein films were formed in a rolling contact than under static conditions [18].

Fan et al. [19] examined an inlet reservoir of viscous material produced from high concentration protein fluids and stated that film formation mechanisms can happen in two distinct ways: boundary and ‘‘gel’’ hydrodynamic deposition.

According to the boundary lubrication mechanism, the proteins adsorb at the CrCoMo surface to form thin, discontinuously deposited films. These films appear to survive rubbing. On the other hand, fluids with high-concentration protein form an inlet reservoir of viscous material that is entrained into the contact forming a separating film of agglomerated-proteins on implant surfaces. In the fluid environment, these deposits act as viscous and possible to easily removed by surface scratches. Once removed from the fluid they dry to form highly-adherent, solid films.

Wear mechanisms occurs through tribocorrosion processes, and with increasing pH the wear of CoCrMo increases as well.

Toshio et al. [21] stated that variation of protein content may affect the pH and viscosity dependency. At low speed the presence of albumin, the lubricant promotes pH dependence and viscosity independence of the tribologicalproperties. On the other hand, globulin promotes pH and viscosity independence at low speed and promotes pH and viscosity dependence at high speed in the lubrication of ultra-high molecular weight polyethylene (UHMWPE) against stainless steel (SUS). Therefore, the effect of constituents and pH variation in the periprosthetic fluid can lead to different lubrication results.

4.6 Composition of formed protein films

From the above discussion, it is clear that the film formation on the joint replacement depends on pH, concentration and viscosity of the solution, but the chemical composition of the formed film remains yet unknown.

The noticeable influence of the electrolyte composition on the passivation behaviour of Co-based alloys has been observed several times. The adsorbed organic species of the SF are assumed attached to this passivation film, altering their surface reactions. In result, adsorption of proteins disrupts the biocompatibility of the metallic materials used and accelerated metal dissolution may occur. At the same time, a protein (or amino acid) biofilm on the surface behaves as a lubricant film reducing friction and thus leads to reduced mass lost [26, 30]. The chemistry of the biofilm it is yet to be clarified.

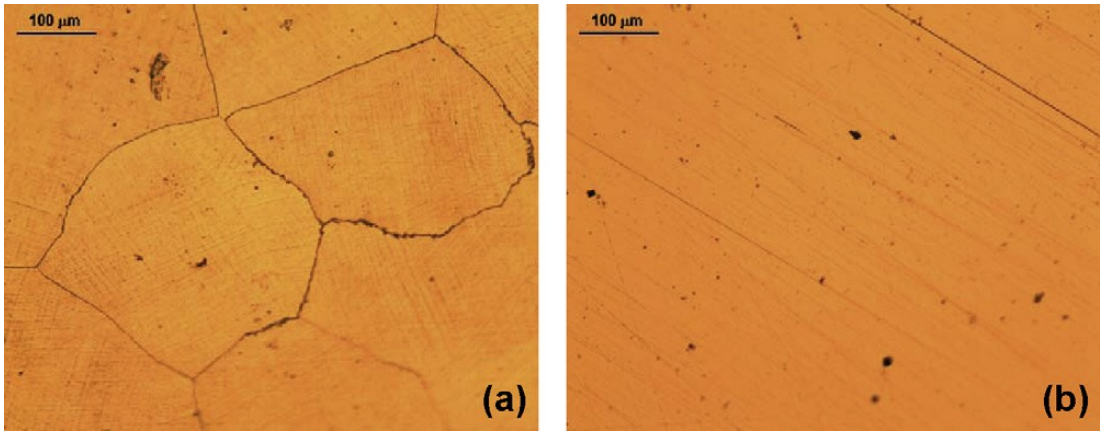

Fig. 4 Optical images of the CoCrMo alloy microstructure: (a) grain boundaries and (b) carbide content [26].

Milosev et al. [25] used electrochemical oxidation to observe the composition, thickness and structure of the oxide layer formed on the CoCrMo alloy in simulated physiological solution at various passivation potentials. Remarkable changes were found in the oxide layer found depending on the applied passivation potential. Presumably, the structure of the SF film could be analysed in a similar way.

Fan et. al. examined an inlet reservoir of viscous material produced from high concentration protein fluids. It is stated that an organic deposit form on the surface, yielding from agglomerated proteins. The organic deposit remains viscous in a fluid environment and it is possible to abolish this layer by surface scratches. After drying, a highly adherent solid film is formed [19].

