Wrocław University of Technology

DOCTORAL THESIS

Mechanical, histological and biological analysis of artificial joint loosening

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Institute of Materials Science and Applied Mechanics

WROCŁAW UNIVERSITY OF TECHNOLOGY

FACULTY OF MECHANICAL ENGINEERING

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Synkowi

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1 Introduction

Total hip arthroplasty (THA) is a very common surgical procedure and is considered to be the greatest achievement of orthopaedic surgery. Total hip replacement (THR) or hip prosthesis replaces a destroyed or damaged joint thus alleviating pain and allowing the patient to return to an active lifestyle. The average lifespan of a hip prosthesis is between fifteen and twenty years [93]. However, due to various reasons a certain number of implants achieve a shorter life-time leading to premature revisions. The most common cause for the revision of total hip replacement is the aseptic loosening. Aseptic loosening is thus an important problem for long-term successful functioning of hip replacements [18, 101]. Development of fibrous tissue and the resorption of bone tissue around the implant can cause the loosening of artificial joint that leads to the necessity of revision surgery.

One of the main causes for the aseptic loosening is the formation of wear debris due to mechanical loading and consequent biological reaction. The wear particles are mostly generated between the frictional surfaces of the artificial joint. With the aid of pseudosynovial fluid, the wear particles can move throughout the joint, reach the periprosthetic tissue and evoke an inflammatory reaction leading to osteolysis and consequent loss of stability of the prosthesis.

The aim of this work was to determine the effects of mechanical and biological factors on the phenomenon of degradation and loosening of total hip replacement.

THR is the composite mechanical system with the mechanical characteristics playing an essential role in its performance. The mechanical properties of the hip replacement and natural hip differ significantly leading to a deformation conflict as well as to the biological response of the surrounding tissues. For that reason, object of the study comprises not only the analysis of the hip prosthesis itself but also the analysis of surrounding tissues, where the release of wear particles and their biological impact was identified.

The subject of the investigation were forty-three hip implants revised due to aseptic loosening and the periprosthetic tissue samples retrieved at revision surgery. Hip joint implants were studied because they are the most frequently replaced joints in human body (Fig. 2.1.1.). The examined implants were Müller type, low friction implants, with the acetabular component made of ultra high molecular weight polyethylene (UHMWPE),

cemented femoral stem component made of titanium alloy and the femoral head made of either ceramic (Al_2O_3) or metal (stainless steel or titanium alloy).

In the past, the majority of hip replacements were cemented, i.e. inserted with the aid of bone cement. The cemented prostheses are implanted at lower cost, however, the operation time is prolonged due to preparation of cement. For implants implanted until 2000, the literature data and the Scandinavian registry show better survivorship of cemented prostheses compared to cementless prostheses [101, 102].

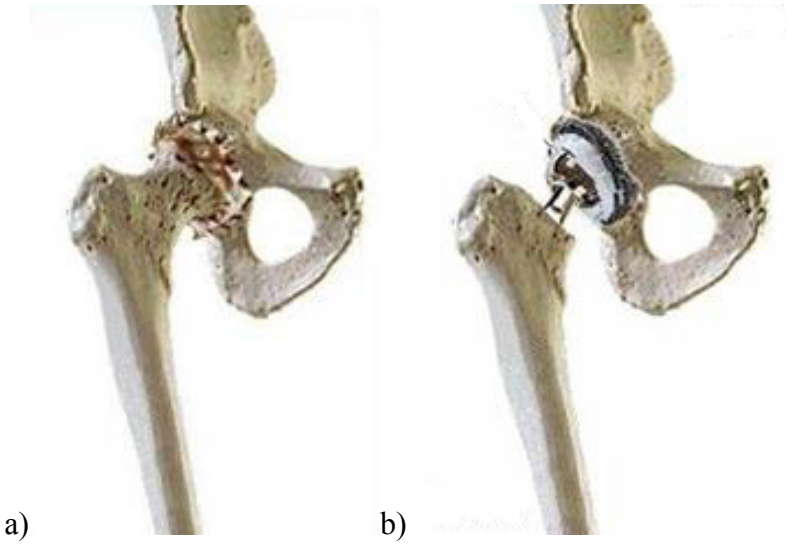
Mechanical and material properties, as well as types of wear patterns due to wear process in vivo were analyzed on retrieved implants. The composition, morphology and size of metallic and polyethylene wear debris particles isolated from periprosthetic tissue were examined. In addition, the biological response to wear particles was studied in samples of periprosthetic tissue of retrieved prostheses and tissue samples cultured in vitro.

Despite a great number of scientific studies, many aspects of the loosening of artificial joints are still to be revealed. Premature revision of the joint replacements is still an important problem for contemporary arthroplasty. By analysis of the material and mechanical properties of the implants, as well as the analysis of the biological reaction of periprosthetic tissue, we approach to the process of implant loosening in a complex manner. Understanding of the process of aseptic loosening contributes to the improvement of implant design and material selection and eventually leads to the prolongation of its long-term in vivo survivorship.

2 Literature review

2.1 Total joint replacements

Natural joint is the junction between rigid bones of the skeleton permitting relative movement and force transmission. The joints have a very complicated anatomical structure. The joint surfaces are covered by a smooth articulating surface – cartilage, which allows pain free movement. Every joint is enclosed by a capsule lining with a smooth tissue called synovium. The synovium releases a fluid, which lubricates the joints. The synovial fluid consists of proteins, salts and other components like lubricin, hyaluronic acid (polysaccharide glycosaminoglycans), which increase the viscosity of the synovial fluid. In a healthy joint the friction is extremely low (μ=0.005÷0.02) [11] and the wear is practically zero.



a) b)

Fig. 2.1.1. Natural arthritic hip joint (a) and total hip replacement (b) [73].

Unfortunately the lubricating system can be disturbed e.g. due to a rheumatic disease, arthritis, infection or injury, causing the increase in friction and wear of joint surfaces. When the articulating cartilage is worn out or damaged, the bones come in contacts rub on each other causing intensive pain and stiffness of the joint. When the

rehabilitation, pain medications and all other treatment cannot relieve the pain and disability in the patient's joint, surgery is needed. During the surgical procedure called total hip arthroplasty (THA), natural joint is replaced by an artificial device (prosthesis, implant) to restore joint movements. The joint replacement helps to relieve pain and increase the mobility of the destroyed joint.

The main function of the prosthesis is to transfer the loads between bones [11]. The most commonly replaced joints are hip and knee joints. The dysfunction of these joints strongly limits the movement capabilities of the patient. Joint replacement can also be performed on other joints including shoulder, elbow, finger and ankle.

Total hip replacement consists of an acetabular cup, femoral stem and femoral head (Fig. 2.1.2.). Cemented or cementless acetabular component is implanted into the pelvis to replace the damaged socket. The femoral head is cut off and the medullar cavity is drilled. The femoral stem is inserted in the femur by the press-fit mechanism or is cemented depending on the patient's bone quality. The femoral head is then mounted on the neck of the femoral stem.

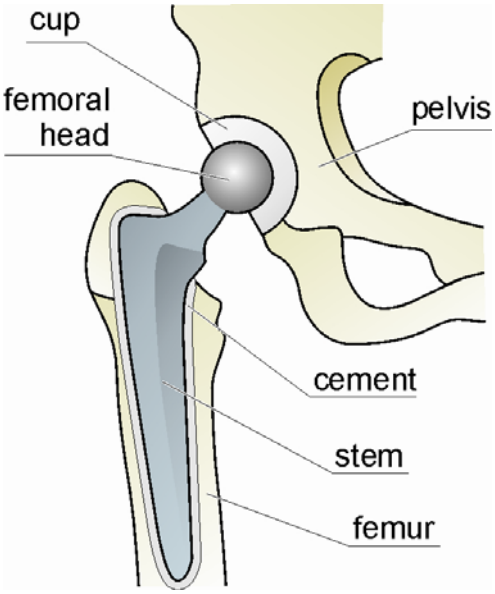


Fig. 2.1.2. Total hip replacement

2.1.1 History of hip implants

The idea of the artificial hip was developed in early nineteenth century, when other surgical techniques failed to relieve the pain associated with the degenerative joints. The first surgery relied on the insertion of different materials between destroyed cartilage surfaces without any stabilization. The fast relocation of those materials and intensive fibrous tissue growth were observed. Different materials were used in the beginning of implantation history including skin, pig bladder, periosteum, zinc, gold, rubber [5] or ivory [51]. Later, more sophisticated materials were applied for implant construction and more complicated procedures as total joint replacement were used. The origin of total hip arthroplasty, in which both femoral and acetabular components are replaced, is usually associated with Philip Wiles [41], who used metal prosthesis fixed by screws in 1938. Later, the implants were designed to be inserted into medullar cavity of the femoral bone. The hip implants composed of metal-on-metal articulating surfaces were introduced by McKee [5]. Several types of metal-on-metal prostheses were developed in the 1960s. The main problem with these prostheses was intensive wear and loosening of implants.

In 1970s, metal-on-metal hip were completely replaced by Charnley's model of implants. Sir John Charnley developed a low friction hip implant with polymer socket and metal femoral head [5], where the friction coefficient was reduced. Attempts to use polytetrafluoroethylene (PTFE) as the bearing material produced intensive wear and fast loosening. Later, high density polyethylene (HDPE) was successfully introduced into orthopaedic service. Sir John Charnley also introduced the use of bone cement (polymethylmethacrylate) in total hip replacement. For the next 30 years, research was directed at improvements of design and implantation technique of prostheses with a metal-on-polyethylene articulation.

The metal-on-metal implants were "rediscovered" by the British surgeons in 1980s [51]. Today, third generation of metal-on-metal bearing implants, as well as ceramic-on-ceramic implants is on the rise. Various modifications of implant design and material are now available for total hip replacement as a consequence of numerous attempts to achieve long-lasting implant. The combination of polyethylene cup and hard - metal or ceramic - femoral head is still treated as a gold standard and is used in more than 90% of currently implanted joints [134].

2.1.2 Desirable properties of biomaterials for total hip replacements

Materials used for the manufacture of implants replacing the natural joints are called biomaterials. Biomaterials have to be biocompatible and execute the prescribed functions, i.e. be mechanically strong, wear and corrosion resistant and have low elastic modulus. Although many available materials satisfy these requirements up to a certain level, there is no ideal material for joint replacement and the choice of material is always a compromise between its properties and clinical performance.

Biocompatibility is the ability of a material to perform a set function in the organism with a suitable host response. The material has to be tolerated by the bone tissue. In order to defend the organism, the physiological environment acts aggressively towards foreign bodies. Materials that are not biocompatible evoke strong tissue reaction, which employs immune system. The healing wound around the implant that is not biocompatible prolongs, evokes inflammation and can act as a source of constant irritation. An implant made of such material does not have any chance to perform the function of load bearing implants. Most materials used today are biocompatible in bulk, but they may excite a variable response when they are present in particulate form.

The total joint replacement is exposed to large loads; hence, the materials have to exhibit high strength to withstand weight-bearing loads. The material should be flexible enough to sustain repeated stresses and must not break or bend.

Wear process is inevitable in artificial joints, as every material wears out. The materials with high wear resistance should be chosen for total joint replacements to minimize generation of wear particles that can evoke strong inflammatory reaction (it will be discussed).

The materials for artificial joints should be corrosion resistant. The physiological environment contributes to the corrosion, releasing the ions and compounds as a result of electrochemical action. Moreover, the interface tissue in loosened implants may become acidic thus accelerating the corrosion. The reaction that occurs during the corrosion is the increase of the valence state of the metal atom [60]:

$$M \rightarrow M^{z+} + ze^{-} \text{ (oxidation)}$$

The oxidation event (loss of the electron and the increase in the valence) results in the release of free ions from metal surface into the circulation, which can cause cell necrosis or evoke an allergic immune response.

All metallic surfaces in contact with the body fluids corrode to some extent thus increasing the concentration of metal ions in blood and body fluids. As wear particles are generated, a new surface area for dissolution of the elements becomes available. Metal alloys used for orthopaedic implants are resistant to corrosion due to the formation of passive films, which prevents the corrosion by limiting the rate at which oxidation or reduction processes can take place. The formation of the passive layer on the metal surface runs according to the reaction [60]:

$$M^{z+} + z/2\ H_20 \rightarrow z/2\ MO + zH + ze^{-}$$

The passive films form spontaneously on the surface of metals and are very thin. In spite of the presence of passive films the corrosion can occur, e.g. when two dissimilar metals are in contact [168]. When the femoral head and the stem are made of different materials the corrosion is very common (galvanic corrosion). Under certain conditions pitting corrosion can also occur on the surface of metal implants leading to the formation of small cavities.

The biomaterial of the implant should have elastic modulus (Young's modulus) close to the value of bone. The modulus of elasticity is a measure of rigidity or stiffness of a material. It is calculated by dividing the load (stress) by the amount of deflection (strain). The big difference in the material rigidity causes a load transmission failure. The implant represents the obstacle in the load transfer between bones. The mechanical stress greatly influences the bone remodelling, which was proved by the experimental and numerical methods [12, 13, 14]. The presence of implant causes the stress shielding of the surrounding bone that is thus not loaded enough and becomes progressively weaker [11]. The implant does not have anchorage in the weak bone and the loosening can develop. The lowest stiffness among the orthopaedic alloys have titanium alloys, although still much bigger than that of bone (elastic modulus of bone is 10-30, while the elastic modulus of titanium alloy is 110 GPa, Fig. 2.1.3.) and due to this property titanium alloys are commonly used as stem materials in total hip replacements.

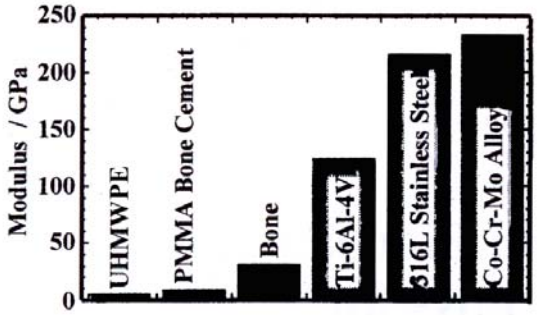


Fig. 2.1.3. Elastic moduli of UHMWPE, bone cement, bone tissue, titanium alloy, stainless steel alloy and cobalt alloy [126].

2.1.3 Biomaterials for total hip replacements

The artificial joints can be composed of metal alloys, polymers and ceramics (Fig. 2.1.4.). Because of their strength, metal and their alloys are commonly used as bearing surfaces in total joints replacements. They are jeopardized by corrosion in the fluid environment but a spontaneous formation of a thin protective oxide layer on the surfaces of metals makes them corrosion resistant. Unfortunately, the oxide layers have low shear strength and low resistance to abrasion and wear out producing hard oxide wear particles. The oxide particles will be removed directly as wear debris or become embedded in the soft UHMWPE, leading to severe third body wear [20, 97, 124].

The principal metal alloys used in orthopaedic practice are:

- stainless steel alloy (316 L),
- titanium based alloys (Ti6Al4V, Ti6Al7Nb)
- cobalt based alloys (Co28Cr6Mo).

Stainless steel is often used for orthopaedic implants. The standard type is stainless steel 316 L type which contains 17-20% wt. chromium, 13-15% wt. nickel, 2-3% wt. molybdenum and balance iron. The notation “L” signifies low carbon content in the steel, which means that intergranular corrosion due to precipitation of Cr-carbides at the grain boundaries will not be present [168]. In stainless steel chromium is responsible for forming

the surface oxide layer (chromium oxide Cr_2O_3) that prevents corrosion. Molybdenum is added to enhance the corrosion resistance on the grain boundaries. The stainless steel is relatively prone to corrosion.

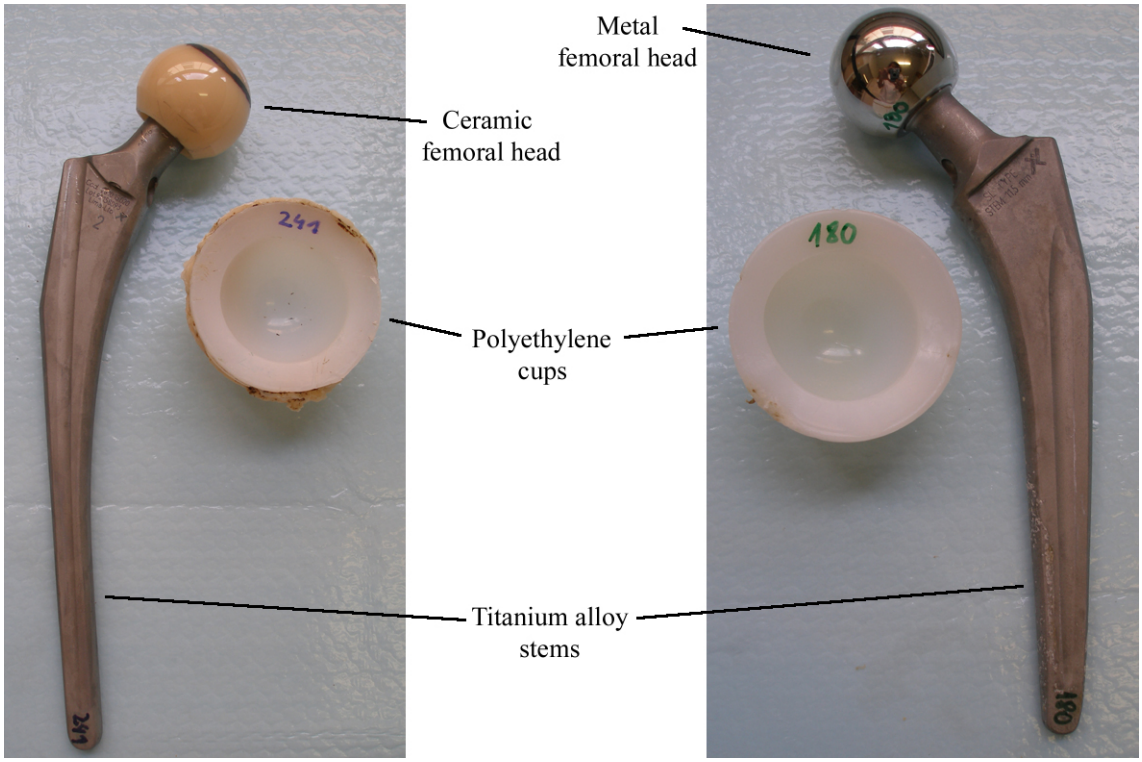


Fig. 2.1.4. Different materials used for artificial joint composition.

Titanium is used both in pure form and alloyed for orthopaedic applications. The pure form of titanium has poor load bearing properties; therefore, the titanium alloys with better mechanical properties are used for this applications. The most commonly used alloy in biomedical applications is Ti6Al4V with 6% aluminium and 4% vanadium. The oxide TiO_2 layer on the surface of titanium alloys protects the alloy from the corrosion and contributes to the enhanced tribological behaviour [36]. The titanium spontaneously forms a passive oxide layer which contributes to its high corrosion resistance and biocompatibility with human tissue.

Cobalt-based alloys are used most commonly as a cobalt-chromium-molybdenum alloy (CoCrMo). The content of chromium is 19 to 30%, which contributes to corrosion resistance. Molybdenum has been found to be beneficial under active corrosion conditions [168]. The high elastic modulus devaluates the cobalt alloy for implantology [126].

Ultra high molecular weight polyethylene (UHMWPE) is widely used in orthopaedics as a bearing material in artificial joints as an articulating surface. The UHMWPE is a polymer composed of repeating ethylene units (Fig. 2.1.5.). Monomeric molecules of ethylene are linked through polymerization in order to compose the polymeric chain of polyethylene.

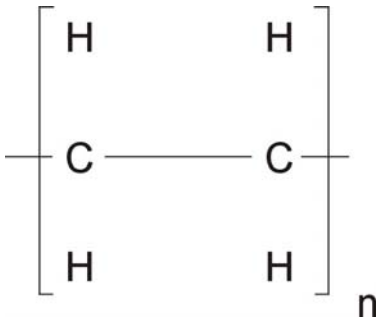


Fig. 2.1.5. Chemical structure of polyethylene.

UHMWPE differs from other polyethylenes in molecular weight and also in impact strength, flow characteristics, abrasive wear and density. The molecular weight of UHMWPE is in the range of 2-6 million, which corresponds to between 71429 and 214286 ethylene groups [139]. UHMWPE has extremely long and highly entangled molecular chains, which makes it more resistant to wear [171]. The density of the UHMWPE is 0.94 g/cm^2. A low-pressure method is used in the manufacturing the UHMWPE. Low friction is the most desirable property of polyethylene; also corrosion resistance and the ability to vibration damping are very important in the implantology. The UHMWPE has a finite lifespan due to intensive wear process limiting implant longevity and generating huge number of wear particles. Although polyethylene in bulk form elicits little tissue reaction, the polyethylene particulate debris can evoke inflammation. This process can lead to aseptic loosening of the implant and subsequently to revision arthroplasty. Wear of UHMWPE can occur through abrasion, adhesion and fatigue mechanisms. Each milligram of polyethylene has been estimated to generate 1.3×10^{10} particles [160].

The methods of sterilization have a significant effect on the wear properties of polyethylene. Sterilization by gamma irradiation in the air environment causes the cross-linking of polyethylene, which modifies the polymer molecular weight, elastic modulus and other mechanical properties.

Ageing of the polyethylene is an important problem in implantology. Physical (heat, light, radiation, tension), chemical (oxygen) and biological factors contribute to faster ageing of polyethylene. During the ageing process, covalent bonds in the main chain of the polyethylene are destroyed, which decreases the molecular weight of the UHMWPE. Due to the ageing process polyethylene loses its elasticity, becomes more brittle and changes its colour.

Polymethylmethacrylate (PMMA) is used to anchor the implant component to the bone tissue. The thickness of the cement mantle should be approximately 2-3 mm. The bone cement acts as a buffering layer, distributing the applied stresses from the stiff implant to the more compliant bone. Cement in solid form is bone-friendly and the cement may become osteointegrated [139].

PMMA is a polymer, which is subjected to polymerization during the implant stabilization. Polymerization is initiated by the mixing a polymer containing an initiator (catalyst) and a liquid monomer containing an activator. The process proceeds by an exothermic free radical reaction. During the polymerization not all monomer is used to build the polymer bulk, which is a clinical problem, because the monomer of the polymethylmethacrylate is very toxic and contributes to tissue degradation. The exothermic polymerization can cause thermal damage to the bone tissue. The PMMA is a very brittle material (sustains little plastic deformation), stronger in compression than in tension, but weakest in shear [139]. During the polymerization, the cement contracts (up to 5%), which produces increased porosity in the cement mantle. The porosity produced by the polymerization shrinkage, the presence of barium sulphate or zirconium oxide added to make the cement X-ray opaque and the roughness of the bone-cement interface provide sites for crack initiation in the bone cement mantle. The fatigue failure is the main mechanical problem for PMMA. Very hard wear particles of PMMA operate as a three-body wear particles thus contributing to the increased wear of other joint components [170]. For example, cement particles can get embedded in the polyethylene surface and contribute to intensive wear of femoral head. Some cement particles can adhere to the femoral head surface and abrade the softer polyethylene cup causing increased material removal [170]. The antibiotics are usually added to the bone cement to decrease the incidence of infection.

Ceramics are extremely hard materials and have high wear resistance. Ceramics displays bioactive properties that produce positive tissue responses and are quite attractive

for the total joint replacements. Its ionic structure creates a hydrophilic surface that has higher wettability than metals and hence facilitates lubrication [150]. The main disadvantage of ceramic materials used for load bearing surfaces is their brittleness. The elastic modulus of the ceramic material is very high (380 GPa), which evokes the problem of stress distribution at the implant-bone interface. Ceramics is most commonly used for the femoral heads and inlays of acetabular components. The most typical ceramic materials used in implantology are alumina Al_2O_3 and zirconia ZrO_2. Their wear behaviour is determined by the properties such as grain size and pore size [92].

Different bearing combinations are used for the hip joint replacement. The most common sliding pairs are:

– metal-on-UHMWPE (metal femoral head coupled with polyethylene cup),
– ceramic-on-UHMWPE (ceramic femoral head coupled with polyethylene cup),
– metal-on-metal (metallic femoral heads coupled with metal acetabular component),
– ceramic-on-ceramic (ceramic femoral head coupled with ceramic acetabular component).

2.2 Failure of total joint replacements

2.2.1 Causes of artificial joints loosening

Total joint arthroplasty is a very successful surgery, relieving pain and giving the patients with destroyed joint surfaces possibility to move and perform their daily activities. Although the clinical results are usually satisfactory, some implants unfortunately develop loosening very fast and the revision surgery is needed. For most implants the rate of revision at 10 years is less than 10% [18]. The loosening of the implants occurs when the implant loses adequate fixation to the bone. Usually the implant loosening is associated with intensive pain. Implant loosening can be seen on an X-ray as a radiolucent line around the implant. The prosthesis does not have a direct contact with the bone tissue. It is surrounded by the connective tissue, which is not visible on the X-ray. The implant becomes unstable and evokes micromotion, which intensify the loosening process.

Implant longevity is becoming more important since younger patients have problems with joints and are doomed to the total joint replacements. They could face multiple revision surgeries during their life span. Unfortunately, each following revision surgery has less satisfactory results and is less successful, because the bone tissue properties are progressively worsened.

The failure of the total joint replacement can be caused by:

- infection,
- fracture of implant component,
- implant dislocation,
- aseptic loosening.

Infection in total joint replacements is quite rare nowadays [10]. Sterile conditions during the surgery, intensive sterilization of implant component and usage of the antibiotics as an addition to the bone cement decrease the possibility of the development of infection [101].

The modern designs of contemporary implants are elaborated in terms of mechanics, wear and strength and hardly ever fracture. Therefore, this reason for implant failure is currently less significant.

Implant dislocation means the disturbance of normal relations in the joint formation. Dislocation weakens the ligament and thus the joint may become loose. Nowadays the implant dislocation is not very common failure.

Aseptic loosening, being the most frequent reason of implant failure, still constitutes a major problem of total joint arthroplasty [18, 101].

2.2.2 Aseptic loosening

Aseptic loosening is characterized by bone resorption, strong inflammation and the formation of connective tissue around the implant without any signs of infection. Clinical features of aseptic loosening of artificial joints are pain and loss of range of motion. Radiologically, osteolysis is indicated by the radiolucent lines at the bone-implant interface (Fig. 2.2.1). The formation of fibrous membrane at the bone-implant interface and infiltration of many inflammatory cells reveal an aggressive nature of the osteolysis process which is related to the production of various mediators of bone resorption.

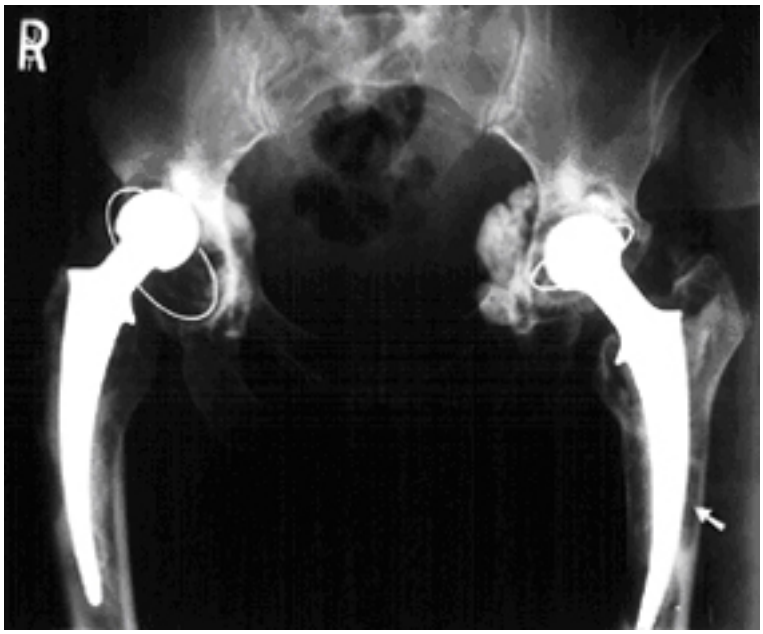


Fig. 2.2.1. X-ray image of failed total hip replacement demonstrating osteolysis caused by polyethylene wear debris (arrow)

There are many factors that influence the aseptic loosening including the exposure of the bone tissue to high stress, implant micromotion, excess fluid pressure, necrosis of bone tissue around cement mantle and the inflammatory reaction to wear particles. All of them work simultaneously leading to the formation of fibrous tissue, bone tissue resorption and subsequently implant loosening.

The aseptic loosening and osteolysis can be mechanically driven, when the bone is under loaded due to a stiff implant material. It was shown that the micromotion between bone and implant plays very important role in the development of the fibrous tissue [49]. Micromotion is recognized as a key factor in the control of bone turnover and is also important in aseptic loosening since it affects the activity of periprosthetic osteoblasts and osteoclasts. The connective tissue creates the healing tissue around the implanted prosthesis. Deposition of extracellular matrix composed of proteoglycans and collagen fibers begin to fill the gap between the bone and implant. Micromotion is reported to induce differentiation of connective tissue fibroblasts into cartilage - or bone-forming cells [6]. The direction of differentiation depends on magnitude of micromotion. When the volume of the movements is greater than 150 μm, the fibrocartilagenous tissue is formed around implant. If the volume is less than 20 μm the tissue differentiates into bone [21] and

the osteointegration can be observed. When the region is fibrous, the adjacent bone has to transfer the load, which leads to the elevated strain in the bone tissue. The fibrous tissue diminishes the contact between bone and implant, which may contribute to the aseptic loosening. Fibrous tissue cannot withstand the pressure to which it is exposed [153] and no osteointegration between bone and implant takes place.

Fluid pressure was also found to cause intensive osteolysis in stable osseointegrated implants [10, 50]. The joint fluid plays an important role in intensification fibrous tissue expansion. With every patient's step the synovial fluid is pumped into soft tissue thus increasing the pressure, which tends to enhance the passage of wear particles.

The cement polymerization is an exothermic reaction. The temperature on the bone-cement interfaces increase significantly causing the destruction of bone tissue. Thick layers of the dead bone cannot be subjected very quickly to the bone remodelling and do not give any support to the cemented implant; subsequently, the fibrous tissue is formed.

Among the factors listed above, the inflammatory reaction to wear particles is regarded to be the principal cause of osteolysis and aseptic loosening [65]. The wear particles generated in every artificial joint evoke intensive inflammatory reaction. Small fragments of different materials are phagocytosed by the defence system cells – macrophages. This process initiates the inflammatory cascade leading to bone resorption and implant loosening. Nevertheless, it is clear that the issue is highly complex and multifactorial.

2.3 Wear of implant components

Wear is the progressive loss of material as a result of mechanical action (due to friction in the contact zones). Wear is the function of use, so wear process is inevitable in total joint replacement. Wear is one of the most important factors that influence the long-term results of total joint replacements. Artificial joints exposed on the large cycling loads wear out intensively generating wear particles. Every bearing surface is a potential source of wear debris. Different materials used to produce artificial joint components wear in different modes, producing particles of different size, different morphology and different volume.

The wear process of artificial joints is multifactorial with a complex interaction of many variables. There are many factors that affect the wear process in total joint replacement including:

- implant related factors,
- clinical factors,
- patient related factors,
- fixation related factors.

Implant related factors comprise type of material, type of sliding pair used for implant construction, type of sterilization and implant design. Different biomaterials have different wear rates. Ceramics are considered to be the most wear resistant material. Differences in the wear mechanisms depend on the combinations of materials used for sliding pairs. The friction coefficient depends on the pair of surfaces in contact. It was shown that polyethylene produce different amount of wear particles (measured as cubic millimeters per year) depending on the femoral head material [5]. In addition, the volumetric wear significantly depends on the femoral head size [42]. With metal ball and polyethylene cup combination, the annual linear polymeric wear ranges from 100 to 300 μm, while the ceramic head paired with polyethylene cup generate less debris, the linear wear varying from 50 to 150 μm per year [4]. The roughness of the implant material influences the wear process. High roughness contributes to intensive wear of the softer material, for example polyethylene, by the hooking of the hard asperities on the softer material.

Sterilization of polyethylene by the gamma irradiation in the oxygen environment contributes to increased brittleness and weakening of the wear resistance of the polyethylene. During the sterilization by the gamma irradiation the cross-linking of the polyethylene is changed. The implant design affects the implant wear as well. It was shown that the diameter of the femoral head was correlated with the size of particles and the rate of particle production [69].

The wear process can be affected by clinical factors. Surgeon's skills are delicate aspects. It seems that more experienced doctors could position the implant more accurately thus decreasing the possibility of wear. It was also suggested that more than the experience of the individual surgeons, more important is the hospital reliability. Highly specialized hospitals may provide more professional health care [18].

Patient related factors like age, activity level, bone quality and gender also have a very important influence on the wear process. Younger (under the age of 55) and patients that are more active are in danger of excessive wear process and aseptic loosening [16, 102]. That is the reason why the surgeons recommend postponing the total hip surgery for young patients. Men are at a higher risk of revision procedures than women [102].

There are also fixation-related factors, which depend on individual patient characteristics. For some patients cementless implants are better, for other cemented fixation contributes to longevity of this prosthesis. However, for older implant generation the cemented prostheses have better survivorship than uncemented implants [102] with the revision rate of 7.4% and 27.3% respectively valid for older implants [101].

The intensive wear of implant components disables bearing loads and contributes to the generation a huge amount of wear particles. This process evokes inflammatory reaction leading to osteolysis and implant failure.

2.3.1 Different types of wear

There are 4 modes of wear in artificial joints. Mode 1 occurs when two primary bearing surfaces rub together as intended, e.g. femoral head and interior of acetabular socket. Mode 2 is present when the primary bearing surface rubs against a secondary non-bearing surface. Mode 3 is related to the third body particles (PMMA fragments, metal particles, bone chips), which are trapped between primary bearing surfaces. This can cause direct abrasive wear. Mode 4 appears between two secondary non-bearing surfaces, which rub together in a manner that is not intended. This may include screw-shell fretting, shell-socket fretting, neck-socket impingement, stem-cement fretting, etc.

The wear process in total joint replacements is caused mainly by three major mechanisms: abrasive, adhesive and fatigue.

Abrasive wear (cutting wear) appears when one surface, usually harder, removes away material from the opposite surface (two body wear) (Fig. 2.3.1. a). This mechanism often changes to third body abrasion (Fig. 2.3.1. b), where particles act like the asperity of the harder material, removing softer material roughness during loading (direct abrasive wear) or roughening the bearing surface with increased rate of wear. The metal particles, cement particles or the fragments of the bone can act as third bodies abrading the bearing surfaces. Increased hardness of the abrasive particles increases the wear loss [179].

The third-body particles can be embedded in a soft material, for example in the polyethylene layer.

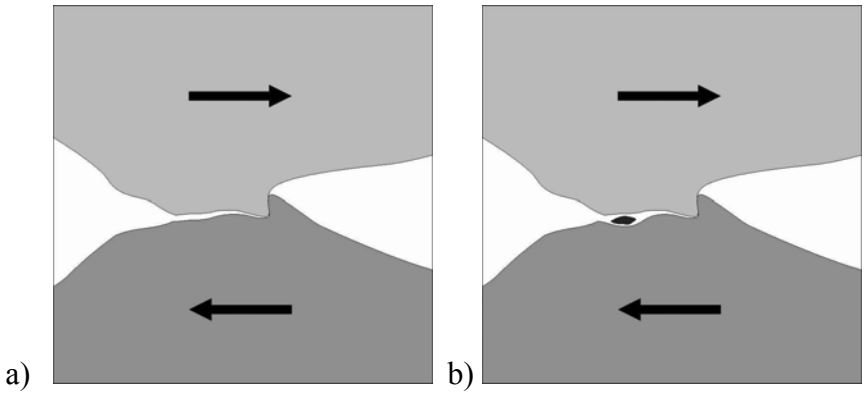


Fig. 2.3.1. Wear types in the artificial joints a) two body abrasion, b) three-body abrasion

Adhesive wear occurs due to strong atomic forces between two surfaces (Fig. 2.3.2. a). Adhesive wear arises when interatomic forces between two surfaces become greater than intrinsic forces between molecules of bulk material. Strong bonding between two surfaces promotes material transfer and loss of material from either surface. A continued motion of the surfaces causes the breaking of the bond junction, which results in the transfer of the softer material to the harder counterface or generation of wear particles. The adhesive wear appears when two materials come into close contact while the lubrication is inadequate, e.g. as in artificial joints [171]. Adhesive wear generates small particles, usually of the weaker material, in the range 0.1 to 10 μm in diameter as well as thin sheets up to about 10 μm in width [45]. In artificial joints, adhesive wear occurs when polyethylene surfaces adhere to opposing metal bearing surfaces. The removal of polyethylene results in very small pits and voids [126]. The adhesive wear can also affect the oxide layer on metal alloys.

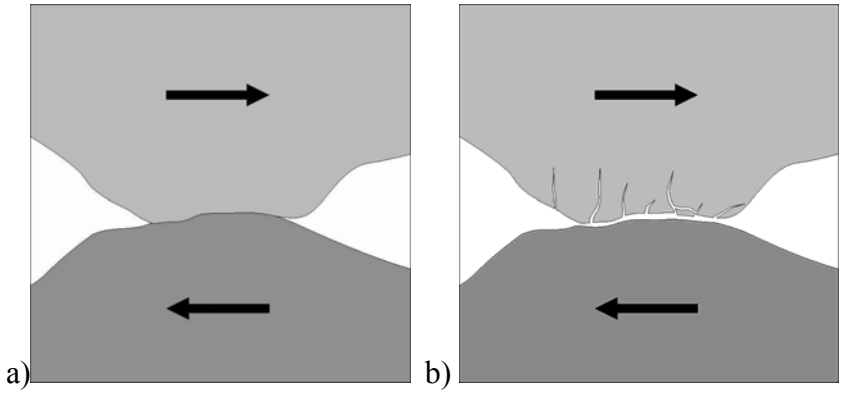


Fig. 2.3.2. Wear types in the artificial joints a) adhesion, b) fatigue.

Fatigue wear occurs through initiation multiple microcracks oriented horizontally under cycling loading when the shear stress or strains exceed the fatigue limit for the material (Fig. 2.3.2. b). Propagation of surface cracks leads to the release of wear particles. Evidence of fatigue wear includes large flake-like wear particles, cracks and delamination on the articulating surface. Fatigue wear occurs with the aging of the material and is characteristic for polyethylene components. Fatigue wear is not measurable until the fragments of material are torn away from the bulk material. There are many factors influencing the fatigue resistance of implant materials. All these wear types contribute to wear particle generation.

2.3.2 Roughness of implant components

Roughness is a measure of a surface texture. It is quantified by the vertical deviations of a real surface from its ideal form. If these deviations are large, the surface is rough; if they are small, the surface is smooth. Roughness can be measured using contact or non-contact methods. Contact methods utilize dragging a stylus across the surface, like a profilometer. Non-contact methods include interferometry, confocal microscopy and electron microscopy.

There are many different roughness parameters in use. The parameters reduce all information about the profile to a single number. Ra is the universally recognised, and

most used, international parameter of roughness [134]. It is arithmetic mean of the absolute departures of the roughness profile from the mean line. Other common parameters include Rt, Rv, Rp, Rpm, etc. Rt (peak to valley height) is the maximum distance between the highest peak and lowest valley height within one evaluation length. It may be used as a measure of counterface imperfections and profile shape. Rt parameter is heavily influenced by very high individual asperities or deep valleys. Rv is the maximum depth of the profile below the mean line within the sampling length. Rp (peak height) is the distance between the mean line and the highest peak within the sampling length. Counterface imperfections with exceptional peaks (especially scratches) above the mean line have been shown to have the greatest effect on the wear of the polymeric surface. Rpm (mean peak height) is the mean distance between the highest peak and the mean line in five consecutive sampling lengths. All these parameters refer to 2D roughness measurements. 3D roughness parameters are indicated with a capital S followed by additional characters e.g. Sa is the arithmetic average of the 3D roughness.

Roughness plays an important role when determining how the surface interacts with its environment. Surface roughness is closely connected with the wear process, especially in artificial joints where sliding surfaces are subjected to intensive and repeating loading. There is scientific evidence that the roughness properties of femoral head surfaces in the hip joint replacements are one of the most important factors that control the wear rate and wear mechanism of polyethylene cups, and determine the polyethylene particles size and volume [43, 59, 80, 112, 134, 157]. Wear of the polyethylene cup was increased in the prostheses which had increased roughness of the femoral head [19, 39, 59, 94, 122, 162]. It was shown that increasing the counterface roughness increased wear volume and also the number of particles produced [116]. Polyethylene wear is proportional to the average surface roughness (*Ra*) of the femoral head [39]. An increase in surface roughness of the femoral head from 0.05 to 0.07 μm would increase polyethylene wear approximately 50%. If the *Ra* increased to 0.17 μm, the wear rate would increase by more than 300% [32]. The femoral head roughness is especially important for more active people [19].

Deep scratches on the femoral head surface play an important role in the wear mechanism of the polyethylene cups. The scratching of the femoral head is currently thought to be a major factor affecting the wear rate of the polyethylene counterface [122]. It has been shown that polyethylene wear particles produced by scratched femoral heads tended to have an increased size and more irregular elongated morphologies [72].

The studies on the pin-on-plate wear simulator showed that scratched counterface contribute to the generation of large polyethylene particles [48].

The polyethylene cup can also have varying roughness, but it does not greatly influence the wear process of implant materials. The asperities of the polyethylene can be worn out by the harder implant surfaces.

Roughness changes during the use of implants. Materials that are less wear resistant reveal increased roughness and generate more wear particles in vivo. High roughness of the femoral head in the artificial joints intensify wear of the softer polyethylene material generating more and more polyethylene wear particles, which evoke inflammatory reaction in the periprosthetic tissue. Therefore, examination of the roughness properties of the femoral head is very important in total hip replacements.

2.4 Wear particles

2.4.1 Methods of isolation and characterization of wear particles

Wear particles are mostly located in periprosthetic tissue. During revision surgery samples of this tissue are removed and can be used for scientific analysis. The tissue samples can be used fresh or frozen. The particles can also be isolated from the tissue samples that are embedded in the paraffin and then sectioned [106, 169], but this method is less common. The first stage in wear particles isolation from the fresh or frozen tissue samples is usually lipid extraction by immersion of the tissue samples in a solution of chloroform and methanol. The wear particles are inorganic and have to be separated from the organic tissue. Several methods of tissue digestion have been reported for the recovery of wear particles from the periprosthetic tissue samples. Strong acids (nitric acid) [106], strong alkali (KOH, NaOH) [1, 23, 93, 117] or enzymes (papain, collagenase, protease) [25, 26] with different time exposure and different temperature of digestion are used to destroy organic material and liberate particles from tissue without etching the wear debris. The solution with wear particles is later centrifuged with a density gradient, usually sucrose. The differences in the density of examined materials are used for their separation. The high density of metal, ceramic, and bone cement particles facilitate their recovery

from the tissue samples. During the centrifugation, they drop at the bottom of the isolation tube. The polyethylene particles that have a density less than that of water (0.9-0.94 g/cm^3) [23], form a white band at the top of the sucrose. Common protocols involve filtration of the digest through filter membrane with a pore size of 0.2 μm, rarely 0.05 μm.

Characterization of the particles can be conducted using the particles counter or scanning electron microscope (SEM). Before SEM analysis, all filters have to be gold or carbon coated by the sputter coater. The SEM pictures can be analyzed by the special graphic program. The size of particles can be presented as area occupied by the particles, the diameter of particles or equivalent circle diameter of particles (ECD), which is defined as a diameter of a circle with area equivalent to the area of the particle. It has the units of length.

The composition of particles can be identified by energy X-ray dispersive spectroscopy (EDS) or by the Fourier transform infrared spectroscopy (FTIR).