Nečas et al. [7, 31] used a model fluid to study the impact of protein content on the lubrication of joint replacements. For lubricant with albumin and γ- globulin content in concentrations 2:1 the film thickness increased with increased sliding speed. The effect was attributed mainly due to the presence of γ-globulin, although the film formation was very complex and both time and distance dependent. The film developed gradually under purely rolling conditions and there was no substantial scatter formation. Under partial negative sliding, where the ball was faster than the disc, the film was generally very thin. Under positive sliding, the most scattered results were obtained when the disc was faster than the ball.

Increase in mean speed usually resulted in an increase of the film thickness, although under positive sliding the effect of speed was opposite. Namely, for the speed of 5.7 mm/s the film thickness was more than 120 nm, while for higher speeds, it was only around 40 nm. The

thickness of the lubricating film can be assumed to scale specifically with the deposited amount of albumin. Presumably, a thin layer of γ-globulin is adsorbed primarily on to rubbing surfaces, enabling subsequent adsorption of albumin to create a layer structure [7]. Thus, this model fluid can be used for chemical composition analysis.

Parkes et.al. [13] also stated that under the rolling condition, protein layers are much thicker compared to statically adsorbed layers. From the study of Fan et al. [19], it was also found that much thicker films were formed in case of γ-globulin occurrence, although by mono or multi-layer adsorbed protein forms a thin residual film. Hence, if the chemistry of γ-globulin film formation is analysed separately, most likely it can provide an interesting result.

Nakashima et al. [14] studied the adsorption of BSA through measurement of friction force and electric potential under open circuit potential (OCP) condition in the rubbing combination of UHMWPE and CoCrMo alloy. The results show the onset of BSA desorption at first rubbing with the gradual decline of desorption. During rubbing, BSA film on t h e metal surface is reconstructed and observed stable with the structure optimally-adapted with respect to the shear force.

Vrbka et al. [32] use the pendulum simulator in combination with optical measurement of film thickness seems to imitate the lubrication processes within artificial hip joints. They show the model able to consider different loading and kinematic conditions, the influence of geometry, clearance and material combination of contact pairs.

Despite all efforts, the role of proteins in the electrochemical behaviour of bio-
metallic surfaces is yet to be comprehended [26]. Addressing this unknown area of biofilm chemistry simultaneously with consideration of mechanical properties could, hence, bring a significant advance in this research field.

5 DISCUSSION

The principal causes of osteoarthritis are assumed to be classified into two main classes. The first one is the mechanical loading change, which leads to the total joint replacement of patients due to the stiffer structure. The other one could be articulated as a change of chemical composition of the bone matrix and the SF. Most likely, the secondary structures of SF proteins and tropocollagen are affected in osteoarthritis [4, 10, 12]. Most of the studies found are concerned with adsorption and desorption of proteins in different kinematic conditions and film thickness in different pH, buffer and concentration of proteins [7, 13, 14, 17-20].

Frictional coefficients, frictional force, shear force, wear measurement, boundary lubrication, surface geometry, rheology of various constituents are also considered in connection with proteins and other SF components to explore the knowledge in this area [2, 3, 8, 14-16, 20]. To take into account the mechanical and chemical environment of human synovial joint, the viscosity of various modal fluids and wettability of various surface materials were also considered [4, 15 -18] with respect to their duration in time and under varying temperature and pressure [2,17].

Esmonde-white et. al. [12] considered the change of chemical structure in SF under osteoarthritis and total joint replacements and concluded that differences occurred in diseased SF compared to healthy SF. In addition, Jemma Kerns et. al. [10] confirmed that the subchondral bone matrix also altered its chemical structure due to osteoarthritis. Both above observations of chemical structural

changes were investigated by means of Raman spectroscopy. Nevertheless, the structural changes of individual protein components must be addressed to understand these chemical changes thoroughly. The sought for information on protein film chemical composition should be revealed through simultaneous consideration of mechanical properties and control of temperature, pressure and frictional force.

The optical method of Raman spectroscopy provides information relying on the change of bond polarizability of a molecule through inelastic probing of its vibrations. Although the cross-section of Raman scattering is low, it is extremely sensitive to intramolecular conditions. In addition, although water is always present as one of the elements of the biological materials, it does not affect the Raman spectroscopic fingerprint of biomolecules too much [11].