2.4.2 Wear particles of different material and their size distribution

Every material that can be used for the manufacture of implant wears out producing wear debris. The wear rate depends on the wear resistance of the material. The higher the wear resistance, the slower the material wears out, producing smaller amount of wear particles. The wide distribution in the size of the wear debris is truly remarkable. However, the majority of particles are very small, which makes them biologically active.

When polyethylene is used as an articulating surface its wear particles dominate in the fibrous tissue (Fig. 2.4.1.). The size of the polyethylene wear particles can range from between 0.1 μm to 100 μm, but 90% of them are smaller than 1 μm [15, 17, 19, 91, 103, 146, 157]. Occasionally, massive polyethylene fibers and flakes (in size bigger than 10 μm) abound in the interfacial membrane, especially in the total knee replacements, but they are rather seldom. Polyethylene particles can be identified in human tissue by polarized light or by tissue staining, using the oil-o-red methods.

Metal particles dye the fibrous tissue into black colour, which can be visible as intensive metallosis (Fig. 2.4.2.). Metal particles can be very small; their size is measured in nanometers. Metal particles can cause apoptosis of periprosthetic tissue cells [148].

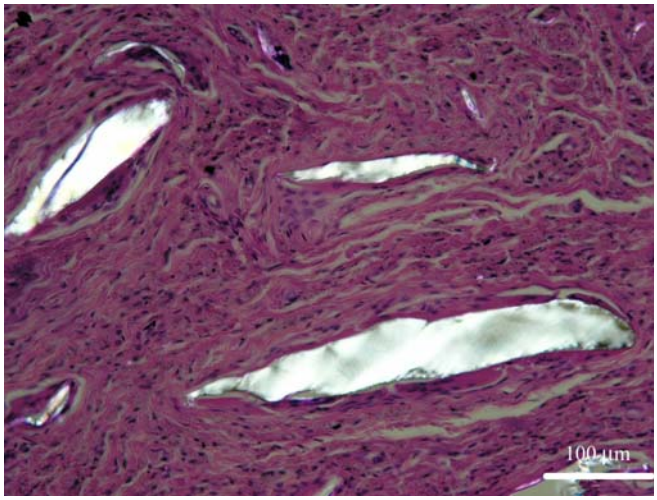


Fig. 2.4.1. Polyethylene particles in fibrous tissue (x 200), (under polarized light).

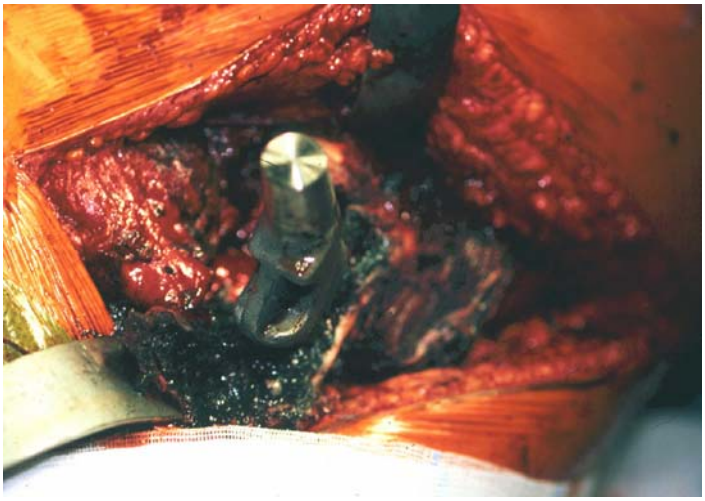


Fig. 2.4.2. Dark tissue around a metal prosthesis.

Polymethylmethacrylate is a very brittle material. When cementation technique is poor, or the fatigue of cement mantle occurs, the cement material crumbles very intensively producing wear particles. Cement particles are the biggest, with mean size of around 100 µm. They can be identified on the basis of the presence of radiopaque agents $BaSO_4$ or ZrO_2.

Wear particles of ceramic materials are rare. The ceramics are very hard materials with significant wear resistance, so the wear process on the ceramic surfaces is almost negligible.

2.4.3 Wear particles migration

Wear particles arise mostly at the articulating surfaces of the artificial joint when two surfaces rub against each other. The biggest concentration of wear particles is found in the pseudosynovial fluid in the joint cavity. The suspended particles can migrate significantly with this fluid. They can be found at the tip of femoral stem or at the dome of the acetabulum. This penetration is possible due to the effective joint space at the interface between bone and implant, which is accessible to pseudosynovial fluid and thus accessible to wear debris.

A huge amount of wear debris is deposited in the periposthetic tissue surrounding implant, where they are phagocytosed by the macrophages or surrounded by giant cells. They evoke strong inflammatory reactions in the tissue leading to the implant loosening.

Massin et al. [107] showed that particles are also able to migrate through cancellous bone due to the three-dimensional microarchitecture of trabecular bone.

Wear particles can be distributed throughout the body by the lymphatic vessels [9]. They can be found in distance organs such as the liver, the spleen, the lungs and the lymph nodes [165]. Approximately 40% of patients with artificial joints are reported to have particles disseminated to the liver or spleen [60].

2.5 Biological response of the periprosthetic tissue to wear particles

In artificial joints wear particles are always present. The intensive wear process is caused by very high loads to which artificial joints are exposed. Each step taken by the patient with a hip implant generates around 40000 particles [58]. Such a large amount of foreign particles cannot remain in the body without causing any effect. The biological reactions to wear debris were first studied by John Charnley in the 1960s. Sir Charnley

suspected that wear particles would cause an inflammatory reaction. He decided to implant them into his own, healthy thigh. After few weeks he obtained painful confirmation of his theory [164]. Later, only bone cement particles were suspected of causing inflammatory reactions and unsuccessful implantations. In 1987, Jones and Hungerford coined the term "cement disease" to describe osteolysis associated with aseptic loosening around cemented total joint replacements [82]. This phenomenon convinced the surgeons to introduce cementless prosthesis. Those implants were based on the press-fit fixation method. Unfortunately, the number of the unsuccessful implants did not decrease significantly. Wear debris was also found in the cementless prostheses, therefore the "cement disease" turned into "particle disease". The inflammatory biological reaction of the periprosthetic tissue is present when there is contact with wear particles of any material used in arthroplasty [153] and is often the cause of aseptic loosening. Aseptic loosening is the implant loosening without any signs of infection, the presence of a periprosthetic inflammatory fibrous tissue and possible osteolysis on the bone-implant interface.

2.5.1 Fibrous tissue

The bone-implant interface is a very dynamic layer. After the implantation, connective tissue creates healing tissue around prosthesis. Mechanical and biological factors influence the quality of this layer. Huge micromotion can contribute to an intensive fibrosis between implant and bone tissue. Additionally, the biological response to wear particles enhances this process, limiting the possibility of bone-implant integration. In well-fixed implants, where the micromotion is low (30-50 μm), the healing tissue can be converted into mature bone and the implant and bone remain in direct contact.

Fibrous tissue had histological and histochemical characteristics of synovium. The inflammatory fibrous tissue can secrete many mediators and enzymes, which can influence bone resorption around the implant. Histological analysis of a fibrous tissue layer showed a number of macrophages, fibroblasts and some lymphocytes.

A macrophage is a type of white blood cell (12-20 μm in diameter) with a monocyte origin. After the entrance of monocytes into the inflamed tissue, they undergo several changes and differentiate into macrophages. They grow and increase the amount of intracellular lysosome, allowing for better phagocytosis. The main function of the

macrophages is the elimination of foreign substances. They engulf foreign bodies, pathogens as well as dead cells. Macrophages produce various cellular mediators and enzymes during inflammation.

A fibroblast is a mesodermally derived cell of connective tissue. Fibroblasts secrete all components of the extracellular matrix (fibrillar procollagen, fibronectin, glycosaminoglycans, reticular and elastic fibers and glycoproteins). Active fibroblasts are characterized by the abundant rough endoplasmic reticulum. Inactive fibroblasts, also called fibrocytes are smaller and spindle shaped. They have a reduced rough endoplasmic reticulum. Tissue damage causes activation of the fibrocytes, which become fibroblast and start to multiply. Fibroblasts are reparative cells, which become active in the periposthetic tissues [153]. Fibroblasts can differentiate into other cell types.

A lymphocyte (6-8 μm in diameter) is a type of white blood cell (agranulocytic leukocyte). There are two types of lymphocytes, namely T cells and B cells. Antigen appearance activates B-cells, which can transform into plasma cells. Plasma cells have large nuclei and produce antibodies.

2.5.2 Tissue reaction to wear debris

Wear particles in the fibrous tissue are recognized by the immune system as an antigen (any substance that stimulates the production of antibodies and elicits an immune response by the organism). This evokes a chronic inflammatory foreign body reaction. The immune system manages to protect the human body from organic antigens. Wear particles in artificial joints are inorganic, indigestible chemical components like polyethylene, which poses a problem during inflammatory reaction.

The presence of antigens in the tissue results in the release of histamine by immune cells. Histamine is a multifunctional substance, which induces vasodilatation (the spreading blood vessels). Amplified blood flow and activated endothelium cells in the vessels walls (increased permeability of blood vessel walls) contribute to intensive migration of monocytes from the peripheral blood to the inflamed tissue. Monocytes differentiate into macrophages in the inflammatory region. Macrophages are able to phagocytosize foreign bodies, including wear debris thus evoking an inflammatory reaction. Macrophages are rich in digestive enzymes capable of breaking down various

organic molecules. Macrophages are the key cell types in biological response to wear debris (Fig. 2.5.1.). There are about twenty five times more macrophages around loose implants compared to the bone surrounding well-fixed implants, since continuous wear requires a constant supply of phagocytes [85]. In the inflamed fibrous tissue there can be more than 4000 macrophages per mm^2 tissue [7].

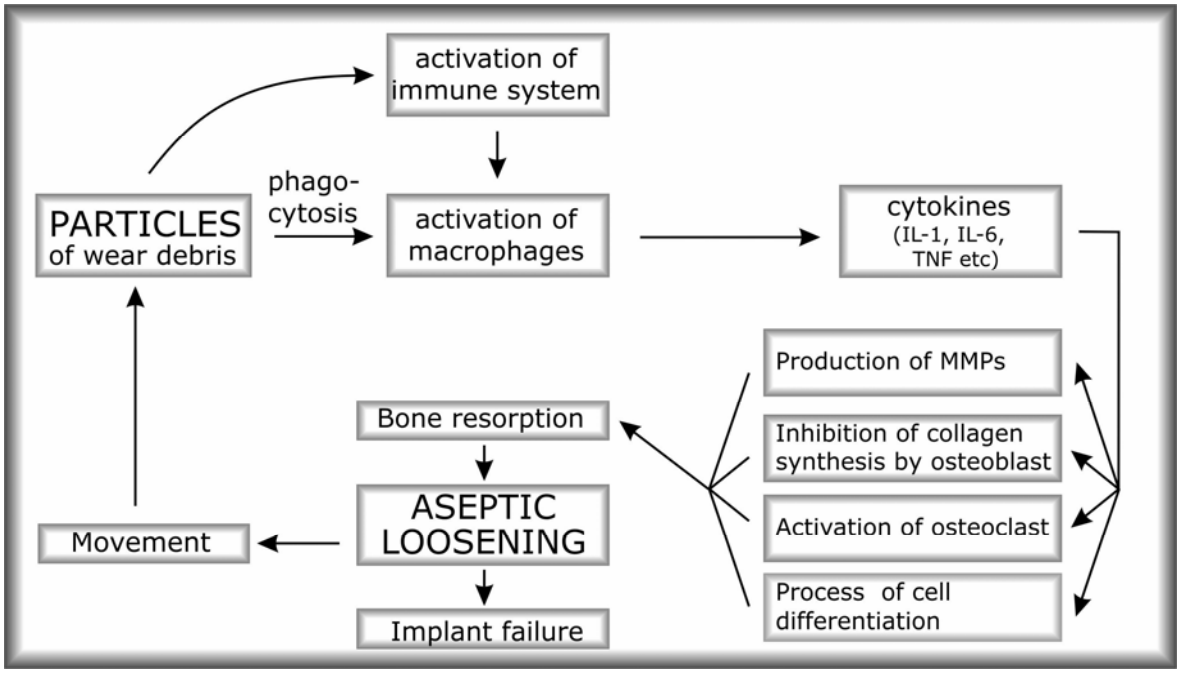


Fig. 2.5.1. Biological response to the wear particles from implant materials in arthroplasty [45].

2.5.3 Phagocytosis

Phagocythosis is a process of engulfing and digestion of a foreign body by the white blood cells (phago = "eating", cyte = "cell"). Phagocytosis begins by the nonspecific binding of particulate wear debris to the receptors on the cell surface [145]. During phagocytosis, the cellular membrane of macrophage invaginates creating a phagosome. Phagosome and lysosome fuse to form phagolysosome. Lysosome contains a large amount of different degradative enzymes for example acid hydrolases, Dnase (deoxyribonuclease), RNase (ribonuclease), proteases, phosphatases and lipases – enzymes that break larger molecules down to their respective subunits. Lysosoms have acidic pH values, which contribute to the effective action of enzymes.

Phagolysosome formation causes oxygen burst. Stored sugar is used to produce nicotinamide adenine dinucleotide phosphate-oxidase (NADPH), which is an electron donor for oxygen. During the reaction of the NADPH oxidase, toxic and reactive oxygen species (ROS) such as peroxide (H_2O_2) and superoxide (O_2^-) are released. All reactive oxygen species can cause extensive chemical damage to contents of the phagolysosomes. Lysosomal enzymes and ROS easily destroy the organic antibodies. Unfortunately, wear particles cannot be digested and are removed from the macrophages without any changes. They can be phagocytized by other macrophages. The presence of nondegradable particles results in a constant state of activation of all cell types present in fibrous tissue.

2.5.4 Cytokines

Once they phagocytozed wear debris, macrophages become activated to release many chemical mediators with inflammatory and degradative action, in particular the cytokines. Cytokines (citos = “cell”, kinesia = “movement”) are small (in the range of 5-20 kDa) and soluble glycoproteins (complex proteins, which contain molecules of carbohydrates). Cytokines often act locally in very low concentration (of 10^{-12} M). The most important role of these molecules is communication among the immune system cells. Cytokines are inflammatory mediators. Cytokines work by bonding to their cell-specific cytokine receptor. Cytokines modulate growth, proliferation and activation of

different cell types. Cytokines operate as a network in which every component controls and regulates the other [90]. The net effect of cytokines is the result of manner of interactions between them, such as redundancy, pleiotropy, synergism and antagonism. Redundancy is the ability of different cytokines to induce the same response from the target cell. Pleiotropy means multiple biological actions. The same cytokine can cause different effects under different circumstances [128]. Synergism is an exaggerated effect of two cytokines, which is greater than the sum of their individual effects. Antagonism is the ability of certain cytokines to cancel the effect of other cytokines.

Cytokines can be divided into 5 subgroups: interleukins, chemokines, the tumor necrosis factors family (TNF), interferons (IFN) and hematopoietic cytokines.

Interleukins are soluble proteins, which are involved in processes of cell activation, cell differentiation, proliferation, and cell-to-cell interactions. The main role of chemokines is to control the chemotaxis, by signalling different cells to move in a specific direction. The cells direct their movements according to the gradient of chemokine concentration. The tumor necrosis factors family limits tumor development and is very important during inflammation. Interferons are proteins, which have a virus-unspecific antiviral activity. Virus-infected cells secrete interferons to protect other non-infected cells against infection. The antiviral activity demands synthesis of RNA and specific proteins and can be limited by the specific inhibitors. Interferons are also involved in antiproliferative and immunomodulating activities. They can also modulate the metabolism, growth and cell differentiation. Hematopoietic cytokines act on cells of the hematopoietic system (hematopoiesis is used to describe blood formation).

Cytokines play a very important role in the aseptic loosening of orthopaedic implants. Based on cytokines function they can be divided into proinflammatory (which cause bone resorption) and antiinflammatory (which contribute to bone formation) cytokines. Around a failed implant, the secretion of proinflammatory cytokines is enhanced. The production of antiinflammatory cytokines is too small to restore the balance. The equilibrium in the periprosthetic tissue is upset and the chronic inflammatory process can be observed. The most important proinflammatory cytokines in implant failure are IL-1, IL-6 and TNF [129]. All of these are involved in bone resorption. They influence the osteoclasts and can indirectly induce synthesis of other cytokines, which contribute to bone resorption.

2.5.5 The most important cytokines in biological response to wear debris.

Interleukin-1

Interleukin-1 (IL-1) affects nearly every cell type in the organism. IL-1 can be secreted by macrophages, monocytes, dendritic cells and others. Interleukin-1 has two types: IL-1 alpha and IL-1 beta. IL-1α is associated with the cell membrane and acts via cellular contact. IL-1β is the prototypical proinflammatory cytokine [130]. The generation of IL-1 can be influenced by other cytokines including TNF-alpha, IFN and by bacterial endotoxins, viruses and other antigens.

IL-1 has a wide range of biological and physiological effects, including evoking fever, T-lymphocyte activation, synthesis of prostaglandin and other cytokines. IL-1 is one of the most important mediators of inflammatory reactions [35] and is associated with bone remodelling. IL-1 stimulates osteoclastic bone resorption by enhancing osteoclast operation [54]. It increases osteoclast survival by blocking apoptosis [81]. IL-1 can also stimulate activation of mature osteoclasts indirectly, through other cells [90]. The production of IL-1α in the periprosthetic tissue of loose implants is positively related to osteolysis around them [149]. IL-1 can also induce the expression of matrix metaloproteinases [88], especially collagenase and gelatinase [95], which degrade the extracellular matrix. Interleukin-1 contributes to osteolysis by the activation the of osteoclast and by the inhibition of the osteoblasts responsible for new bone tissue deposition.

Interleukin-6

Interleukin-6 can be secreted by many cell types but mainly by macrophages, fibroblasts, osteoclasts and endothelial cells. Interleukin-6 may be considered a prototypic pleiotrophic cytokine [8]. IL-6 has overlapping activity functions with IL-1 and TNF. The synthesis of IL-6 can be enhanced by interferon, TNF-alpha and viral infections. Interleukin-6 plays a very important role during the inflammatory process around loosened implants inducing the osteoclast formation [155]. IL-6 influences the differentiation of lymphocytes B into plasma cells and antibody secretion. IL-6 also contributes to extensive matrix metaloproteinases production [95]. It has been described as both a pro-

inflammatory and an anti-inflammatory molecule [8]. The IL-6 gene is directly regulated by sex steroids (estrogen and androgen protect skeleton, because they inhibit IL-6 production and bone degradation this way) [105].

Tumor necrosis factor

Tumor necrosis factor (TNF) is produced by many cells as macrophages, monocytes, neutrophils, T-cells and also by tumor cells. TNF can be generated in response to a wide variety of stimuli, including viruses, bacteria, parasites, cytokines and foreign antigens. TNF influences growth, differentiation and many functions of healthy and tumor cells. TNF-alpha is a growth factor for normal cells and for tumor cell it has necrotizing effect. TNF is an extremely pleiotrophic cytokine, activating multiple signal transduction pathways. TNF is a proinflammatory cytokine that provides a rapid form of host defence against infection. TNF induces the synthesis of IL-1 and prostaglandin E2. It also stimulates phagocytosis and the synthesis of superoxide dismutase in macrophages.

TNF takes part in bone remodelling. TNF stimulates differentiation of osteoclasts, but not their activation [163]. The addition of an anti-TNF antibody inhibits bone resorption by supernatants from particle-stimulated macrophages [3]. Osteoblasts under the influence of IL-1 produce TNF. TNF is one of the most important cytokine involved in wear particles induced osteolysis [76].

The influence of each cytokine should be considered as a part of the vast network of interacting mediators.

2.5.6 The role of cytokines in bone resorption

Normally, bone mass is controlled by the delicate balance between formation and bone resorption. Both processes are governed by mechanical signals, which operate in conjunction with local and systemic factors. Osteoblastic cells of mesenchymal orgin synthesize bone matrix and are entrapped in the bone tissue as osteocytes. Osteoclastic cells are large, multinucleated phagocytes of hematopoietic origin that resorb bone. During the aseptic loosening, the equilibrium is upset and the osteolysis is observed. Cytokines are the most important mediators in developing osteolysis around loosened implants. The wear

particles that are present in every artificial joint evoke bone resorption (dissolution of the mineral phase and matrix degradation) by induction cytokine synthesis. Cytokines can contribute to bone resorption in a direct or an indirect way. The most important are: induction of matrix metaloproteinase synthesis, inhibition of new bone formation, osteoclast activation and osteoclast differentiation.

Matrix Metaloproteinases

Matrix metaloproteinases (MMPs) are enzymes, zinc and calcium dependent endopeptidases. They control physiological and pathological connective tissue remodelling, wound healing, inflammation etc. MMPs are able to break down any extracellular matrix component [154]. There are twenty three known human metaloproteinases [120]. Matrix metaloproteinases can be divided into 4 subgroups: collagenases (MMP-1, MMP-8, MMP-13 and MMP-18), gelatinases (MMP-2 and MMP-9), stromelysins (MMP-3 and MMP-10) and membrane-type metaloproteinases (MMPs-14-17) [109].

MMP are secreted as inactive forms, zymogens (proMMPs). The zymogens are activated by other enzymes (proteinases). The activity of MMP is strictly regulated at several levels, such as the activation of proenzymes or the inhibition of active enzymes by tissue inhibitor of metaloproteinases (TIMP-1, -2, -3, and -4).

The matrix metaloproteinase activation can be enhanced by the inflammatory cytokines. MMPs show the first signs of inflammatory response to wear debris [151] and they are involved in bone resorption. Wear particles of orthopaedic biomaterials induce the release of matrix metaloproteinase by activated macrophages [123]. Osteoblasts and osteoclasts also produce MMP-13 (collagenase 3), MMP-2 and MMP-9 (gelatinases A and B) [98, 131].

Matrix metaloproteinases can degrade the connective tissue and the nonmineralized osteoid of the bone tissue, which exposes mineralized bone to osteoclasts. Imbalance between the levels of MMPs (overproduction in response to inflammatory cytokine) and their inhibitors is one of the most important causes of total joint failure. The degradation of bone matrix without a compensatory increase in bone matrix synthesis results in net bone loss.

Inhibition of new bone formation

Normal bone formation is essential for implant osseointegration. Disruption of osteoblast function could be important in implant loosening. Proinflammatory cytokines are able to inhibit new bone formation reducing the osteoblasts activity. Osteoblasts are responsible for synthesis and secretion of the organic matrix of bone, including type I collagen (a protein that constitutes 90% of the bone matrix). Exposure of osteoblast to TNFα and IL-1β and other cytokines results in suppressed proliferation of osteoblasts [167], suppressed procollagen mRNA expression and subsequently can lead to reduction of type I collagen synthesis [110], which is crucial for formation of osteoid. Wear particles can interact directly with osteoblasts, limiting synthesis of procollagen [133]. In the bone-implant interface the equilibrium between new bone formation and bone resorption is unsteady. Osteoblasts cannot compensate for bone loss.

Direct influence on osteoclasts

The main function of osteoclasts is bone resorption. Osteoclast are multi-nucleated (3-20 nuclei), large (20-100 μm in diameter) cells. Osteoclasts arise after the fusion of bone marrow-derived mononuclear phagocyte precursors.

Many cytokines released in response to wear debris stimulate the survival of osteoclasts, prevent osteoclast apoptosis [81] and contribute to their activation. IL-1 acts on osteoclast directly [81]. Cytokines can also activate osteoblast to stimulate osteoclasts.

Osteoclasts are also able to engulf particles, which results in their activation [172]. They can also acidify subosteoclastic space. At the low pH values bone hydroxyapatite $Ca_{10}(PO_4)_6(OH)_2$ is dissolved and the bone loss occurs.

Differentiation of osteoclasts

Proinflammatory cytokines can cause differentiation of the osteoclast precursors into mature osteoclasts capable of bone resorption. Macrophages can also be able to resorb bone directly by the release of oxide radicals and hydrogen peroxide [58]. Macrophages associated with wear particles are also capable of differentiation into multinucleated cells, into osteoclast-like cells, that have capacity to carry out extensive bone resorption [128, 135, 136]. In vivo studies have shown that the number of osteoclast is increased (twenty times or more) in tissue around a loose implant when compared with bone surface in a well-fixed implant [85].

Wear particles are responsible for the development of osteolysis at the bone-implant interface. Wear debris induces the release of cytokines thus negatively affecting bone turnover. The osteolysis at the bone-implant interface causes a loss of implant support. The implant becomes loose, which leads to serious failure. During the revision surgery, removal of the loose prosthesis and re-implantation of a new prosthesis takes place. Unfortunately, the results of revision arthroplasty are never as good as the primary arthroplasty. Bone tissue after implant loosening is not well vascularized and has a lot of fibrous scar tissue and may contain wear particles, so it is substantially weakened. Therefore, it is very important to limit the possibility of primary implant loosening.

2.6 Summary

The scientific researches concerning aseptic loosening of artificial joints concentrate on three aspects:

- mechanical and biomaterial aspects influencing wear process,
- wear particles analysis,
- biological response to implant and wear particles.

Mechanical and biomaterial aspects influencing wear of orthopaedic implants and also quality of materials and susceptibility to wear are examined on:

- pin-on-disc or pin-on-plate simulators (by Dong et al [36], Święszkowski et al. [153], Turell et al. [162]),
- joint simulators (by Bowsher et al. [19], Elfic et al. [43], Tipper et al. [158]),
- retrieved implants (by Hall et al [59], Kusaba et al. [94], Najjar et al. [122], Tipper et al. [157]).

Each of these methods gives other information. Pin-on-disc or pin-on-plate simulators examine only the material qualities. Joint simulators have similar forces distribution as in natural joint and allow excluding the influence of other aspects on wear process. While the investigations on retrieved implants give the opportunity to identify the conditions contributing to wear in natural joints. All of these researches confirmed the influence of implant surfaces quality on friction and wear of implant components.

Wear particles generated due to intensive wear process appearing in artificial joints are object of many studies. Number of particles, morphology of particles, size distribution of wear particles and other features are investigated on particles:

- generated on pin-on-disc or pin-on-plate simulators (by Affatato et al. [1], Besong et al. [17] and others [48, 57]),
- generated on joint simulators (by Besong et al. [17], Kowandy et al. [93], Tipper et al. [158] and others [43, 137]),
- isolated from human tissues (by Campbell et al. [23, 25, 26], Kobayashi et al. [87], Maloney et al. [103], Shanbhag et al. [146], Tipper et al. [157, 160] and others [72, 91, 96,169]).

Most of studies confirmed that majority of wear particles are smaller than 1 μm, however there is no agreement about size distribution of particles. Some researches overestimate big particles fraction (above micron) [96], while others underestimate particles with submicron size range [74].

Wear particles themselves are not harmful, but the reaction they cause is very harmful leading to implant loosening. The biological reaction of wear particles concentrate on:

- in vitro studies (by Green et al. [55], Konttinen et al. [89, 90], Shanbhag et al. [145, 146] and others [119, 123]),
- animal studies (by Goodman et al. [53], Kadoya et al. [84], Ohashi et al. [127]),
- retrieved human tissue studies (by Maloney et al. [104], Revel et al. [132], Kadoya et al. [84] and others [10, 37, 61, 64, 153]).

In vitro studies give the opportunity to investigate separately the impact of wear particles excluding other factors. Animal studies examine influence of wear particles of specified size, material and concentration on animal tissues. The examination of wear particles isolated from human tissue allows evaluating the influence of particles on periprosthetic tissues. All these studies confirmed the potential of wear particles to evoke inflammatory reaction. However, still many aspects of biological response are not clear.

The literature review confirmed complicity of process of artificial joint loosening. The studies usually concentrate on one of aspects leading to implant failure, while considering all above mention aspect give the opportunity to precisely describe the process of aseptic loosening.

3 The aim and range of the study

The literature review has shown that the process of aseptic loosening in artificial joints is very complex. The mechanics of friction, wear of materials, differences in deformation ability of the materials, structure of the surface layers, differences in roughness together with many other factors act synergistically and can cause instability of a joint replacement and subsequent need for a revision surgery.

The results of this work ought to help to estimate the influence of all of the above-mentioned factors on the bone-implant tissue integration process. The algorithms of this integration are significant in the explanation of the mechanical and the biological processes taking place in the complicated structures of artificial joints.

Enlightenment with regards to the biophysical processes present in the artificial joint may also play a utilitarian role in the healing efficacy and in the extending the survival rate of the implants within the human body, optimization of alloplasty, as well as in the elaborating new solutions for the arthroplasty.

The disintegration of the periprosthetic tissue is very important issue in the contemporary implantology.

The main scientific problem of this study is the description of the mechanisms proceeding around orthopaedic implants and contributing to implant loosening.

Author, having wide group of homogenous implants, undertook histological, biological as well as wear analysis, which determine the osteointegration of implant with bone tissue.

The elucidation of this scientific problem demands the realization of the following objectives:

a) The characterization of the mechanical properties of the implant materials

The mechanical properties of the materials used in artificial joints have a significant influence on the wear process. A considerable amount of data in the literature confirms that surface roughness determines the wear process in orthopaedic prostheses. The aim of this study will be the tribological analysis of the cooperating surfaces of the hip implant by 2D and 3D methods to estimate the wear process. The analysis by the scanning electron microscopy will be used to determine the traces of the wear process on the surfaces of loosened femoral heads and loosened acetabular components. Detailed examination of the material structure of the ceramic femoral heads will provide information about the pore and grain size.

The subjects of this study are forty-three loosened hip prostheses implanted with the use of bone cement. Only cemented implants are taken into account because they comprise an important percentage of all hip implants. Bone cement has Young moduli very similar to bone tissue, which limits the stress shielding and contributes to better osteointegration. Progressive improvements in the cementation techniques provided very good results, which are reflected in the statistics of the implant survival rate. All examined implants have a femoral stem made of titanium alloy (Ti6Al4V) and the acetabular cup made of ultra high molecular weight polyethylene (UHMWPE). The prostheses differ in the material of the femoral head. They are made either of ceramic material (Al_2O_3) or metal (Ti6Al4V or stainless steel). All the examined implants were revised due to aseptic loosening.

b) The characterization of wear particles

Campbell et al. [24], Tipper et al. [157], Howie et al. [70, 71] along with other studies have shown that one of the most important causes of the aseptic loosening of orthopaedic implants are wear particles. Wear particles are generated primarily between the frictional surfaces of the artificial joint. They can be suspended in the pseudosynovial fluid and with this fluid they move to the periprosthetic tissues, where they evoke an inflammatory reaction. The aim of this study will be focused on the material and structural characterisation of wear particles, which determine the aseptic loosening.

The wear particles will be isolated from the fibrous periprosthetic tissues surrounding the loosened implants. The material analysis will be conducted using the scanning electron microscopy (SEM) and the energy dispersive X-ray spectroscopy (EDS). The size distribution of the wear particles will be examined with two methods, by the laser particle analyser and on the basis of the scanning microscopy pictures.

c) The histological analysis of the periprosthetic tissues

The formation of fibrous tissue around loosened orthopaedic implants is the subject of many scientific studies [7, 50]. The fibrous tissue obstructs the osteointegration of the implant or cement mantle with the bone tissue and is an undesirable aspect of the arthroplasty. The acute and chronic inflammatory reaction in the fibrous tissue causes the osteolysis and subsequently leads to the implant loosening.

Histological analysis of the periprosthetic tissues will reveal the information about the type of the inflammatory reaction (quality of the dominated cells, presence of the wear particles etc.).

d) The characterization of the biological reaction proceeding at the bone-implant interface

The in vitro studies of the influence of the wear particles on the cell cultures give the opportunities to anticipate the effect of these particles on the periprosthetic tissues in the loosened orthopaedic implants.

The in vitro studies will be carried out on the peripheral blood leucocytes and synoviocytes cell cultures, which will be subjected to the polyethylene wear particles. The influence of the wear particles will be examined on the basis of the activity of proinflammatory cytokines and activity of the matrix metaloproteinase's in the conditioned media from the tissue cultures. The experiments should explain the impact of the wear particles on different kinds of cells in respect of the release of the mediators of the inflammatory reaction.

e) Hypothetic sequences of the loosening process of artificial joint

The intention of this study is to explain of the process of the artificial joint loosening based on mechanical and biological aspects. On the basis of literature review and conducted experiments the hypothetical schema of aseptic loosening will be created.

4 Experiments

4.1 The characteristics of materials used for femoral heads and acetabular cups

4.1.1 Material

The subject of this study were forty-three loosened hip implants collected during revision surgeries mainly for aseptic loosening at the Valdoltra Orthopaedic Hospital in Slovenia. The mean survivorship of hip prostheses was 11.3 years (range 3.84 to 18.9). Thirty-two patients were female and eleven were male. The patient's mean age during primary implantation was 61.4 years (range 35.4 to 75.3) (Tab. 4.1.1.).

All the implants had a Műller-style straight stem made of titanium alloy, titanium-aluminium-vanadium (Ti6Al4V) (Lima-Lto, Udine, Italy or Bioimplants, Milan, Italy) that followed a biomechanical self-locking mechanism (Fig. 4.1.1.) Femoral component were cemented using bone cement (polymethylmethacrylate, PMMA). Acetabular cups were made of ultra high molecular weight polyethylene (UHMWPE). Polyethylene cups were either cemented cups (Lima, Milan, Italy and Cremascoli, Milan, Italy) or uncemented isoelastic cups (R. Mathys, Bettlach, Switzerland). Three types of material were used for the femoral heads. There were twenty-one implants with stainless steel femoral heads (AISI 316L) (nine coupled with cemented cup and twelve with uncemented cups). Among the investigated implants, seven prostheses had a titanium alloy femoral heads (Ti6Al4V) (four coupled with cemented and three with uncemented cups). Fifteen retrieved implants had a femoral head made of ceramics (Al_2O_3), all of which were used with cemented acetabular cups. These fifteen hip implants were divided into two groups based on the period of implantation. The first group comprised, six implants implanted from 1986 to 1989 (denoted as "old ceramic"). The second group comprised nine implants implanted from 1990 to 1998 (denoted as "modern ceramic"), (Table 4.1.2.). The diameter of the femoral heads in all groups was 32 mm.

Table 4.1.1. Details of forty-three patients with loosened total hip replacements (M – male, F – female, R – right, L – left, 316L/cem – stainless steel femoral head group with cemented cup, 316L/uncem – stainless steel femoral head group with uncemented cup, TiAlV/cem – titanium alloy femoral head group with cemented cup, TiAlV/uncem – titanium alloy femoral head group with uncemented cup).

Group name	Implant number	Gender	Side	Age at 1.op	In situ / years	SL stem	Head	Polyethylene cup	Reason for revision
316L/cem	9	M	R	62,18	10,24	Lima	316L	cemented	loosening acetabular part
	40	F	R	67,93	9,67	Lima	316L	cemented	loosening acetabular part
	113	F	R	67,21	8,12	Lima	316L	cemented	loosening femoral part
	339	F	R	66,84	12,20	Lima	316L	cemented	loosening femoral part
	504	F	R	67,43	9,44	Lima	316L	cemented	loosening femoral part
	61	M	R	57,87	14,28	Bioimplants	316L	cemented	osteolysis femoral part
	180	F	L	54,04	16,39	Bioimplants	316L	cemented	loosening acetabular part
	286	M	L	65,29	4,18	Bioimplants	316L	cemented	loosening femoral part
	548	F	R	74,37	6,73	Bioimplants	316L	cemented	loosening both parts
316L/uncem	32	M	L	56,82	8,48	Lima	316L	izoelastic R Mathys	loosening femoral part
	53	M	L	53,95	10,01	Lima	316L	izoelastic R Mathys	loosening femoral part
	121	F	R	71,63	8,25	Lima	316L	izoelastic R Mathys	osteolysis both parts
	122	F	R	60,43	8,76	Lima	316L	izoelastic R Mathys	loosening acetabular part
	184	F	L	64,47	9,71	Lima	316L	izoelastic R Mathys	loosening both parts
	201	F	R	65,98	7,76	Lima	316L	izoelastic R Mathys	loosening femoral part
	472	M	L	61,45	11,22	Lima	316L	izoelastic R Mathys	loosening femoral part
	523	F	R	58,57	11,27	Lima	316L	izoelastic R Mathys	loosening femoral part
	549	F	R	54,14	11,74	Lima	316L	izoelastic R Mathys	loosening femoral part
	106	F	L	63,05	14,68	Bioimplants	316L	izoelastic R Mathys	loosening both parts
	252	F	R	56,13	12,65	Bioimplants	316L	izoelastic R Mathys	loosening femoral part
	484	F	L	54,55	14,32	Bioimplants	316L	izoelastic R Mathys	loosening femoral part
TiAlV/cem	3	F	R	61,72	14,37	Bioimplants	TiAlV	cemented	loosening both parts
	255	F	R	49,77	18,67	Bioimplants	TiAlV	cemented	loosening both parts
	298	F	R	66,43	14,93	Lima	TiAlV	cemented	loosening both parts
	361	F	R	56,35	18,92	Bioimplants	TiAlV	cemented	loosening both parts
TiAlV/uncem	114	F	R	51,28	15,72	Bioimplants	TiAlV	izoelastic R Mathys	loosening acetabular part
	393	M	R	75,34	5,60	Bioimplants	TiAlV	izoelastic R Mathys	loosening both parts
	563	F	R	54,27	15,85	Bioimplants	TiAlV	izoelastic R Mathys	loosening femoral part
Old ceramic	24ok	F	R	68,25	8,80	Cremascoli	ceramic	cemented	instability
	125ok	F	R	65,17	13,94	Cremascoli	ceramic	cemented	loosening both parts
	192	M	L	35,36	17,42	Cremascoli	ceramic	cemented	loosening both parts
	494	F	R	56,28	18,36	Cremascoli	ceramic	cemented	loosening both parts
	84	F	L	65,69	15,50	Cremascoli	ceramic	cemented	loosening acetabular part
	164ok	F	L	55,47	13,88	Cremascoli	ceramic	cemented	loosening both parts
Modern ceramic	15	M	R	56,43	12,00	Cremascoli	ceramic	cemented	loosening both parts
	50	F	L	64,35	11,67	Lima	ceramic	cemented	loosening both parts
	87	F	R	70,75	12,57	Lima	ceramic	cemented	loosening femoral part
	52ok	M	R	63,67	5,21	Cremascoli	ceramic	cemented	loosening femoral part
	79ok	F	L	74,82	3,98	Cremascoli	ceramic	cemented	pain
	241	F	R	59,62	5,59	Lima	ceramic	cemented	loosening both parts
	461	F	L	64,37	9,06	Lima	ceramic	cemented	loosening femoral part
	143ok	M	L	64,69	8,92	Cremascoli	ceramic	cemented	loosening femoral part
	113ok	F	R	55,37	8,99	Cremascoli	ceramic	cemented	loosening both parts

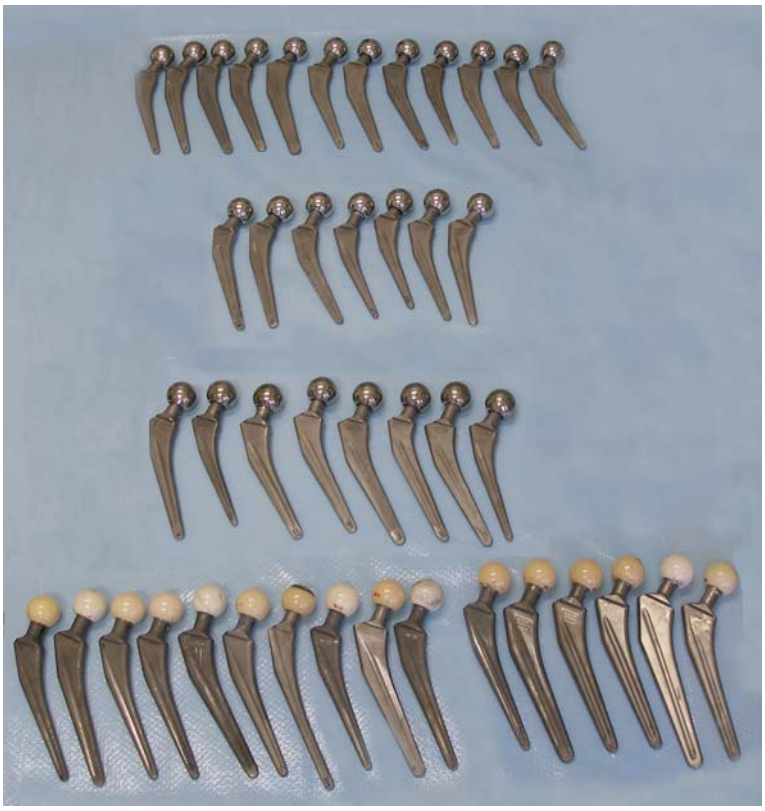


Fig. 4.1.1. The examined hip implants retrieved during revision surgeries mainly for aseptic loosening.

Although there are other types of bearings available on the market (ceramic-ceramic, metal--metal etc.), 80% of hip prostheses still comprise polyethylene acetabular cup coupled with a metal or a ceramic femoral head [47]. In this study, prostheses with stems inserted using bone cement were examined. Fifteen of the studied implants had an uncemented acetabular component, which provided a good opportunity to examine the influence of fixation method on the implant loosening.

4.1.2 Methods

The analysis of wear

Wear traces on the surface of femoral heads and the interior of polyethylene cups were examined using a scanning electron microscope, SEM (Hitachi S-2600) with a magnification of 50 to 2000 x. From each group, one femoral head was analyzed. Prior to SEM analysis, the femoral heads and polyethylene cups were washed in distilled water and dried.

Microstructure of ceramic femoral heads

From each groups of ceramic implants two femoral heads were analyzed in regards to microstructure. The fragments of the femoral heads were grinded down to a size of approximately 0.5 mm and polished with diamond pastes. Scanning electron microscope (Hitachi S-2600) was used to examine the microstructure of the ceramic heads.

Roughness measurements of the femoral heads (2D method)

The surface roughness of the femoral heads was measured on all examined implants with a diamond stylus profilometer (Talysurf Hobson Form Talysurf series 2, Leicester, UK) using a 1 mm evaluation length. The results were expressed as an arithmetic mean of the absolute departures of the roughness profile from the mean line (average roughness of the surface – *Ra*) and total amplitude roughness (the parameter *Rt*). The *Rt* parameter showed the distance between the highest peak and the lowest valley within the measured area and was used to present the imperfections at the surface.

The roughness measurements were carried out at the dome area, unworn area (chosen by the naked eye) and at the worn area located 90º to the dome area, where polishing and scratches were dominant. For each area, at least four measurements were taken and the results were presented as an average value.

The areas of macroscopic damage visible by the naked eye were also measured. Care was taken not to include damage done by surgical instruments. The average

roughness was calculated for the dome, the unworn and the worn areas and for the whole femoral head being the average of all these areas.

Roughness measurements of the femoral heads (3D method)

The 3D roughness measurements were carried out by the scanning profilometer (TalyForm) for one femoral head from each examined group. The roughness was measured at the dome area. The measured area had a size of x=1 mm and y=0.5 mm. The sampling step in the x direction was 1 μm and 5 μm in the y direction. The perpendicular resolution of the measurements was 16 nm. The 0.08 mm cut-off filter was used for the measurements.

The TalyMap Expert 3.1.10 software was used to define 3D roughness parameters. The following roughness parameters were used to describe the examined surfaces (according to the report EUR 15178 EN): Sa – arithmetical mean height of the surface, Sq – root mean square height of the surface, Sp – maximum height of peaks, Sv – maximum height of valleys, Sz – maximum height of the surface, average of five highest and five lowest points of the surface.

Besides the height roughness parameters, hybrid, area and volume parameters were also measured. These parameters describe the geometrical structure of the examined surface in a complex way and are very significant in the tribological problems. The following parameters were assigned: Ssc – mean curvature of the summits and Sdc – area section height difference between 20 and 80%.