Within the Raman spectrum, there are several qualitative and quantitative indicators of a peptide conformation. In particular, the hydrogen bond stabilised secondary structural motifs of peptides such as α-helix or β-sheet structures have well- defined imprints on the Raman spectrum. As an example, the p osition of Amide I band (describing the C=O stretching, contributions from C-N stretching and N-H deformation) for the α-helical peptide is 1645-1660 cm -1 , while for β-sheet peptide the band is located at 1665-1680 cm-1. In addition, Amide III band (describing the vibrational coupling between adjacent C-H and N-H deformation) is observed at 1265-1300 cm-1 for α-helix, while β-sheet structure shifts the band to 1230-1240 cm-1. Therefore, the changes of a peptide conformation, that through chemical reactions or denaturation can even be the reason of loss of biological activity of proteins [33], are easily monitored using Raman spectroscopy.

Indeed, Raman spectroscopy was successfully used in the study of Depciucha et al. [11] to evaluate the balance between phospholipids and proteins in blood serum with the conclusion that the method provides quick measurement results for analysis of biological material.

Using Renishaw inVia Raman spectrometer the below spectra have been obtained for solutions of γ-globulin, albumin and BSA before and after the tribological experiment. In addition, optical images of the film formed on CoCrMo metal ball were taken. The tribological experiment was conducted within pendulum hip simulator with the ball on the disc configuration by metal prosthesis head of CoCrMo ball (28mm) ISO 5832-12 [17, 31, 34]. The first experiment was conducted with 25% BSA as the lubricant. Then 28mg/ml of albumin solution and 11mg/ml of γ-globulin were respectively prepared as the lubricant with phosphate buffer solution (PBS) and the experiments continued.

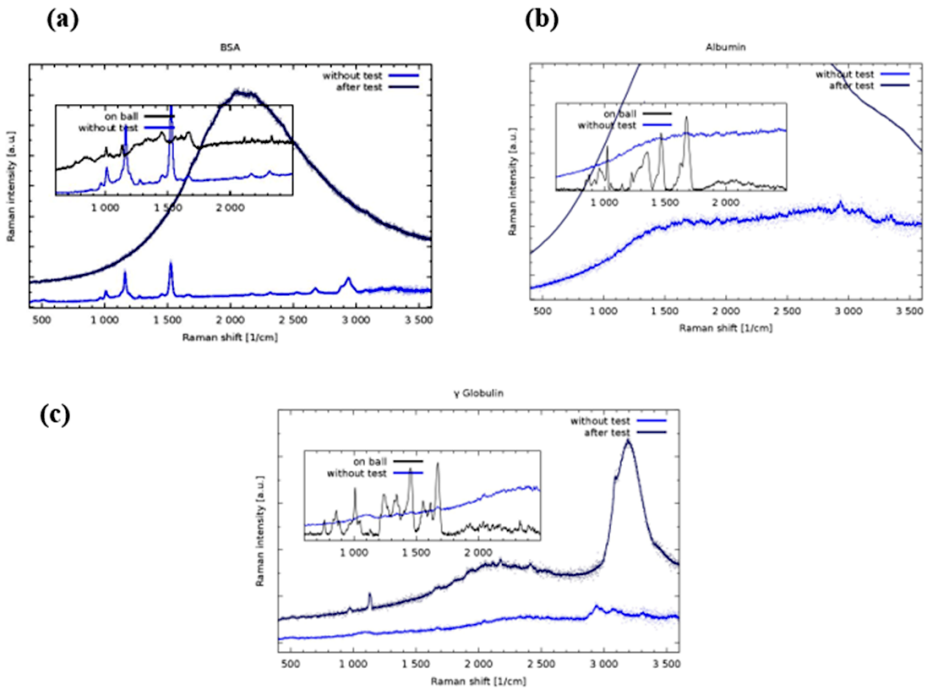

Fig. 5 Raman fingerprint spectra of solutions without tribological test and after tribological experiment together with Raman spectra of the film formed on CoCrMo ball with lubricants (a) BSA, (b) albumin, (c) γ-globulin.

In the above Raman spectra, the buffer correction was applied. The spectra found on the metal ball for all lubricants were quite different from the spectra of without-test liquid of the same protein. The reason for the difference could be due to the crystalline form of the protein on the metal ball (see Fig. 6). There could also be a chemisorbed transition state within protein lubricants and metal surfaces. The after-experiment protein solution spectra for BSA and albumin have similarities but did not show appropriate spectra, although after-test γ-globulin shows well-defined peaks. Most probably the β-sheet structure of γ-globulin is affected by chemical reactions. The α-helical structure of BSA and albumin become distorted. The result of the tribological effect of joint replacement can be more evident if we can use mixtures of different proteins for the experiment.