Statistics

The data distribution was checked by the Shapiro-Wilk test (STATISTICA, version 6. StatSoft, Inc.). The data was normally distributed, followed Gaussian distribution, so the parametric test, unpaired Student t-test was used to compare results of surfaces roughness. In all tests statistically significance was used judged based on a p value of less than 0.05.

4.1.3 Results

Implants wear analysis

All examined hip prostheses retrieved during revision surgeries revealed intense tribological wear traces. On metal heads (stainless steel and titanium alloy), abrasive wear was observed indicating by numerous multi-directional scratches (Fig. 4.1.2.).

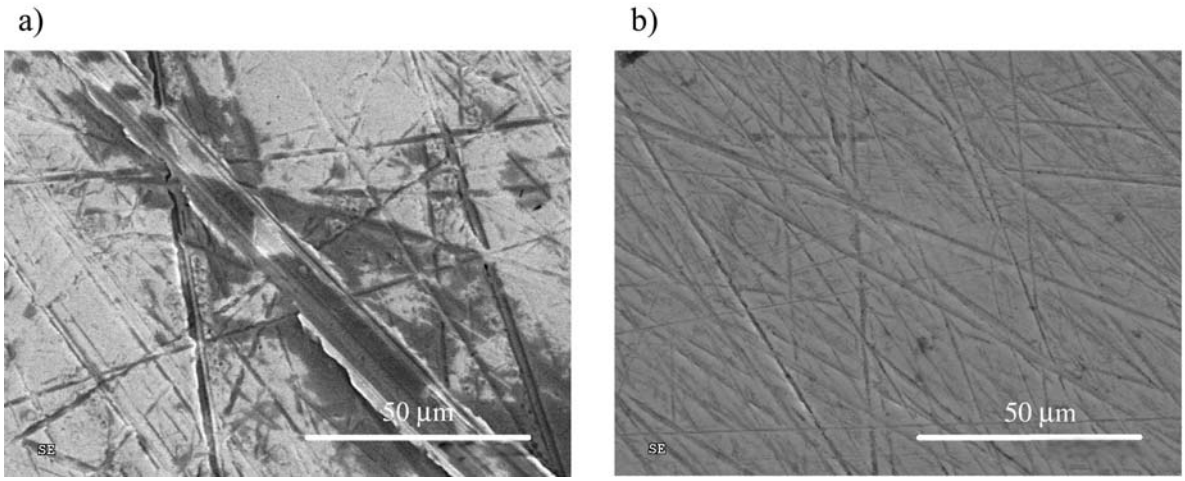


Fig. 4.1.2. SEM picture of tribological worn surfaces of metal heads. Intense multi-directed scratches and grooves were observed a) surface of stainless steel femoral head, b) surface of titanium alloy femoral head, SEM (x 1000).

On the stainless steel femoral heads traces of adhesive and fatigue wear were observed (Fig. 4.1.3.). Ceramic heads from both groups revealed adhesive wear traces of polyethylene material (Fig. 4.1.4.). At the surfaces of ceramic heads pits (or cavities) due to removal of ceramic grains were evident (Fig. 4.1.5.). There were more pits present at the femoral heads form the old ceramic group than from the modern ceramic group.

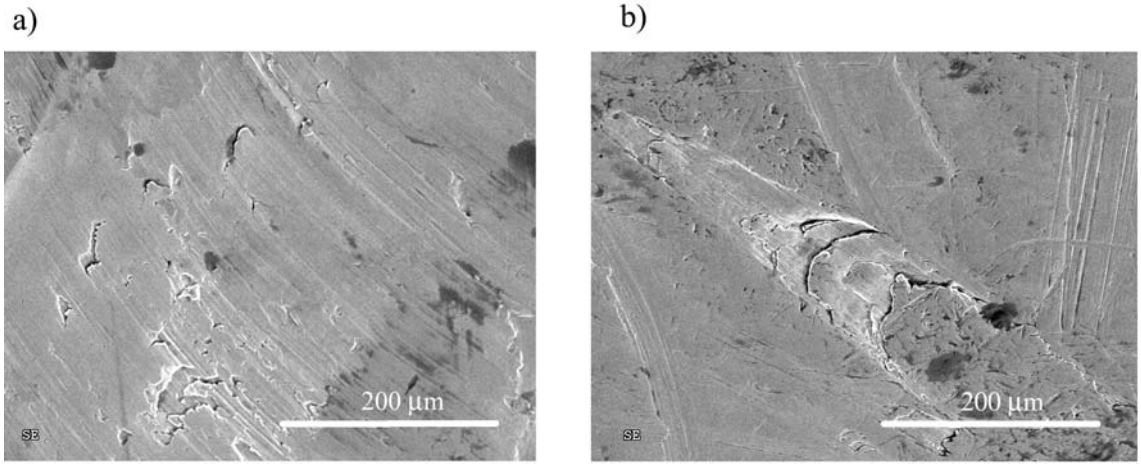


Fig. 4.1.3. Wear traces on the stainless steel femoral head a) adhesive wear b) fatigue wear, SEM (x 250).

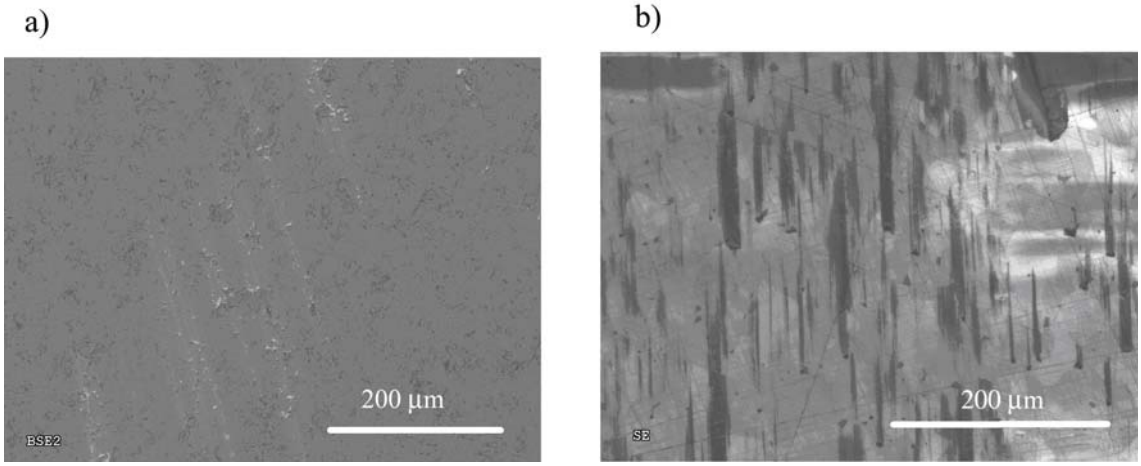


Fig. 4.1.4. Adhesive wear on the ceramic femoral heads a) old ceramic, SEM, (x 200) (backscattered contrast), b) modern ceramic, SEM (x 1000).

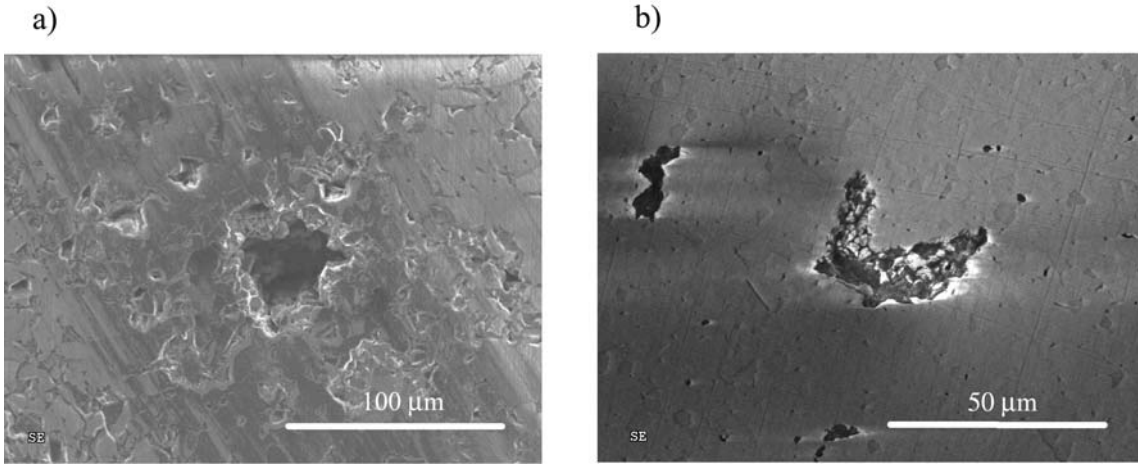


Fig. 4.1.5. Pits at the surfaces of ceramic femoral heads a) old ceramic group, SEM (x 500), b) modern ceramic group, SEM (x 1000).

All examined polyethylene acetabular components showed intensively worn interior (Fig. 4.1.6., Fig. 4.1.7.). SEM analysis revealed different wear modes of polyethylene material. In the interior of polyethylene cups significant traces of abrasive wear were visible – scratches and grooves (Fig. 4.1.7.). The evidence of adhesive and fatigue wear process was also observed (Fig. 4.1.8.). A fibrillar structure was noticed at the interior of the polyethylene cups articulating against ceramic heads (Fig. 4.1.9.).

Fig. 4.1.6. Polyethylene acetabular component retrieved during revision surgery.

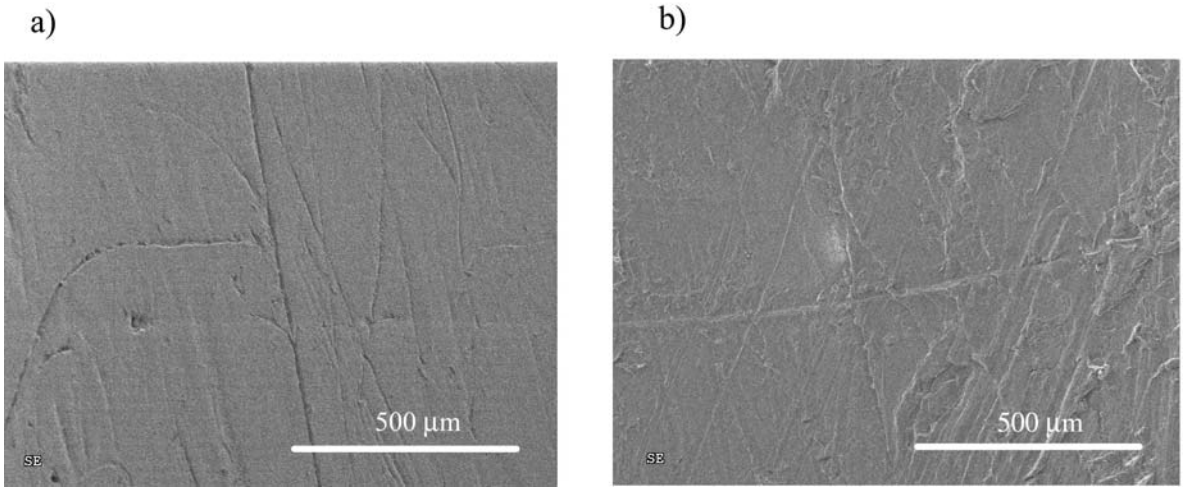


Fig. 4.1.7. The interior surface of polyethylene acetabular component articulating against stainless steel, SEM (x 100).

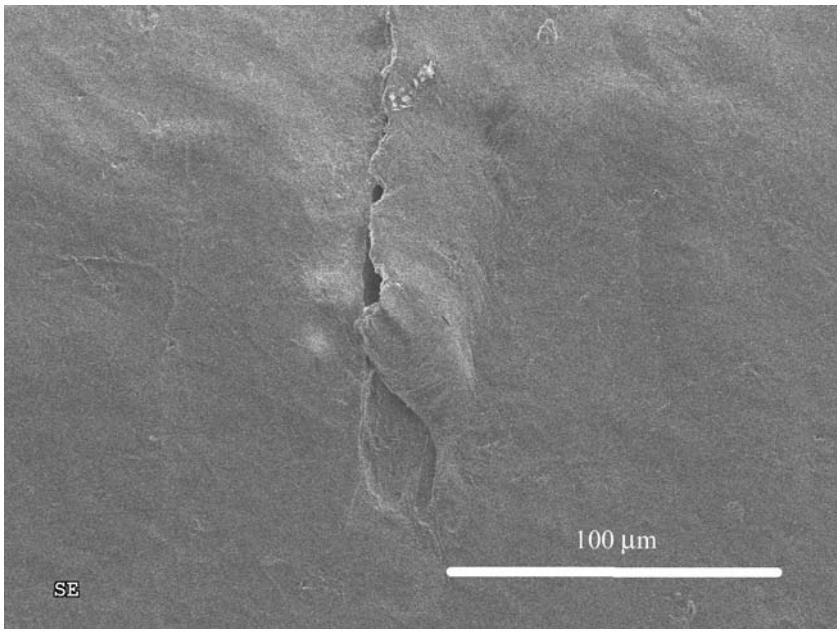


Fig. 4.1.8. Evidence of adhesive and fatigue wear process on the interior surface of polyethylene cup articulating against stainless steel femoral head, SEM (x 100).

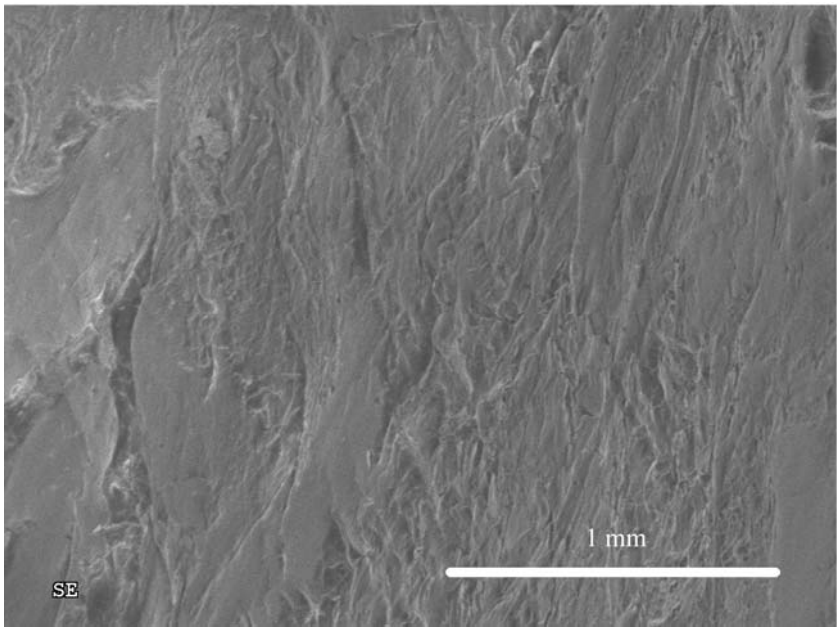


Fig. 4.1.9. Fibrillar structure of the interior of polyethylene cup articulating against ceramic femoral head, SEM (x 50).

Microstructure of ceramic femoral heads

The SEM analysis of polished sections of ceramic heads showed significant differences between the two groups. Femoral heads from the old ceramic group exhibited bigger size of grains and pores (Fig. 4.1.10.) than heads from the modern ceramic group. The differences in the pore size were also visible in the SEM images taken at the femoral heads which were not polished (Fig. 4.1.11.).

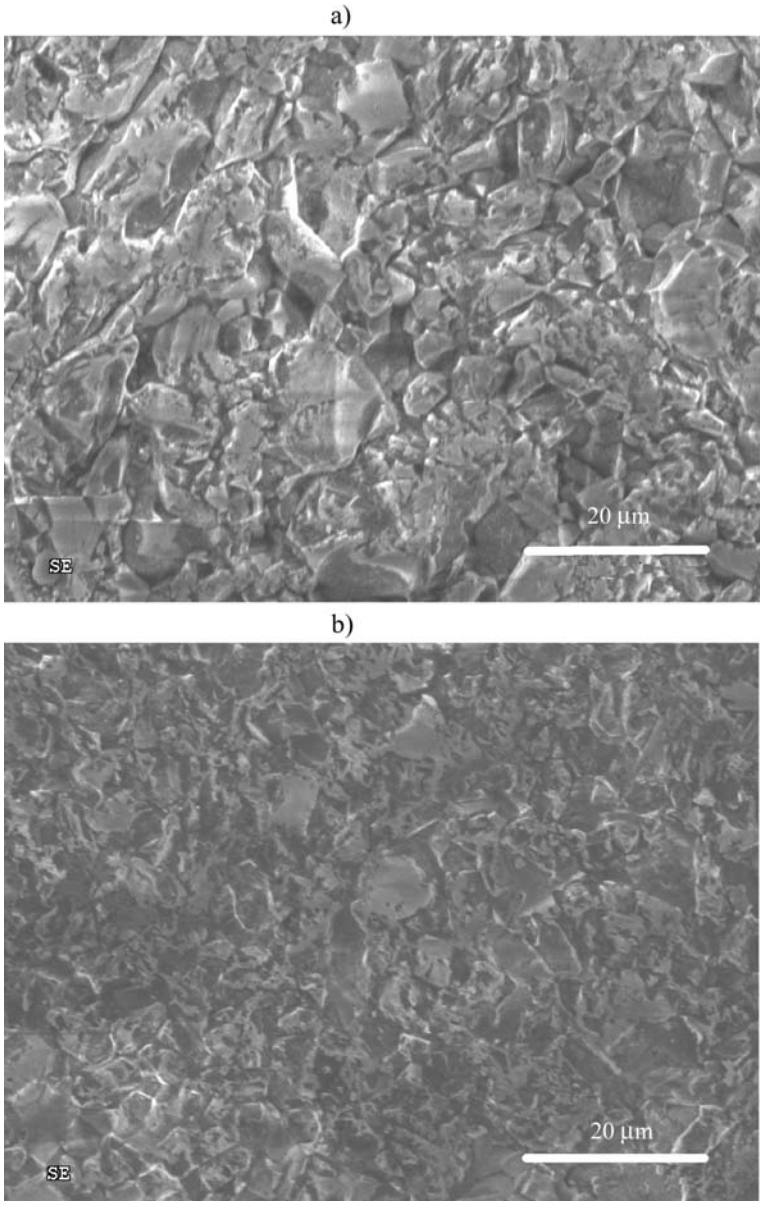


Fig. 4.1.10. Microstructure of ceramic femoral heads on the polished sections a) old ceramic, b) modern ceramic (SEM, x 1500).

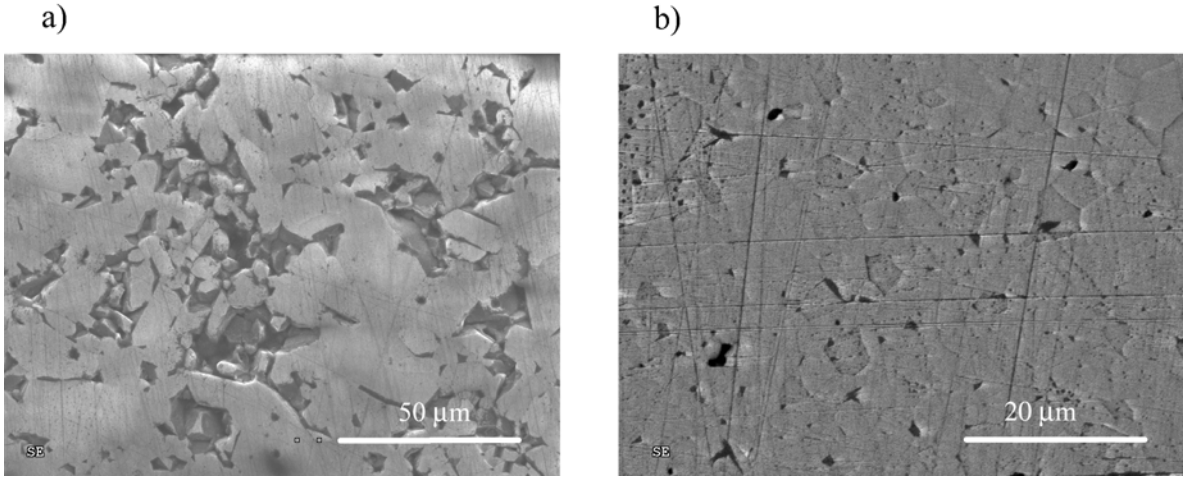


Fig. 4.1.11. Microstructure of ceramic femoral heads at the non-polished sections a) old ceramic (SEM, x 1000), b) modern ceramic (SEM, x 2000).

In the table 4.1.2. the implantation period of examined prostheses was presented. Implants from the old ceramic group were implanted in the eighties, whereas implants from the modern ceramic group were implanted in the nineties.

Table 4.1.2. Periods of ceramic heads implantations.

Ceramic implants	Old ceramic	Modern ceramic
Implantation period	1986 to 1989	1990 to 1998

Roughness measurements of the femoral heads (2D method)

Roughness measurements of the surface of the femoral heads enabled the analysis of wear process, which intensively proceeds in the artificial joints. The examined femoral heads differed significantly in the roughness depending on material of the femoral head. The biggest differences were evident among the ceramic femoral heads. In spite of the same chemical composition of the material used (Al_2O_3), the differences in roughness were significant. Femoral heads from the old ceramic group exhibited the biggest values of considered roughness parameters, Ra (Fig. 4.1.12., Fig. 4.1.14.) and Rt (Fig. 4.1.13., Fig. 4.1.15.). The smallest roughness was detected in the group of modern ceramic. The roughness is presented as an average of all sites examined (dome, unworn and worn areas) and denoted as average roughness of the whole femoral head. There was no significant difference in roughness for metal heads coupled with cemented or uncemented polyethylene cups, respectively. All the metal heads had similar average values of examined roughness parameters.

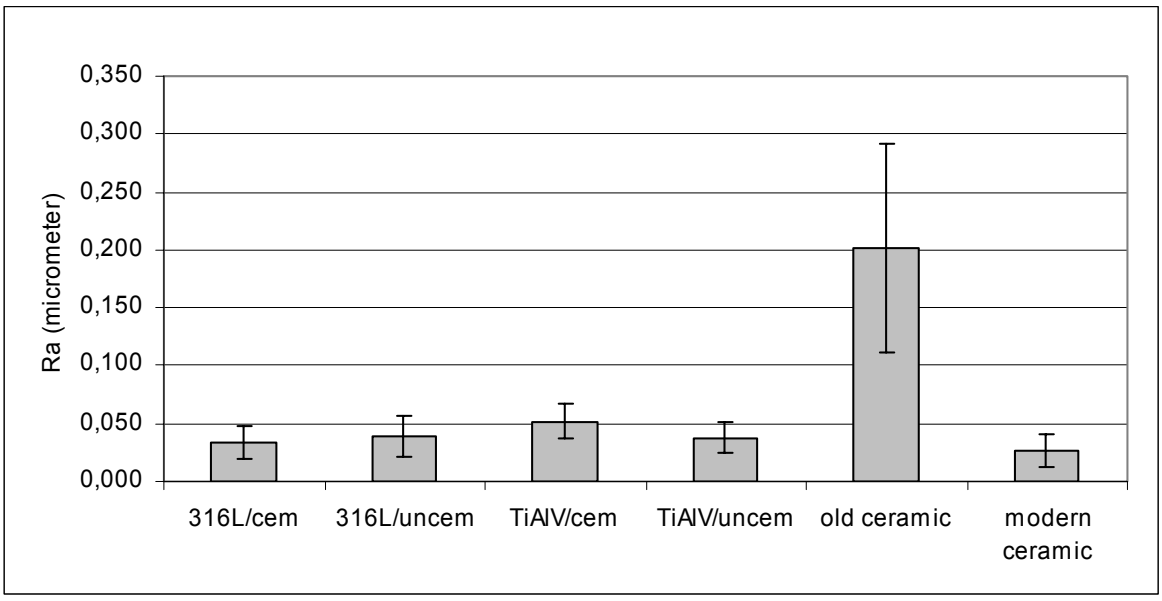


Fig. 4.1.12. The average roughness of the whole femoral heads for different head materials and cups fixation (results with standard deviation)
(316L/cem – stainless steel femoral head group with cemented cup, 316L/uncem – stainless steel femoral head group with uncemented cup, TiAlV/cem – titanium alloy femoral head group with cemented cup, TiAlV/uncem – titanium alloy femoral head group with uncemented cup).

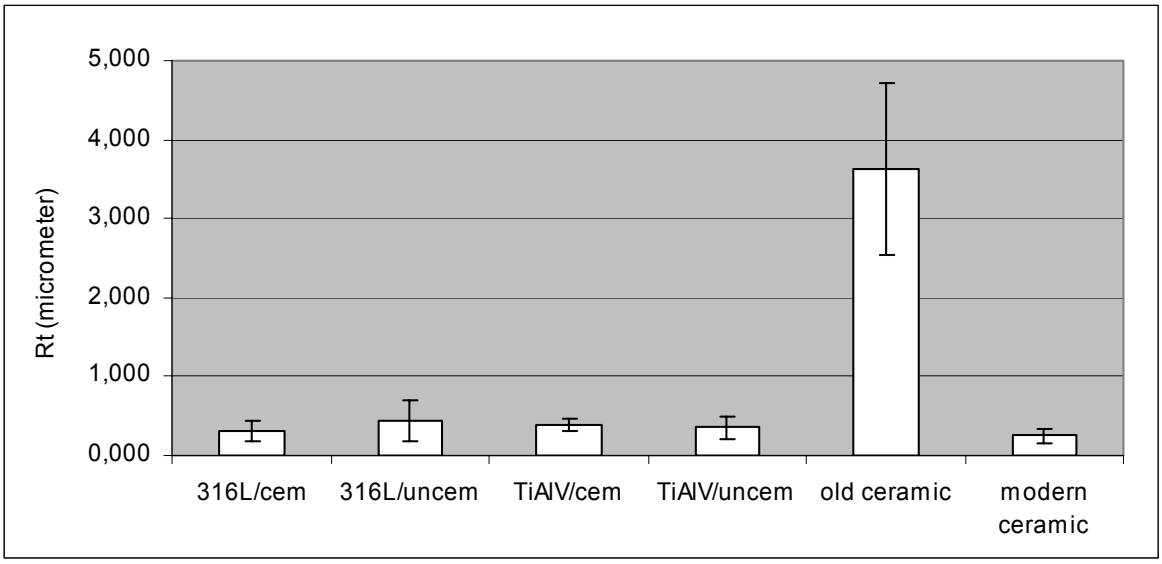


Fig. 4.1.13. The total amplitude roughness of the whole femoral heads for different head materials and cup fixation (results with standard deviation) (316L/cem – stainless steel femoral head group with cemented cup, 316L/uncem – stainless steel femoral head group with uncemented cup, TiAlV/cem – titanium alloy femoral head group with cemented cup, TiAlV/uncem – titanium alloy femoral head group with uncemented cup).

Results of roughness measurements carried out at different sites at the femoral heads are presented in figures 4.1.14. and 4.1.15. The differences in the average roughness among different areas of the individual femoral heads were visible only in the group of old ceramic materials, however they were statistically insignificant ($p>0.05$). The areas chosen as the unworn areas revealed roughness similar to that in the worn and dome areas.

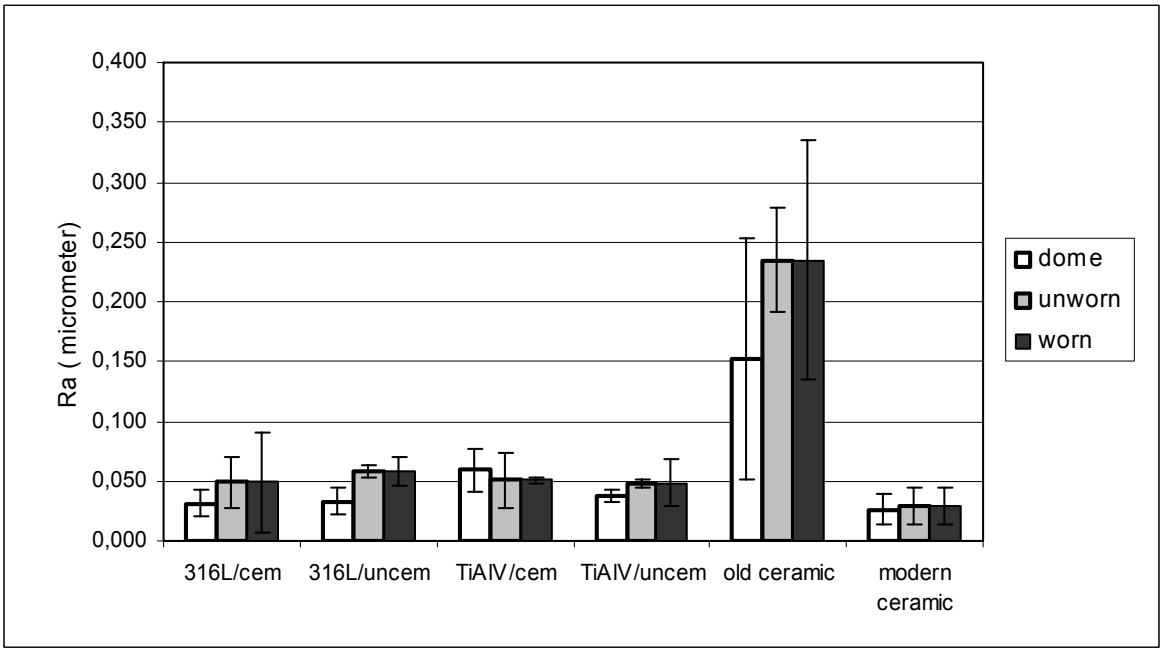


Fig. 4.1.14. The average roughness for different femoral head areas for different head materials and cup fixation (results with standard deviation)
(316L/cem – stainless steel femoral head group with cemented cup, 316L/uncem – stainless steel femoral head group with uncemented cup, TiAlV/cem – titanium alloy femoral head group with cemented cup, TiAlV/uncem – titanium alloy femoral head group with uncemented cup).

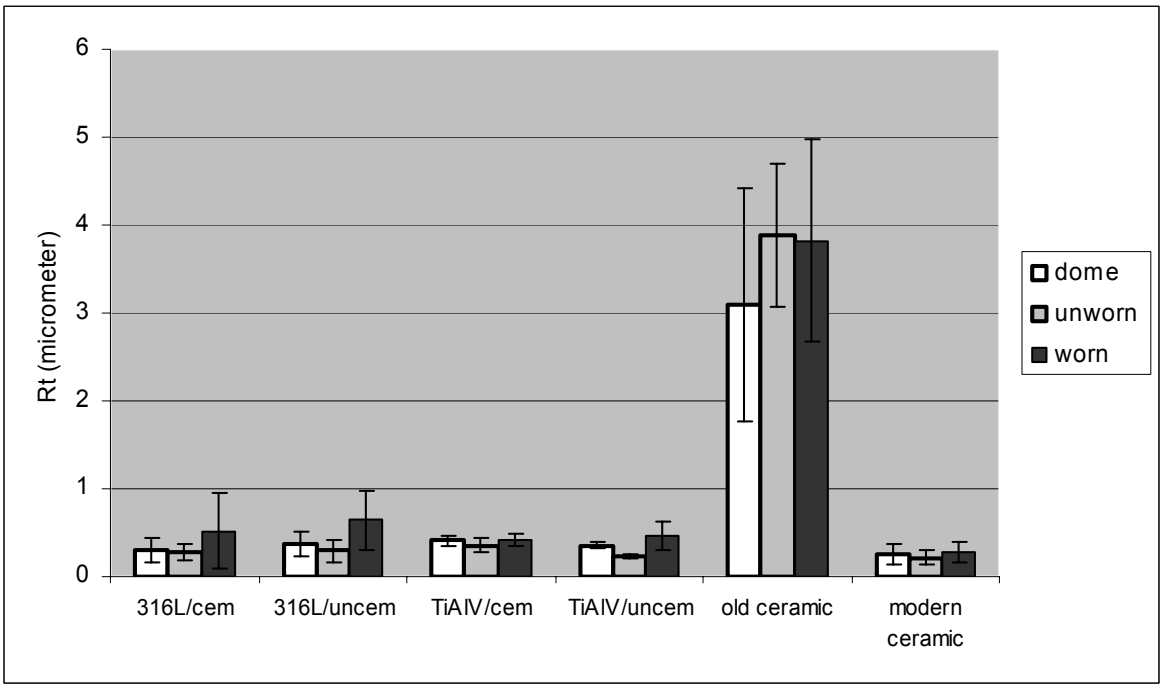


Fig. 4.1.15. The total amplitude roughness for different femoral head areas for different head materials and cup fixation (results with standard deviation) (316L/cem – stainless steel femoral head group with cemented cup, 316L/uncem – stainless steel femoral head group with uncemented cup, TiAlV/cem – titanium alloy femoral head group with cemented cup, TiAlV/uncem – titanium alloy femoral head group with uncemented cup).

Roughness measurements of the femoral heads (3D method)

Based on the 3D roughness measurements a precise characterization of the wear traces on the retrieved femoral heads was enabled.

The table 4.1.3. shows the amplitude parameters of 3D measured on the surface of retrieved femoral heads. All the examined parameters showed the highest values for the femoral heads made of old ceramic (statistically significant in Student t-test $p<0.01$). On the other hand, the lowest value exhibited the femoral heads made of modern ceramic material. The metal heads revealed similar roughness properties; however, the surface of the stainless steel heads had deeper valleys and deeper scratches (statistically significant in Student t-test $p<0.05$). For ceramic implants the height of the picks (Sp) was smaller than the depth of the valleys (Sv).

Table 4.1.3. Amplitude parameters of 3D roughness measured on retrieved femoral heads
(Sa – arithmetical mean height of the surface, Sq – root mean square height of the surface, Sp – maximum height of peaks, Sv – maximum height of valleys, Sz – maximum height of the surface, average of five highest and five lowest points of the surface.) (316L/cem – stainless steel femoral head group with cemented cup, TiAlV/cem – titanium alloy femoral head group with cemented cup).

Material of femoral head	Amplitude parameters				
	Sa	Sq	Sp	Sv	Sz
	[µm]	[µm]	[µm]	[µm]	[µm]
316L/cem	0.074	0.084	0.38	0.84	0.73
TiAlV/cem	0.078	0.099	0.53	0.45	0.78
Old ceramic	0.165	0.295	0.955	4.61	4.685
Modern ceramic	0.029	0.041	0.185	0.48	0.575

Three-dimensional images of the surfaces of the stainless steel femoral head (Fig. 4.1.16.) and of the modern ceramic femoral head (Fig. 4.1.17.) showed the characteristics of examined surfaces. Please note that the scale of these two charts is different.

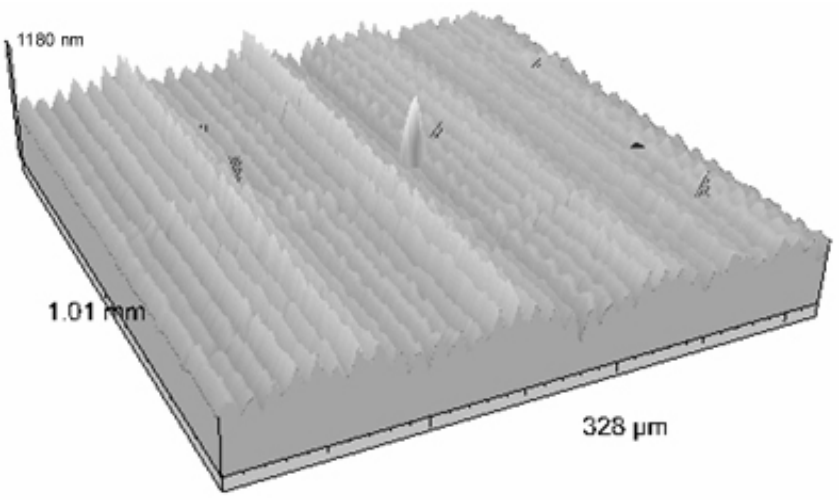


Fig. 4.1.16. The 3-D image of the surface of stainless steel femoral head.

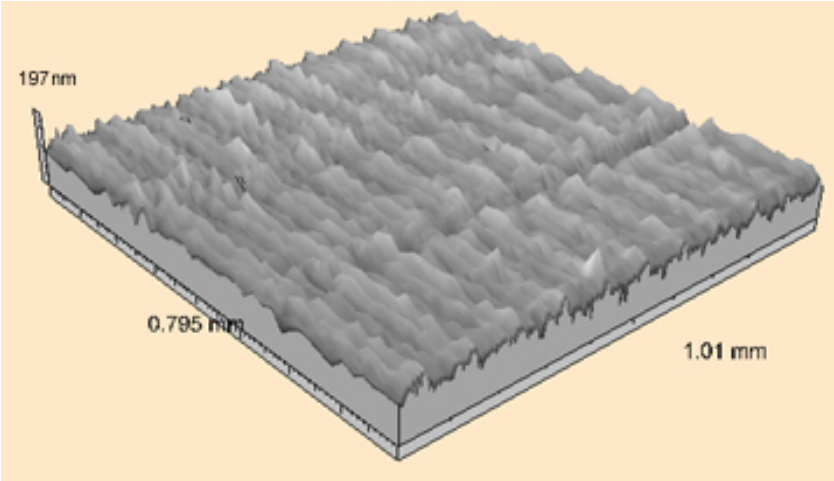


Fig. 4.1.17. The 3-D image of the surface of modern ceramic femoral head.

Hybrid roughness parameters were presented in the table 4.1.4. The value of mean curvature of the summits was the biggest for modern ceramic materials, which indicates of big radius of rounding of the peaks on the counterpart surfaces (Tab. 4.1.4.).

Table 4.1.4. Hybrid parameters of 3D roughness measurements on loosened femoral heads
(Ssc – mean curvature of the summits, Sdc – area section height difference between 20 and 80%.) (316L/cem – stainless steel femoral head group with cemented cup, TiAlV/cem – titanium alloy femoral head group with cemented cup).

Material of femoral head	Hybrid parameters	
	Ssc [1/μm]	Sdc 20-80% [μm]
316L/cem	0.018	0.161
TiAlV/cem	0.023	0.153
Old ceramic	0.024	0.198
Modern ceramic	0.032	0.05

The significant differences were evident in the parameter Sdc 20-80% (area section height difference between 20 and 80%) (Fig. 4.1.18.). The area in this section height is responsible for the contact with polyethylene acetabular component. The lowest value of this parameter was measured for the modern ceramic group and the highest value for old ceramic femoral heads (Tab. 4.1.4.) (statistically significant in Student t-test $p<0.05$).

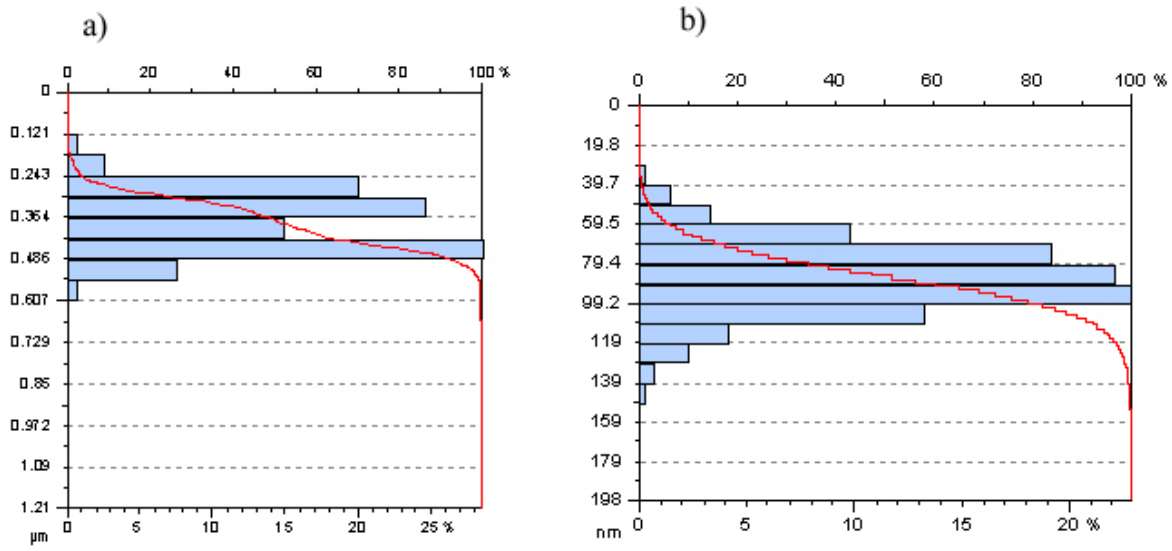


Fig. 4.1.18. The curve of load capacity (Abbot curve) a) stainless steel femoral head (Sdc 20-80%=0.161 µm), b) modern ceramic femoral head (Sdc 20-80%=0.05 µm).

The roughness profiles showed anisotropy in all examined surfaces (Fig. 4.1.19. and Fig. 4.1.20.). Stainless steel revealed many little irregularities, less than for modern ceramic femoral heads. On the roughness profile of stainless steel femoral head sharp peaks and one big scratch were observed. Please note that the scale of two presented profiles is different.

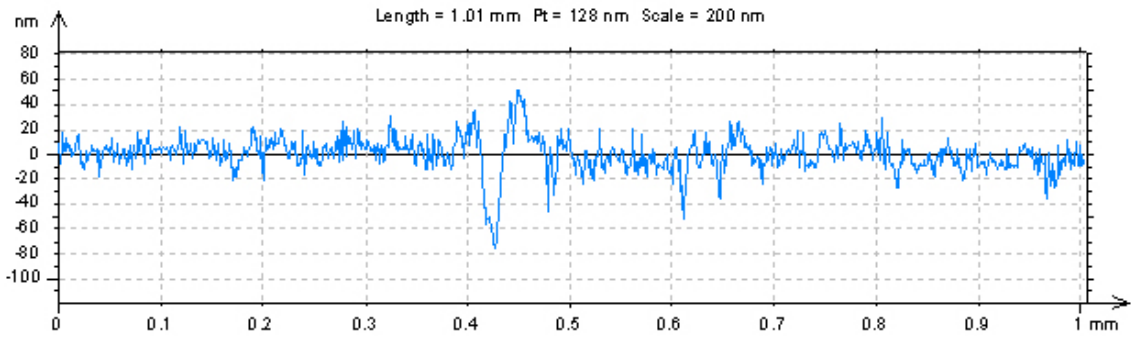


Fig. 4.1.19. Roughness profile of stainless steel femoral head.

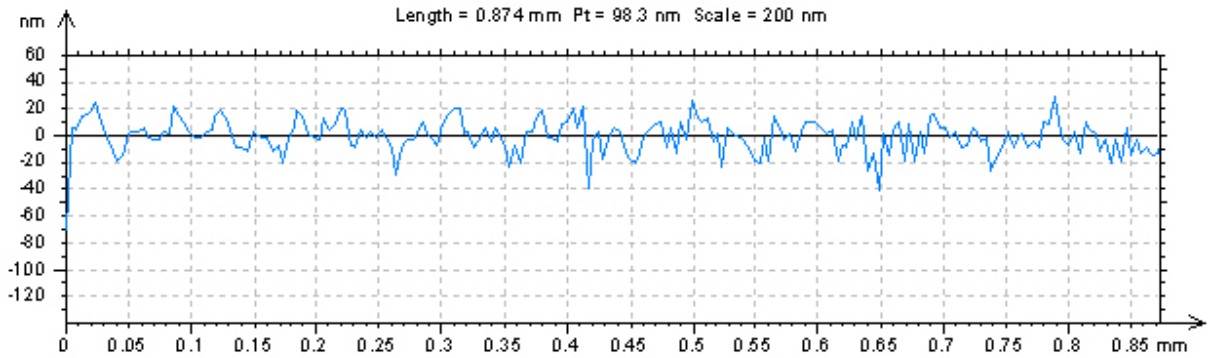


Fig. 4.1.20. Roughness profile of modern ceramic femoral head.

4.1.4 Discussion

The analysis of wear

The artificial hip joints are exposed to large and repetitive loads. Additionally, biological environment is aggressive thus contributing to the implant degradation and wear. The SEM examination of the femoral heads retrieved during revision surgeries revealed different wear modes.