To study the protein changes in more detail, one of plasmonic-enhanced Raman modalities could be used. In particular, surface-enhanced Raman scattering (SERS)-based biosensors are developing rapidly for the applications of chemical analysis, nanostructure characterization as well as for biomedical sectors [35].

Making use of high increase of Raman signal after protein binding to the metallic nanoparticle(s), SERS is a very effective technique [35-37]. In another SERS study of protein-nanoparticle interaction, bio-conjugated silver nanoparticles were used by Laruinaz et al. [38]. In summary, SERS has been recognized as a very successful operative analytical tool due to its high sensitivity, high selectivity, and fluorescence-quenching properties [35, 38].

The formed film structures on CoCrMo ball surface for BSA, albumin and γ-globulin were observed during the Raman experiment in order to investigate the biochemical influence of protein lubricant on the metal surface.

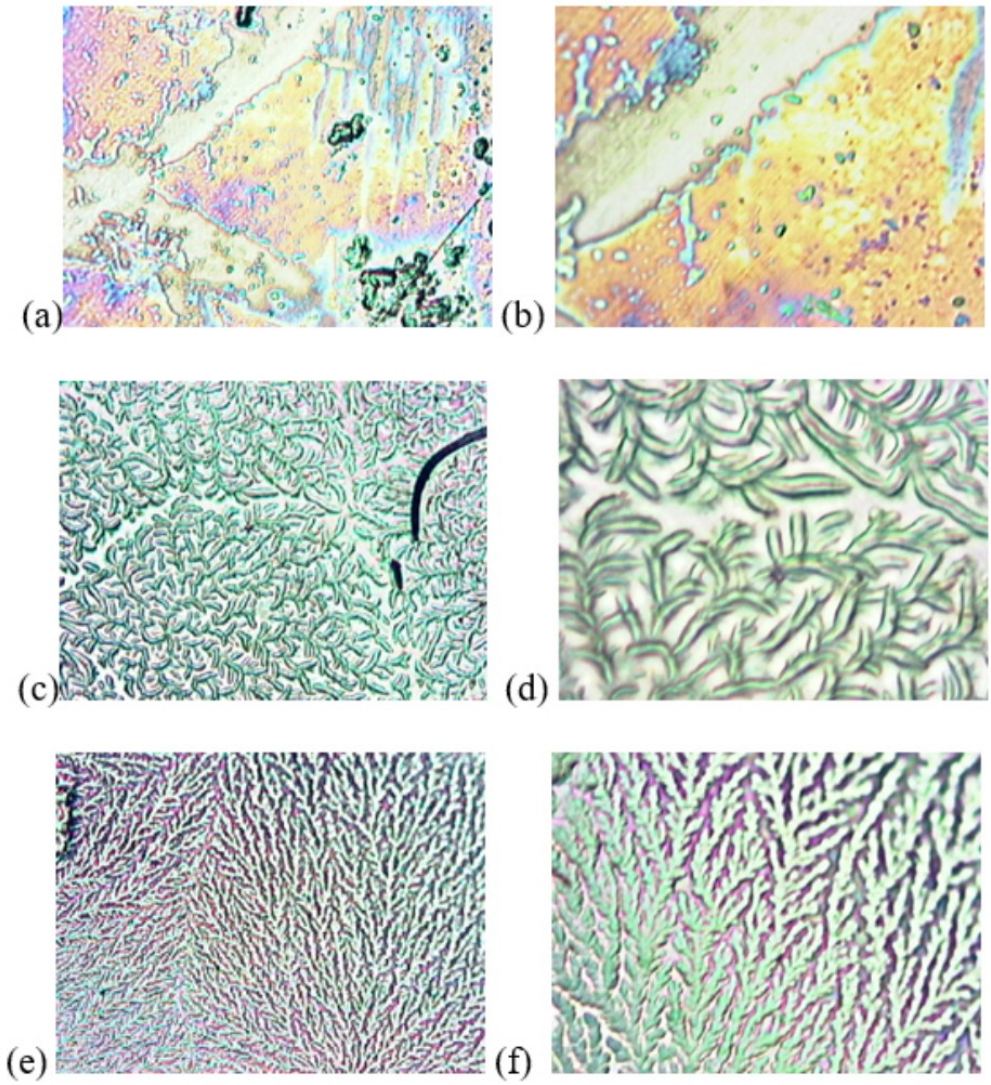

Fig.6 Film formed viewed with Raman microscope on the ball by respectively (a) BSA and (b) BSA enlarged view, (c) albumin and (d) albumin enlarged view and (e) γ-globulin and (f) γ-globulin enlarged view. The horizontal size of panels is 180 μm (a, c, e) and 70 μm (b, d, f).