The abrasive wear is characteristic for ceramic material [147], but on the examined ceramic surfaces no abrasive wear traces were observed. It was shown that ceramic femoral heads are more wear resistant than metal femoral heads [137] and are not susceptible to abrasive third-body wear [116].

At the surfaces of all ceramic femoral heads a material loss was observed (Fig. 4.1.5.). The areas of material removal (pits or cavities) can be connected to a grain boundary fracture mechanism, which contributes to grains excavation [2, 159, 178]. The grain boundary fractures can be caused by the material defects or by the material impurities. The gaps formed after grain dislodgement can initiate and then lead to the propagation of microcracks [2], which could even lead to the fracture of the implant. It was shown that wear particles can be trapped in the pits formed at the surface of ceramic femoral head [63] and lead to the increase in abrasive wear of coupled polyethylene acetabular component.

The performed research showed that more pits at the surfaces were formed in the old ceramic materials, which can be caused by the presence of more impurities in the material. In the 1980s (Tab. 4.1.2.), when these implants were implanted, the material requirements were not strict as today. Removal of grains left the surface with numerous cavities with sharply defined edges thus contributing to intensive polyethylene wear by abrasive and adhesive mechanisms.

At the surfaces of metal femoral heads the abrasion wear process prevailed (Fig. 4.1.2.), in accordance with literature data [49, 58, 175]. Scratches on the metal femoral heads could be made by metal or cement particles operating as third-body wear particles. They became trapped between artificial joint surfaces and rub against them. It was shown that metal femoral heads differ in the type of abrasive wear (Fig. 4.1.2.). On the titanium alloy femoral heads small scratches dominated and covered the whole

surface articulating against polyethylene cup. On the stainless steel femoral heads areas without virtually any scratches and areas with many deep scratches were observed. The differences in the type of abrasive wear among metal heads result from the difference in the material properties. Titanium alloy is less wear resistant than stainless steel; therefore, every contact of titanium alloy surface with hard wear particles can cause the formation of new scratches.

On the stainless steel femoral heads adhesive (the lumpy film transfer of UHMWPE) and fatigue wear (fractures perpendicular to the direction of wear traces) were observed (Fig. 4.1.3.).

Polyethylene is a material which is very susceptible to wear. Due to the strong friction present in the artificial joints polyethylene wears out intensively producing a large amount of wear particles.

All examined polyethylene acetabular components showed wear traces characteristic for abrasive wear. Polishing and scratches were observed in the interior of polyethylene. The scratches formed due to friction against rough femoral heads or due to third-bodies (e.g. cement particles). Under the influence of strong, repeating loads, polyethylene is subjected to adhesive and fatigue wear as well. It cracked and delaminated, as observed in the interior of polyethylene cups (Fig. 4.1.8.).

Wear is not a property of a material but rather a result of the tribological conditions. Thus, the wear of polyethylene strongly depends on the material of the femoral head. The interior of polyethylene cup coupled with metal femoral heads revealed mostly scratches (Fig. 4.1.7.), while polyethylene cup coupled with ceramic femoral heads showed a fine, fibrillar appearance (Fig. 4.1.9.). Highly oriented fibrillar structure is very brittle and highly susceptible to fractures when the friction occurs perpendicular to the fibrils [171].

Saikko et al. showed that the fibrillar structure of polyethylene indicated the domination of adhesive wear process [99]. The traces of adhesive wear process of polyethylene were evident on the surfaces of all examined ceramic femoral heads (Fig. 4.1.4.). Strong adhesive bonding can be formed between ceramic and polyethylene. The polyethylene transfer layer can occupy large areas of femoral heads [38]. The initial ceramic-polyethylene contact is replaced by the polyethylene-polyethylene contact, which decreases the surface hardness and abrasive wear [29].

On the other hand, Jasty et al. [79] suggested that the polyethylene fibrillar structure arose due to abrasive and adhesive wear. The authors indicated that polyethylene material from the interior of polyethylene cup could be pulled out and drawn into fine fibrils lying parallel to each other.

In this investigation both abrasive and adhesive wear traces on polyethylene cup were observed confirming the hypothesis proposed by Justy et al.

The SEM investigation showed that all examined loosened implants revealed intensive wear traces, which were formed during normal usage of the artificial joint. It was shown that on metal femoral heads mostly scratches were observed, while on the ceramic femoral heads mainly material excavation were present. These features influence the wear mechanisms of polyethylene cups.

Microstructure of ceramic femoral heads

The literature data show that the wear process of polyethylene acetabular components is 50 times smaller for ceramic-on-polyethylene than for metal-on-polyethylene bearings [143]. On the other hand, Devane et al. reported no differences in the polyethylene wear coupled with metal and ceramic heads, respectively [34]. The results of the experiments in this work proved that the quality of ceramic material can affect the degree and mechanism of polyethylene wear.

The quality of ceramic materials is still being improved. In last few decades the purity of material, ceramic grain and pore size, and consequently the mechanical properties of medical-grade ceramic have been changed [66, 99, 156, 176]. The ceramic microstructure - grain and pore size - significantly influence the wear process of polyethylene cups articulating against ceramic femoral heads. The wear rate decreased with decreasing grain size of the ceramic material [31, 156, 173]. Ceramic materials with large grain size are susceptible to wear due to an increased possibility for crack propagation. The cracks usually appear at the grain boundaries [86], which are longer in the ceramic from the older generation. The sharp edges of microcracks on the ceramic femoral heads rub against soft polyethylene cups causing abrasive wear. In the old generation ceramic grains had size around 7 μm. In the 1990s the ceramic grain size requirements were restricted to 4.5 μm [66, 78].

Examined ceramic prostheses differed in the implantation period (Tab. 4.1.2.) Implants from the old ceramic groups were implanted in the eighties whereas the implants from the modern ceramic group were implanted in the nineties.

It was assumed that examined implants differ in microstructure of ceramic material and it was confirmed by the SEM analysis. The SEM images of polished (Fig. 4.1.10) and unpolished sections (Fig. 4.1.11) of the ceramic surfaces revealed big difference in the grain and pore size.

The SEM images of the unpolished surfaces of ceramic femoral heads showed differences in the pore sizes (Fig. 4.1.11.). The porosity plays a significant role in the wear process of polyethylene cups. Pores and gaps after grains removal can act as a source of slip and twinning and increase wear of polyethylene cups [56]. Pores are the weak point for the propagations of microcracks. In the group of old ceramic the pores were much bigger than in the modern ceramic group thus contributing to the increase in wear of polyethylene cups.

The obtained results showed that the quality of ceramic materials significantly affect the wear process. Old ceramic materials exhibited bigger grains and bigger pores in the microstructure, which increased the wear process of polyethylene cups and contributed to intensive osteolysis by producing many polyethylene particles evoking inflammatory reaction in the periprosthetic tissues.

Roughness measurements of the femoral heads

The roughness of the femoral head influences the wear of polyethylene acetabular component articulating against it [43, 59, 80, 112, 134, 157]. The higher the roughness of the femoral head, the more intensive is the wear of polyethylene cup [19, 32, 39, 59, 94, 116, 122, 157, 162]. Based on the roughness parameters of femoral heads surfaces we can learn indirectly more about the wear process of polyethylene cups.

The results of this work revealed that the examined femoral heads differ in the roughness. Consequently, different wear modes of polyethylene cups were observed. The obtained roughness was a resultant of an initial roughness, wear resistance of the material and the amount of third-bodies scratching the femoral heads surfaces.

The roughness parameters were measured at the dome area of femoral heads (2D and 3D roughness measurements) because in this area randomly moving wear particles accumulate and contribute to intensive wear [94]. The roughness was also measured at the equatorial area being ninety degrees to the dome area (2D roughness measurements), where the wear process is intensive [121]. The 2D parameters were also examined at the unworn areas chosen by the unaided eye. Unfortunately, unworn areas chosen in this way exhibited roughness values similar to that of worn surfaces indicating that it is very hard to locate unworn areas on the retrieved femoral heads.

The 2D roughness was measured using Ra and Rt parameters. The average roughness of the surface (Ra) is the most often chosen parameter that describes the topography of artificial joints surfaces [134]. The total amplitude roughness (Rt) shows the scratches and grooves on the examined surfaces.

Despite the same chemical composition the examined ceramic femoral heads differed significantly in the roughness. The old ceramic femoral heads revealed the biggest values of all roughness parameters for both 2D and 3D parameters (Figs. 4.1.12. - 4.1.15., Tab. 4.1.3., Tab. 4.1.4.).

Femoral heads from the old ceramic group were implanted in the eighties, when the standards of femoral heads surface finishing had not been very restricted. The initial roughness was probably much higher, further increasing during years of wear in vivo. This work showed that the old ceramic heads had bigger grains and pores in the surfaces (Fig. 4.1.10., Fig. 4.1.11.) and more pits left by the grain removal (Fig. 4.1.5.). When the roughness is measured in the areas of these surfaces imperfections, the values can be significantly increased.

The roughness properties of old ceramic femoral heads contributed to an intensive wear process of coupled polyethylene acetabular components. Modern ceramic prostheses exhibited very small roughness, which was especially confirmed in the 3D analysis (Tab. 4.1.3.). It resulted from the improved wear resistance of ceramic material, as showed by Buford et al. [22]. A decreased pore size and amount of cavities after grain removal played an important role in decreasing of roughness.

The defects in ceramic femoral heads surfaces were all recessed (cavities and pits), they do not stand out the surfaces, while metal surface defects tend to protrude. The 3D roughness measurements showed that at the surfaces of ceramic femoral heads mainly holes and material loss were visible. The Sv parameter - maximum height of valleys in

both ceramic groups exhibited bigger values than Sp parameter - maximum height of peaks (Tab. 4.1.4.) (statistically significant in Student t-test $p<0.05$). The ceramic materials are very wear resistant and appearance of scratches is very seldom. The deep valleys are connected rather to grains excavation than to abrasive wear. The differences in the wear traces among ceramic and metal heads significantly determined the wear mode of polyethylene acetabular components in the contact with femoral heads. Protruding groves on the metal femoral heads can scratch and cut unevenness of the inner surface of polyethylene cups (Fig. 4.1.7.), so the abrasive wear dominated in the metal-on-polyethylene implants. The gaps in the ceramic femoral head contribute to sliding of polyethylene and adhesive wear. The abrasive wear of polyethylene cup in these implants could be connected to third-body mechanism and to sharp edges of the gaps that can rub the polyethylene surface.

The hybrid parameters of 3D roughness measurements revealed that the modern ceramic femoral heads had best friction properties. The values of Ssc parameter – mean curvature of the summits were the highest (Tab. 4.1.4.). Roughness profiles showed rounded peaks and small amount of irregularities (Fig. 4.1.20.). These features are beneficial from the mechanical point of view. They decrease the wear process of polyethylene acetabular components because the conditions contributing to cutting process do not appear.

The area section height difference between 20 and 80% (Sdc) was the lowest for the modern ceramic femoral heads (three times less than for metal heads and four times less than for old ceramic femoral heads) (Tab. 4.1.4.) (statistically significant in Student t-test $p<0.05$). The Sdc parameter describes the surface area which takes part in the friction against the acetabular cup. Smaller value of this parameter indicates bigger factual contact area during friction and smaller pressure on counterpart surface, which subsequently decrease wear of the material.

Since the roughness measurements of the metal femoral heads did not show the difference between the cemented and uncemented groups, the 3D measurements were conducted only for the cemented cups groups. All metal femoral heads revealed similar values of average roughness (Fig. 4.1.12.).

The 3D roughness profiles revealed strong anisotropy of examined surfaces (stainless steel Fig. 4.1.16. and modern ceramic Fig. 4.1.17.). The directions of cut

processing were visible. The roughness profiles differed approximately for an order of magnitude.

The presence of bone cement may affect the roughness of the femoral heads. Bone cement is very brittle material, producing many hard wear particles that can get trapped between articulating surfaces. Hall et al. [58] showed that radiopaque cement particles act as third-bodies intensifying abrasive wear of femoral heads and polyethylene cups. The impact of bone cement particles is less evident in the ceramic-on-polyethylene bearings than in metal-on-polyethylene because ceramic femoral heads are not susceptible to abrasive wear connected with third body wear [116]. No differences in the roughness among implants with cemented and uncemented polyethylene cups were observed. In all groups stems were fixed using bone cement, which produces cement particles. The amount of cement particles produced from acetabular component fixation did not additionally contribute to the increase in roughness of femoral heads.

The results of this work proved that it is worthwhile and important to examine surfaces of retrieved implants in order to study the wear traces, analyze roughness parameters and, finally, to define the wear mechanisms of polyethylene cups in contact with femoral heads.

It was revealed that from the mechanical point of view the modern ceramic femoral heads showed the best properties among the tested implants. They had the smallest roughness, rounded peaks on the surface and big factual area of contact with polyethylene cup. All these features significantly decrease the wear process of polyethylene acetabular components.

The performed experiments have shown that the ceramic femoral heads do not always decrease the wear mechanism of polyethylene cups, as has been reported in literature [34, 47, 156]. The ceramic microstructure, initial roughness and susceptibility to grain removal are very important in the polyethylene-ceramic contact and determine its wear.

4.2 The characterization of wear particles

4.2.1 Material

The subject of this study were wear particles isolated from periprosthetic tissues surrounding loosened hip implants. The tissue samples were retrieved during revision surgery of loosened hip prostheses in which the stems were made of titanium alloy, the acetabular components were made of ultra height molecular weight polyethylene and the femoral heads were made of metal (stainless steel or titanium alloy) or ceramic (two groups differentiated on the basis of the period of implantation). The implants also differed in the way of acetabular component fixation. There were both cemented and uncemented prostheses.

The sites of tissue sampling were chosen at the discretion of the surgeon. From the retrieved periprosthetic tissues metal, acrylic and polyethylene particles were isolated. Polyethylene wear particles were analyzed in respect of size distribution.

4.2.2 Methods

Wear particles isolation

The protocol used for the wear particle isolation was a modified version of Campbell et al. [24]. The retrieved fibrous tissue samples were frozen and then later used to isolate wear particles. Periprosthetic tissue samples were shaken overnight on the shaker in a mixture of chloroform and methanol (2:1) to extract lipids. Then the tissues were digested in 5 M sodium hydroxide for four hours at 65°C. The digest was then centrifuged at 6000 rpm (revolution per minute, the number of full rotations completed in one minute) for one hour over sucrose gradient (5% sucrose). The dense upper layer that contained polyethylene particles was collected and hydrolyzed for one hour at 80°C and then centrifuged with isopropanol (one hour at 6000 rpm). The metal, organic and cement particles settled on the bottom of the tube. Polyethylene particles formed a thin white band on the top of the isopropanol solution. All four types of particles were collected and stored in cool place.

SEM analysis

After ultrasonication for five minutes, isolated particles were filtrated through membrane nucleopore polycarbonate filter papers (Costar, Pleasanton, California) with a pore size of 0.2 μm. Filters were sputter coated with gold or graphite and observed under a scanning electron microscope SEM (JEOL JSM 5800, Tokyo, Japan) at 15 kV. The metal, organic and cement particles were coated with carbon in order to determine the composition of the examined particles by an energy dispersive X-ray spectroscopy (EDS, LINK, ISIS 300, Oxford Instruments, Oxford UK).

Polyethylene particles size distribution

To perform the analysis of size distribution of isolated polyethylene particles the SEM pictures of polyethylene particles taken at the magnification of 10000 and 20000 were analyzed using the computer program Image Tool (version 3, UTHSCSA). At least three SEM pictures were analyzed per one tissue. Firstly, each individual particle was pointed manually. This step was time-consuming but was required due to a strong agglomeration of the particles. Once all the particles were manually recognized, the image was scanned and then analyzed using graphic software Image Tool (version 3, UTHSCSA).

Particle size was defined by the equivalent circle diameter (ECD): a diameter of a circle with an area equivalent to the area of the particle. It has the units of length. The percentage number of particle in each size range was calculated.

Polyethylene particles volume distribution – laser analysis

The isolated polyethylene particles were analyzed using an automated laser scattering particle size distribution analyzer (Horiba LA-920). The laser scattering method is particularly adapted to measuring particle size distribution of the small particles in dispersion. This method relies upon the fact that the diffraction angle is inversely proportional to particle size. Horiba LA-920 measures particles diameter lying within the size range of 0.02-2000 micrometers. Within the instrument, particles were suspended in the distilled water. Before the measurement, ultrasound was turned on for five minutes.

Laser scattering particle sizing instruments calculate particle size distribution based on the assumption that all particles are spherical. The volume of particles of different size (diameter) was presented in the diagram.

Statistics

The data distribution was checked by the Lilliefors test (STATISTICA, version 6. StatSoft, Inc.). Because the normality assumption was not met for the data, the non-parametric Kruskal-Wallis test was used. In all tests statistically significance was used judged based on a p value of less than 0.05.

4.2.3 Results

The isolation of wear particles gives the opportunity to observe the particulate debris of almost all materials comprising the artificial joint. Cement (Polymethylmethacrylate, PMMA), metal and polyethylene articles were isolated from periprosthetic tissues. Metal and cement particles were detectable by back-scattered SEM analysis and verified by the energy dispersive X-ray analysis. No ceramic particles were found in the examined tissue samples.

The cement particles were identified on the basis of $BaSO_4$ or ZrO_2 content (Fig. 4.2.1., Fig. 4.2.2.), which were added as radiopaque agents to the bone cement. Evidence of cement particles was detected in all examined tissue samples (100% of tissues samples). They were usually visible among calcium phosphate particles (Fig. 4.2.2.).

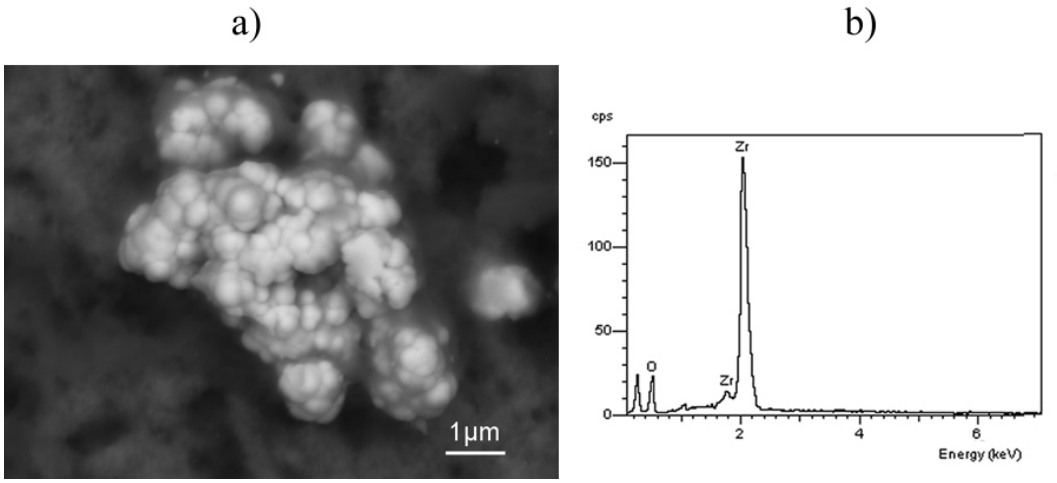


Fig. 4.2.1. Cement particle a) isolated from tissue sample (SEM, Back-scattered contrast, x 4000), b) EDS spectrum indicating presence of ZrO_2, which is used for labelling cement.

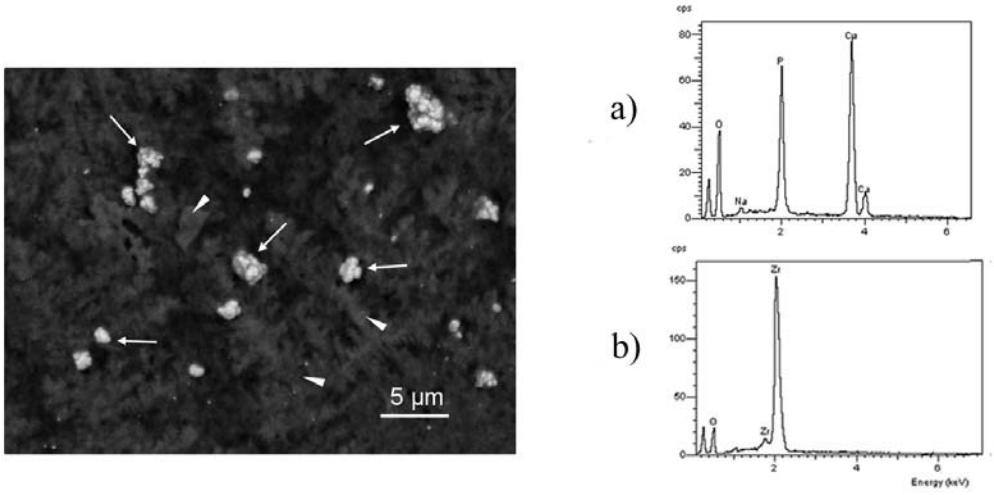


Fig. 4.2.2. Cement particles (arrows) and organic particles (arrow heads) isolated from tissue samples (SEM, Back-scattered contrast, x 1000), a) EDS spectrum of organic particles, b) EDS spectrum of cement particles.