The different structures of the formed film produced by different proteins are clearly visible from Fig 2. These observations can also play an important role in the biochemical explanation of adsorption takes place on the metal surface by protein molecules during film formation.

From the above discussion, it could be assumed that the CoCr femoral head could act as a heterogeneous or contact catalyst by forming synovial protein films as chemisorbed transition state and changing the secondary structural composition of protein molecules within artificial joint replacement. That is, the activation barriers might be reduced by the process of heterogeneous catalysis [39-42].

6 CONCLUSIONS

From the systematic review of last ten years (2006-2016) contribution it has been observed that research on protein film formation of synovial fluid beneath the artificial joint is mostly concentrated on mechanical properties such as kinematic conditions, film thickness, frictional coefficients, frictional force, shear force, wear, boundary lubrication, surface geometry, rheology and wettability of different modal fluids and different surface materials. The influences of some physical chemistry parameters like pH, buffer, viscosity and concentration of proteins, temperature, pressure and time dependence were also investigated. However, analysis of chemical changes induced within artificial joint and chemical composition of the formed films are yet to be revealed.

- Therefore, to describe the condition of joint replacement and to increase the longevity of the replaced area, the chemical changes taking place in the proteins of synovial fluid need to be observed to explain the biochemical behaviour of the materials using in joint replacement.

- Raman spectroscopy is a good technique to understand clearly the changes of the chemical structure, as the vibrational fingerprint of each compound reveals the specific chemical structure of the compound within the formed film in the Raman spectrum.

- The Raman spectroscopic differences obtained for each of the protein lubricants before use and after experiment within the simulator of the ball on disc set up can explain the chemical reactions that are occurring withinartificial joint replacement.

- Also, by observing the Raman spectra of lubricant film formed on the ball, one can address the chemical structural changes that are taking place due to bond breaking or formation within the proteins of synovial fluid.

- There could also be a possibility of heterogeneous catalysis process occurring among proteins within joint replacement in case of using CoCrMo alloy as a metal prosthesis.

- Furthermore, using mixtures of different synovial proteins in appropriate ratios as lubricants may show significant spectroscopic differences and thus it would be possible to understand the lubrication chemistry and mechanism of synovial liquid proteins within artificial prosthesis more explicitly.

7 ACKNOWLEDGEMENT

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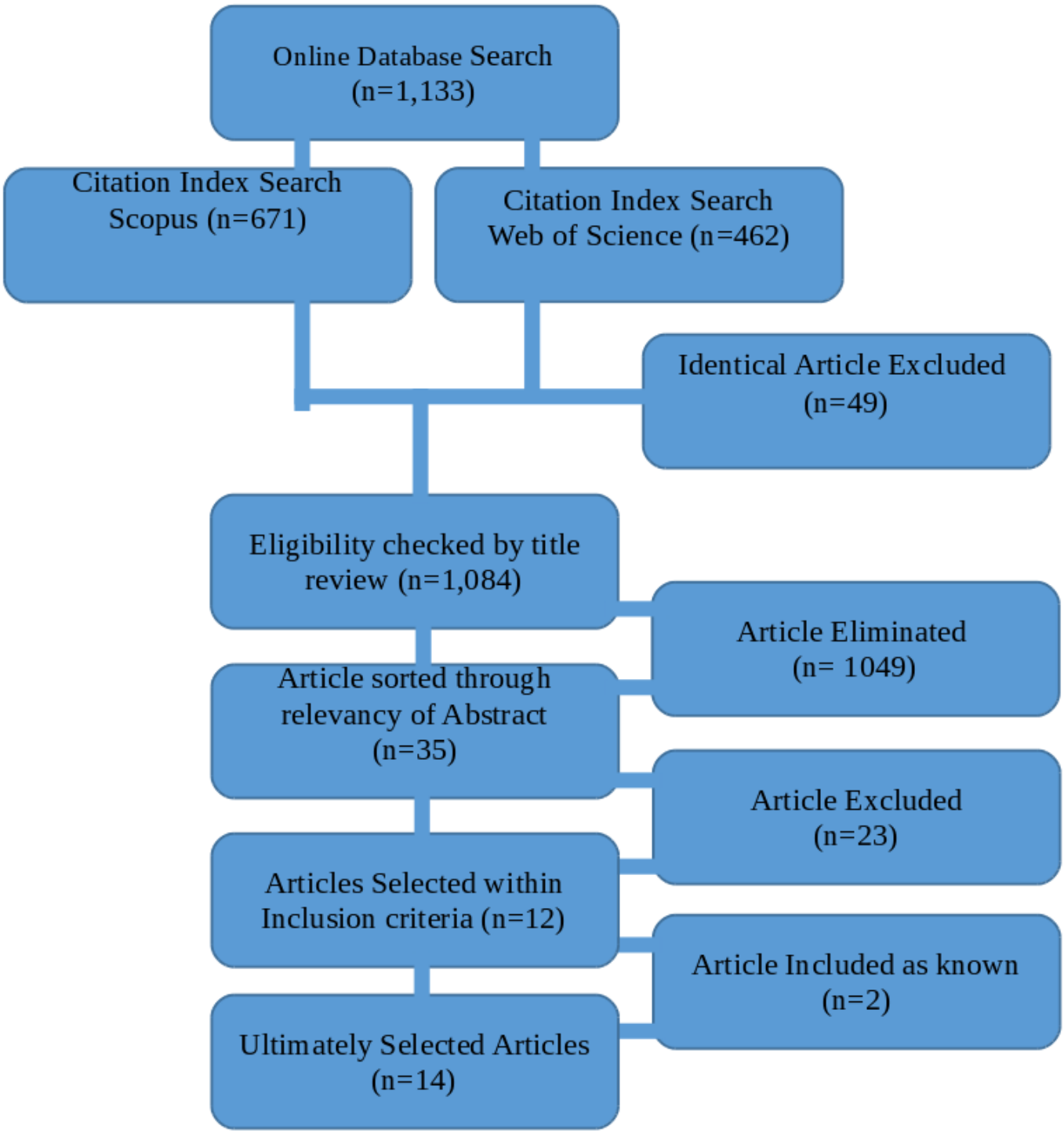

Table 1: Inclusion and exclusion process of article selection for the systematic review.

Table 2: Summary of the systematic review, with characteristics of the eligible studies

Characteristics of the eligible studies (Continued)				
Reference	**Area of research**	**Conditions of analysis**	**Focused parameters**	**Key results**
David Nečas et al. -2016 [7].	The thickness of lubricant film for specific proteins on hip joint replacements and Interfacial lubrication process.	Thin film colorimetric interferometry, fluorescent microscopy in combination with the optical method were used to measure film thickness. Metal femoral head and Glass disc were used in different conditions. Test lubricants were Albumin and γ Globulin (2:1) with saline solution.	Kinematic conditions such as, under pure rolling, partial negative and partial positive sliding on film formation and film thickness.	γ Globulin forms a thin protein layer, Albumin absorbs onto that layer. In most cases, Albumin contributes to increasing the total film thickness.
Adela Galandáková et al. – 2017 [4].	Differences of compositions and the constituents of synovial fluid extracted from respectively, patients of primary arthroplasties and revision arthroplasties and without joint replacement.	Synovial fluid collected from 152 patients were categorized into four different groups depending on their conditions of osteoarthritis and joint replacements. Kinsley method, Quantitative colorimetric kit, Enzyme-linked immunosorbent assay and Vibro-viscometer SV-1A were used respectively to determine the concentration of proteins, phospholipid, hyaluronic acid and viscosity of synovial fluids. For statistical analysis IBM SPSS Statistics 22 software was used.	Concentration of components, viscosity, statistical analysis of constituents for diversified patient characteristics.	Albumin and γ Globulin readily adsorbed on artificial materials, whereby have an influence on the frictional properties of the lubricating surface. The γ Globulin concentration found remarkably higher and concentration of phospholipid observed significantly lower with the revision of joint replacement patients and with patients without joint replacements. Hyaluronic acid and viscosity of different groups were observed unchanged.
Maria Parkes et al. -2015 [13].	Film formation impact of the protein content of model synovial fluids under the static and rolling condition and correlation with changing properties.	Adsorption of Bovine serum albumin and Bovine gamma globulin on silica and chromium surface was observed at pH 7.4 and 8.1. Quartz Crystal Microbalance and ball on the flat device were used for adsorption measurement in the static condition. Under the pure rolling condition, optical interferometry was used to observe the effect of protein content on lubricant film thickness on silica/CoCrMo interface.	Film thickness, pH, adsorption, under static and rolling conditions, different composition of proteins.	Protein layers are thicker under rolling than the statical condition. Film formation depends on protein content and the pH of the solution in both conditions. Bovine gamma globulin and mixed protein bounds more strongly than Bovine Serum albumin. Bovine gamma globulin forms much thicker deposited layers compare to bovine serum albumin.

Characteristics of the eligible studies (Continued)				
Reference	**Area of research**	**Conditions of analysis**	**Focused parameters**	**Key results**
Nakashima Kazuhiro et al. -2015 [14].	Adsorption and frictional property of Bovine Serum Albumin in the rubbing condition of ultra-high molecular weight polyethylene and CoCrMo alloy.	Reciprocating pin-on-disk tribometer with the electrochemical cell was used to measure the frictional property during friction. Open circuit potential condition technique was used to cover friction force and electric potential. But -0.2 applied potential condition also measured electric current and friction force.	Adsorption and desorption behaviour of the protein, frictional property, the coefficient of friction during rubbing condition, shear force, protein concentration. The transition of potential, frictional force and normal loading force, current measurement was recorded.	BSA film is reconstructed during rubbing on CoCrMo alloy surface, under rubbing condition. The absorbed film formed become strong and stable and optimally adapted structure for shear force by receiving shear force.
Jong Bong Park et al.-2014 [16].	Boundary lubrication ability of retrieved CoCrMo head within Hyaluronic acid and phospholipid.	Atomic force microscopy technique was used, including a rectangular silicon cantilever integrated with sharp silicon tips. Hyaluronic acid and phospholipid of various concentrations were used with retrieved CoCrMo head.	Boundary lubrication, Microscale frictional coefficients and Concentration.	Retrieved CoCrMo head's The microscale frictional response has the dependency on the concentration of HA and phospholipid. The optimal concentration that ranges to maximize frictional behaviour is similar to the concentration of HA and phospholipid of human synovial fluid.
Martin Vrbka et al. -2014 [17].	Observation of film formation and measurement of film thickness of bovine serum through various test configurations.	Ball on disc and lens on disk configuration were used for metal and ceramic ball with silica and chromium layer on glass respectively, for bovine serum lubricant considering as a function of the artificial hip joint. Film thickness was measured by optical interferometry as a function of time.	Film thickness, hydrophilicity, hydrophobicity, surface geometry, concentration, wettability, conformity, kinematic condition, temperature, material, time, hydrodynamic effect.	Glass disc covered by a chromium layer showing hydrophobic behaviour formed a thicker lubricating film, on the other hand, silica layer in the same condition showed hydrophilic behaviour. Due to hydrodynamic effect, a thin film was formed on the silica layer. Wettability has no effect on protein film formation, while kinematic condition and conformity of contact surface has the fundamental effect. Due to protein aggregation film formed within ball on disc configuration. On the contrary ball on lens configuration, hydrodynamic effect leads to film formation.

Characteristics of the eligible studies (Continued)				
Reference	**Area of research**	**Conditions of analysis**	**Focused parameters**	**Key results**
Maria Parkes et al.-2014 [18].	Buffer selection and pH impact on the protein adsorption kinetics and film formation respectively under static and rolling conditions.	Bovine serum albumin with respect to different buffer solutions was utilized to observe the adsorption properties by means of Quartz Crystal Microbalance. Under static condition adsorption of proteins were investigated on silica coated quartz crystal. Under the rolling condition, the ball-on-flat device was used and film thickness or protein solution was measured by a thin film optical interferometry.	Solution buffer, pH, film thickness, viscoelasticity, adsorption, static and rolling condition.	The aspect of the buffer depends on pH within static condition. Both in static and rolling conditions pH has an impact on film formation. At lower pH, protein adsorption observer initially rigid and relaxed, with time rate increased. At pH 7.4 or lower, thicker protein film formed in rolling condition. The film found non-uniform and irregular deposition of protein. At higher pH initially, viscoelasticity is higher, also uniform and a consistent film formed at static condition.
Jemma G Kerns et al.-2014 [10].	The molecular structural change occurrence, in the subchondral bone matrix, due to osteoarthritis.	By using Raman spectroscopic technique, differences of molecular structures between tibial plateaus of respectively healthy joints and joints with total replacements due to osteoarthritis were compared. Also, comparison of medial and adjacent compartments of subchondral bones was observed as different load bearing sites.	Molecular structure, Load baring.	Medial and lateral for both bone matrix significant chemical structural changes appear due to osteoarthritis. Whereas spectral differences were not found between the medial and lateral compartment of the subchondral bone matrix.
Zhang Z et al. – 2014 [3].	Interfacial rheology and bulk rheology of model synovial fluid effect by the proteins and hyaluronic acid for respectively various shear rates, strains and frequencies.	A model synovial fluid was utilized for the experiment that contained hyaluronic acid, bovine serum albumin and γ globulin within pH 7.4. A double wall ring geometry and AR-G2 stress controlled TA instruments rheometer were used to measure interfacial rheology and bulk rheology respectively.	Interfacial rheology, bulk rheology, pH of the solution, oscillation shear rate, strain and frequency.	In the steady and oscillatory condition of the model synovial fluid composed of hyaluronic acid, bovine serum albumin and γ globulin interfacial rheology occur due to protein adsorption at the interface are rejected. On the contrary, bulk rheology is controlled wholly by the hyaluronic acid of model synovial fluid in the difference of shear rate, strains and frequencies.

Characteristics of the eligible studies (Continued)				
Reference	**Area of research**	**Conditions of analysis**	**Focused parameters**	**Key results**
Duong Cong Truyen et al.-2012 [8].	The concentration levels of Bovine serum albumin and γ globulin influence on the lubricating ability of CoCrMo hip prostheses.	Sections of the retrieved CoCrMo femoral head from revision surgery were lubricated with several concentrations of bovine serum albumin and γ globulin. Atomic force microscopy was used to measure applied normal force and surface roughness. Friction coefficients were calculated afterward.	Frictional coefficients, the concentration of lubricant, Boundary lubrication, applied normal force and surface roughness.	A maximum level of bovine serum albumin and optimal concentration of γ globulin leads to effective boundary lubrication. Thus the concentration of bovine serum albumin and also γ globulin control the friction of CoCrMo head.
Fan Jingyun et al. -2012 [19].	Film thickness and wear measurements for various model synovial fluids solutions of different pH.	Thin film optical interferometric test device was used to analyse film thickness and wear tests. The experiment conducted within glass disc and CoCrMo surface for a range of model synovial fluids of the variety of pH.	Film thickness, pH, wear measurement.	Film formation signifies two distinct mechanisms: the boundary lubrication mechanism and high viscosity gel mechanism. Wear mechanism is designated through the tribocorrosion process, with higher pH, wear increases. The Chemistry within synovial fluid has a significant effect on wear and failure of joint replacements.
George W Greene et al. – 2011 [2].	Frictional experiment on porcine cartilage and enzymatic digestion of hyaluronic acid to disclose the lubrication mechanism.	The effect of enzymatic digestion of hyaluronic acid with hyaluronidese was investigated. Using surface force apparatus SFA 2000 equipped with friction device attachment normal and frictional force was measured under various loading conditions.	Friction, wear, lubrication, pressure, time duration, shearing force, concentration.	hyaluronic acid and glycoprotein lubricin complex as HA-LUB, trapped at the interface to form a cross-linked network, which plays a role of boundary lubricant and prevent wear mechanism.
Karen A Esmonde-White et al. -2009 [12].	Investigation of differences between biochemical compositions of healthy joint synovial fluids and synovial fluids of osteoarthritis patients.	Drop deposition/Raman Spectroscopy protocol (DDRS) was utilized as an evaluation tool to distinguish between osteoarthritis patients' synovial fluid and healthy joint synovial fluid. The light microscope was used for coarse separation from the dried drop. From knee joint x-rays Kellgren/Lawrence (K/L) scores were used, for grouping individual patients.	Chemical structural analysis, K/L score, Microscopic image	DDRS method can be used as an appropriate detector of synovial fluids of osteoarthritis patients, namely the Raman band describes protein secondary structure. Raman data clarified that disorder within normal electrostatic interactions of synovial fluid occurs due to osteoarthritis.

Characteristics of the eligible studies (Continued)				
Reference	**Area of research**	**Conditions of analysis**	**Focused parameters**	**Key results**
Tannin A Schmidt et al. -2007 [20].	The contribution of Boundary lubrication of various constituents of synovial fluids separately and also collectively.	ELF 3200 (Bose EnduraTEC, Minnetonka, MN) equipment set up was used for the performance of cartilage boundary lubrication tests with fresh bovine osteochondral samples. Effect of graded dilutions was determined by the test lubricants prepared in PBS and different percentage of SF.	Boundary lubrication, frictional coefficients (Static and Kinetic) and Concentration.	Both at physiologic and pathophysiologic concentrations, constituents of SF contribute to the boundary lubrication of the apposing articular cartilage surface. The contribution found individually and also in combination with the constituents of SF.