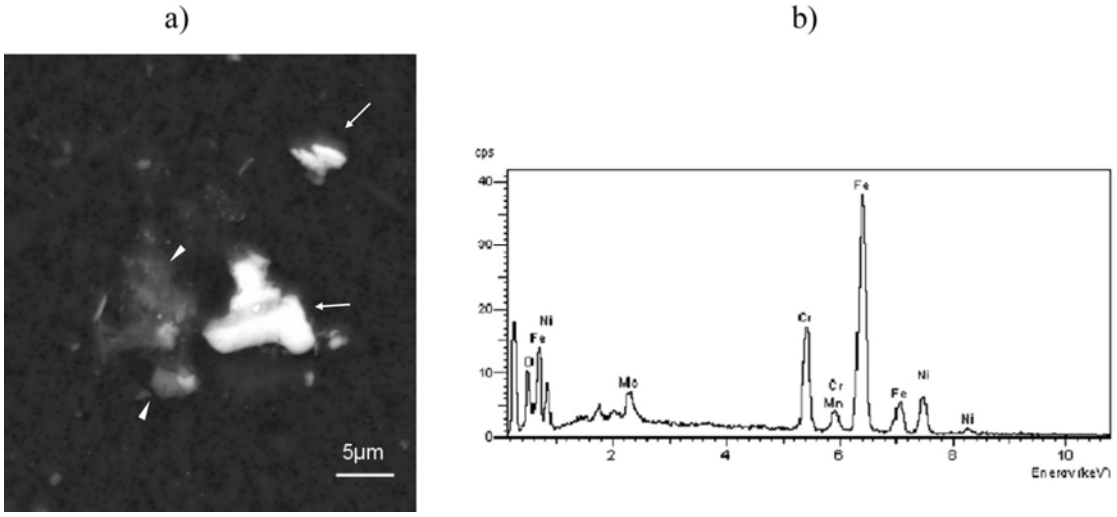


Fig. 4.2.3. a) Particles isolated from periprosthetic tissue: stainless steel particles (arrows) and organic particles (arrow heads) (SEM, back-scattered contrast, x 2000) b) EDS spectrum of stainless steel particles.

Metal particles were identified by energy dispersive X-ray spectroscopy (EDS). Stainless steel particles originated from femoral heads (Fig. 4.2.3.), while titanium alloy particles either originated from femoral heads or from femoral stems (Fig. 4.2.4.). Metal particles were identified in 23.4% of all examined tissue samples.

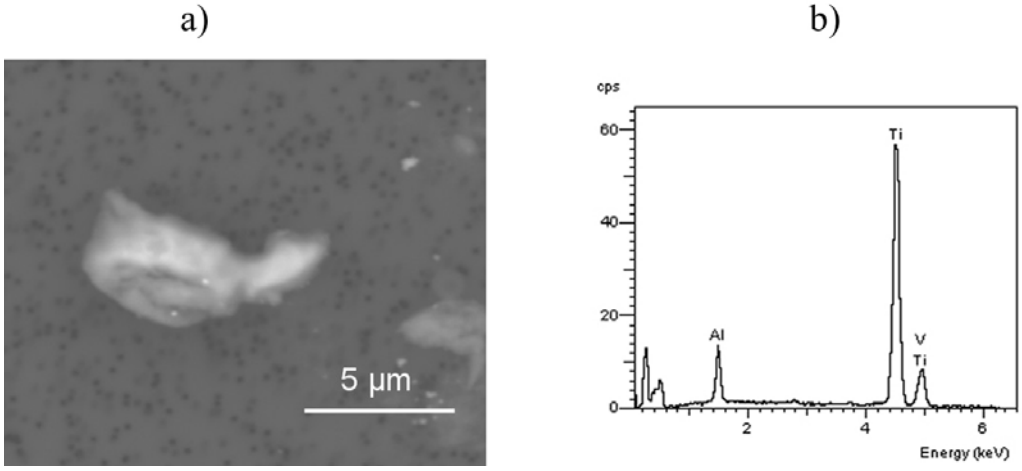


Fig. 4.2.4. a) Titanium alloy particle isolated from periprosthetic tissue (SEM, x 5000) b) EDS spectrum of titanium alloy particles.

The lighter polyethylene particles (density approximately 0.94 g/cm^2) were effectively separated from other, heavier particles by differential centrifugation. Polyethylene particles appeared in different sizes and shapes in all tissue samples (100% of tissue samples) (Fig. 4.2.5.). Very large particles (bigger than 10 µm) were observed in small magnifications (Fig. 4.2.5. a). Elongated particles were present either in submicrometer and also micrometer fraction (Fig. 4.2.5. b and Fig. 4.2.5. c). Very small, submicrometer particles were usually agglomerated (Fig. 4.2.5. c, Fig. 4.2.5. d) forming for example cauliflower-like aggregates (Fig. 4.2.5. b).

SEM images of polyethylene particles showed differences in size of particles between examined groups of implants. The biggest differences were visible among large polyethylene particles. From periprosthetic tissues surrounding old ceramic implants more big polyethylene particles were isolated than from other groups (Fig. 4.2.6.). The morphology and size of polyethylene particles isolated from three groups of implants with metal heads were similar (Fig. 4.2.7.).

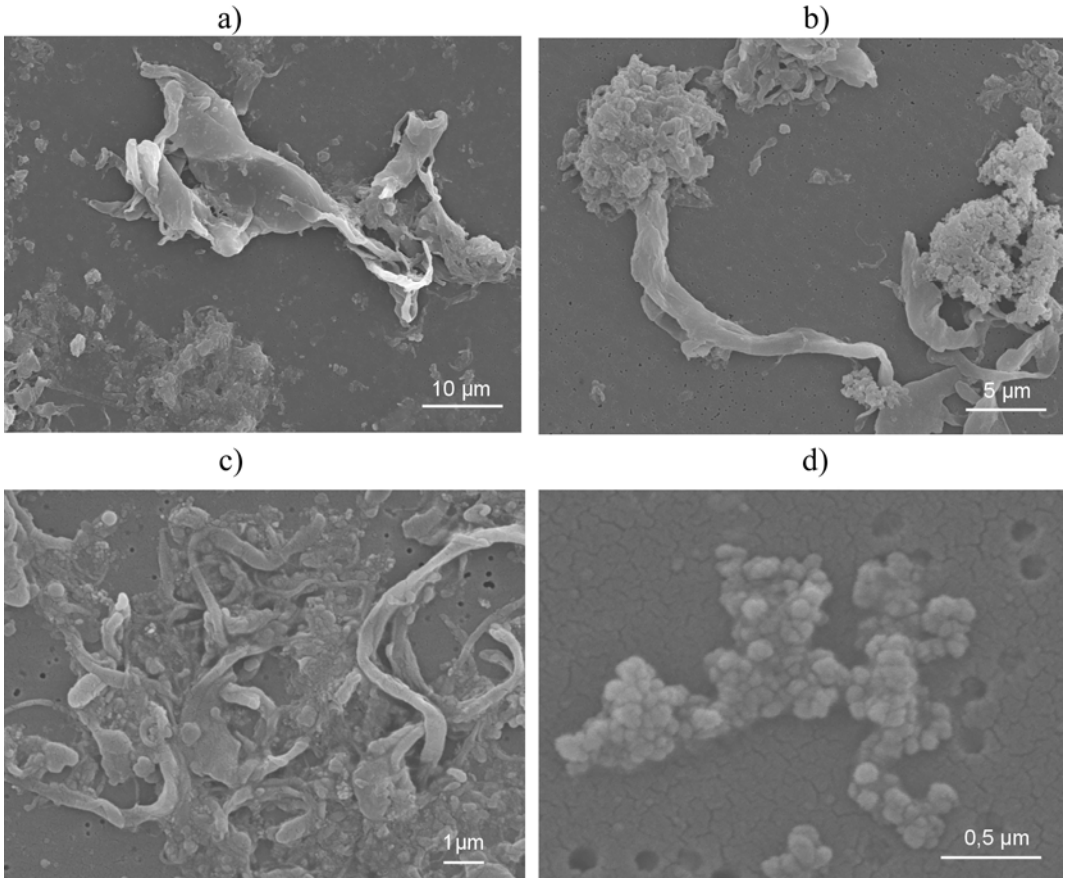


Fig. 4.2.5. Scanning electron micrographs of UHMWPE wear particles of different size and morphology a) x 2000, b) x 4000, c) x 10000, d) x 50000.
The 0.2 µm pores of the filter, on which the particles were collected, are visible in the background.

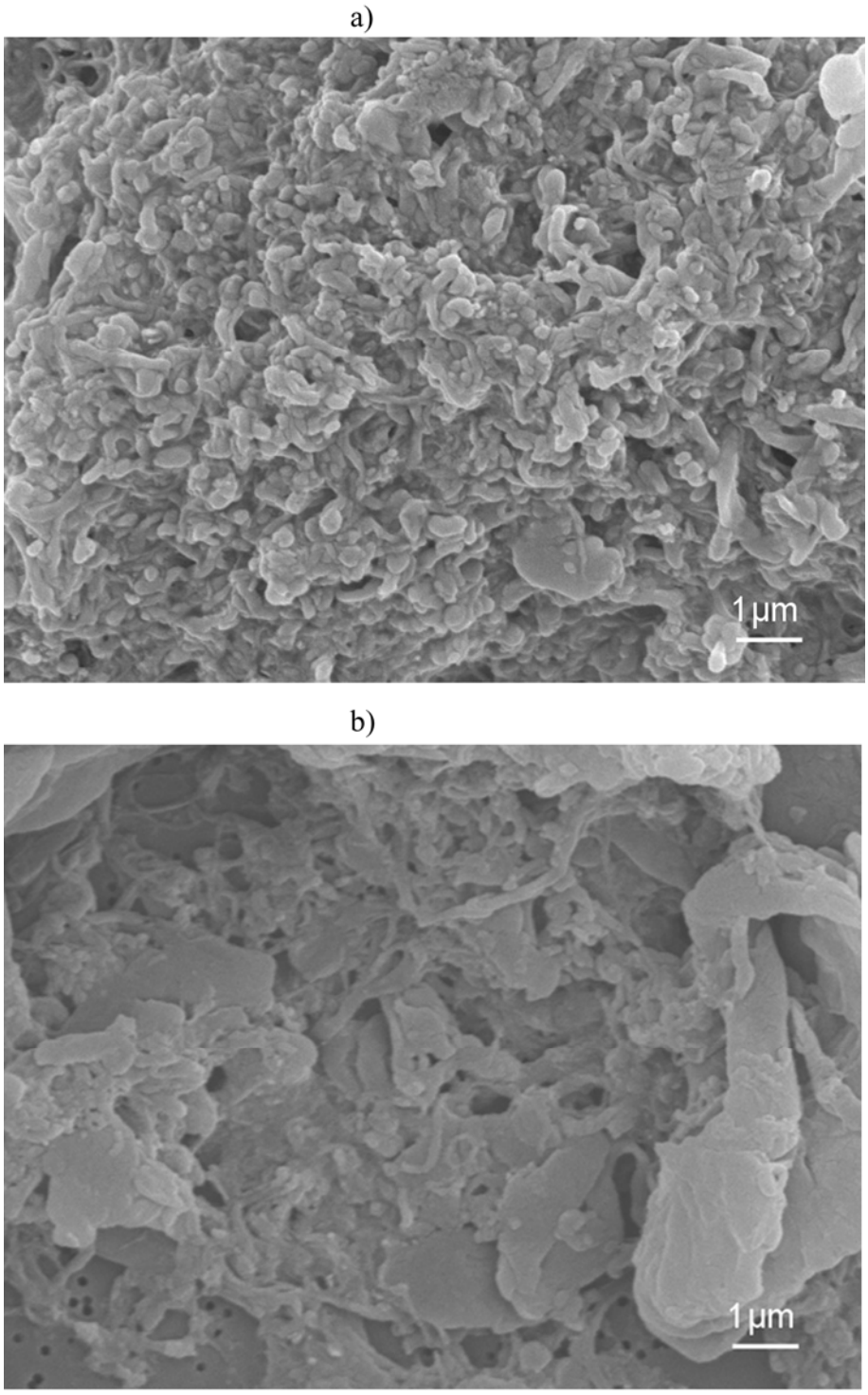


Fig. 4.2.6. Scanning electron micrographs of UHMWPE wear particles isolated from explanted tissues surrounding a) modern ceramic-UHMWPE implant, b) old ceramic-UHMWPE implant.
Sections of the membrane polycarbonate filter were sputter coated with gold and observed at 15 kV (x 10000).

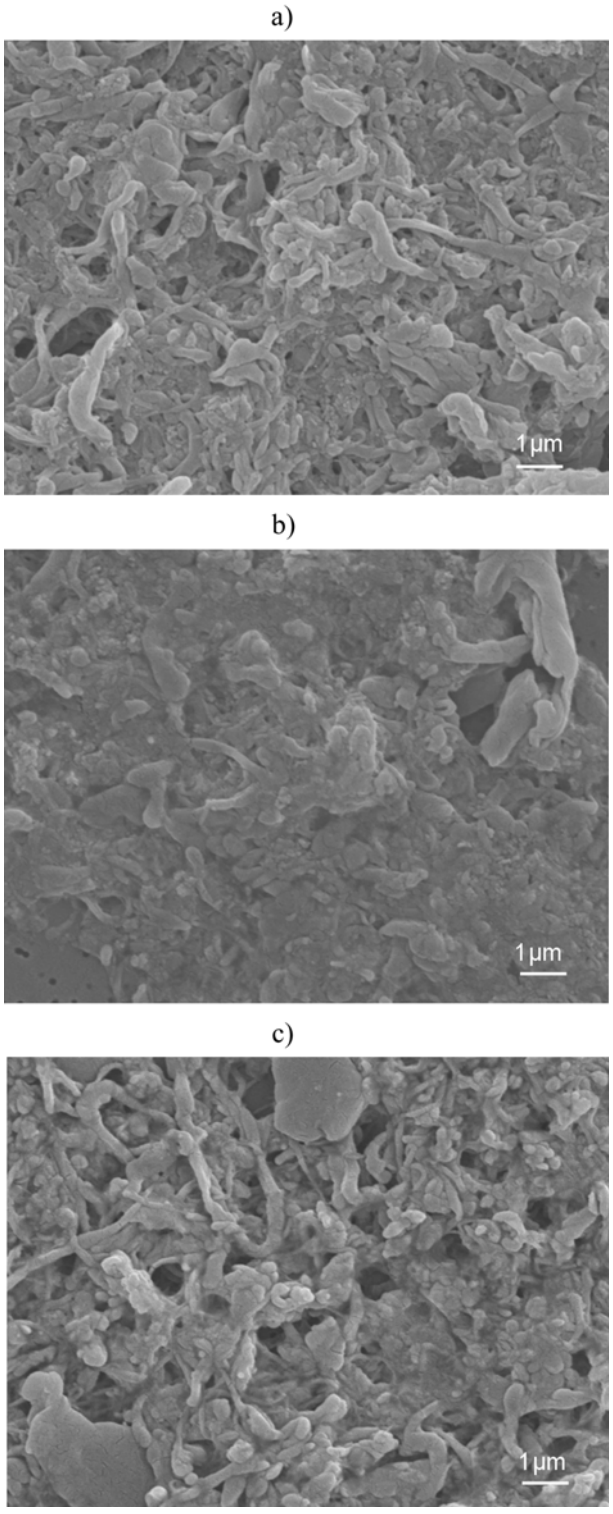


Fig. 4.2.7. Scanning electron micrographs of UHMWPE wear particles isolated from periprosthetic tissues surrounding a) stainless steel-UHMWPE implant with cemented cup, b) stainless steel-UHMWPE implant with uncemented cup, c) titanium alloy-UHMWPE implant with cemented cup (x 10000).

To precisely examine the differences in the size of polyethylene particles isolated from periprosthetic tissues surrounded different implants SEM images analysis was performed. Equivalent circle diameter (ECD) was used to present the data. The average size of polyethylene particles and the percentage number of particles as a function of size in each size range were shown in Fig. 4.2.8. and Fig. 4.2.9.

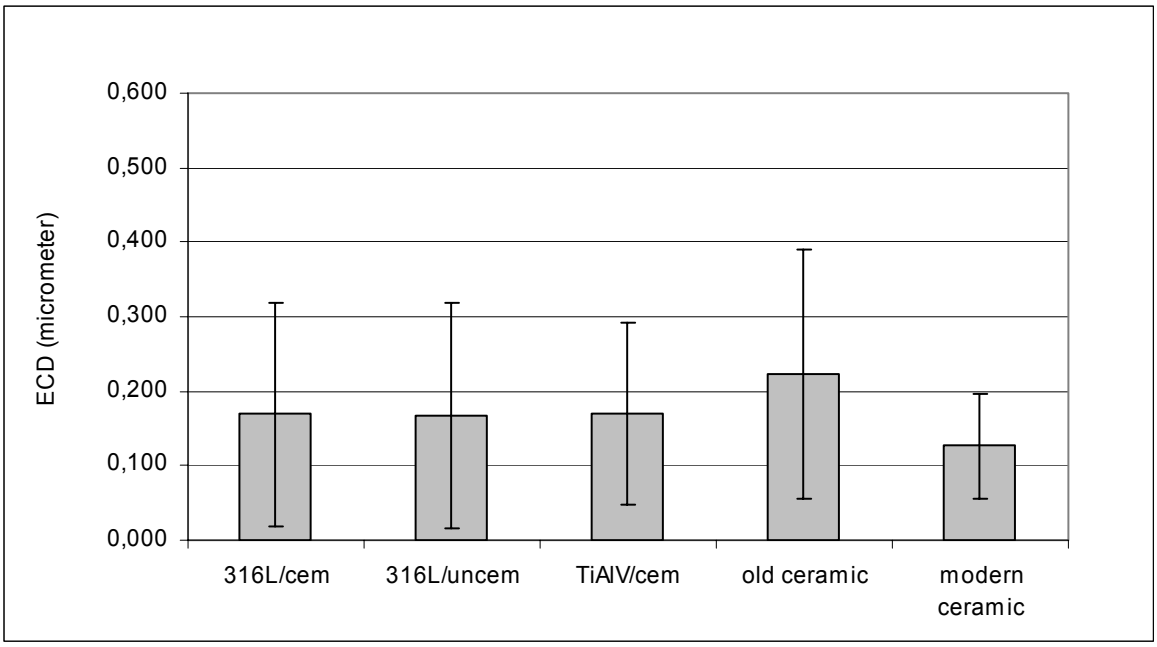


Fig. 4.2.8. The average size of polyethylene particle isolated from periprosthetic tissues of different implants.
Average value with standard deviation. (316L/cem – stainless steel femoral head group with cemented cup, 316L/uncem – stainless steel femoral head group with uncemented cup, TiAlV/cem – titanium alloy femoral head group with cemented cup).

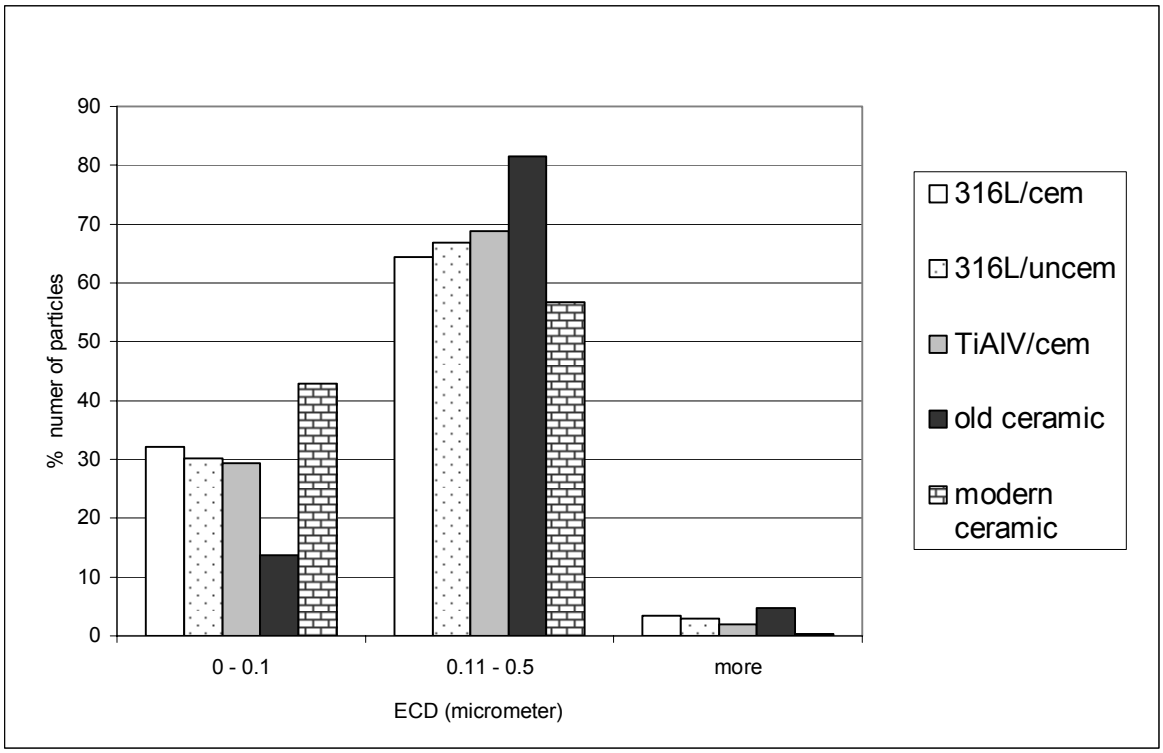


Fig. 4.2.9. Size distribution of polyethylene particles (ECD – equivalent circle diameter), (316L/cem – stainless steel femoral head group with cemented cup, 316L/uncem – stainless steel femoral head group with uncemented cup, TiAlV/cem – titanium alloy femoral head group with cemented cup).

The results confirmed that more than ninety percent of polyethylene particles were smaller than 0.5 μm. The average size of examined particles in all groups was smaller than 0.5 μm (Fig. 4.2.8.). The examination revealed that many particles were smaller than 0.1 μm (32% for stainless steel cemented group, 30% for stainless steel uncemented group, 29.2% for titanium alloy cemented group, 13% for old ceramic and 42% for modern ceramic group). The biggest intensity of such a small particles was observed in the modern ceramic group (statistically significant $p<0.01$ in Kruskal-Wallis test). The biggest number of particles in all groups was in the 0.11-0.5 micrometer size range. Large particles were observed in limited amount, in bigger concentration in group with old ceramic femoral heads (Fig. 4.2.9.) (statistically significant $p<0.01$ in Kruskal-Wallis test). In the group

with modern ceramic femoral heads the smallest number of large particles were found. For all materials very few particles were observed above 1 μm in size.

The percentage volume of polyethylene particles as a function of size is presented in the Fig. 4.2.10. The data obtained using the laser scattering particles size distribution analyzer appeared usually duo modal, having two picks, corresponding to particles with diameter below 1 μm and around 10 μm (Fig. 4.2.11.).

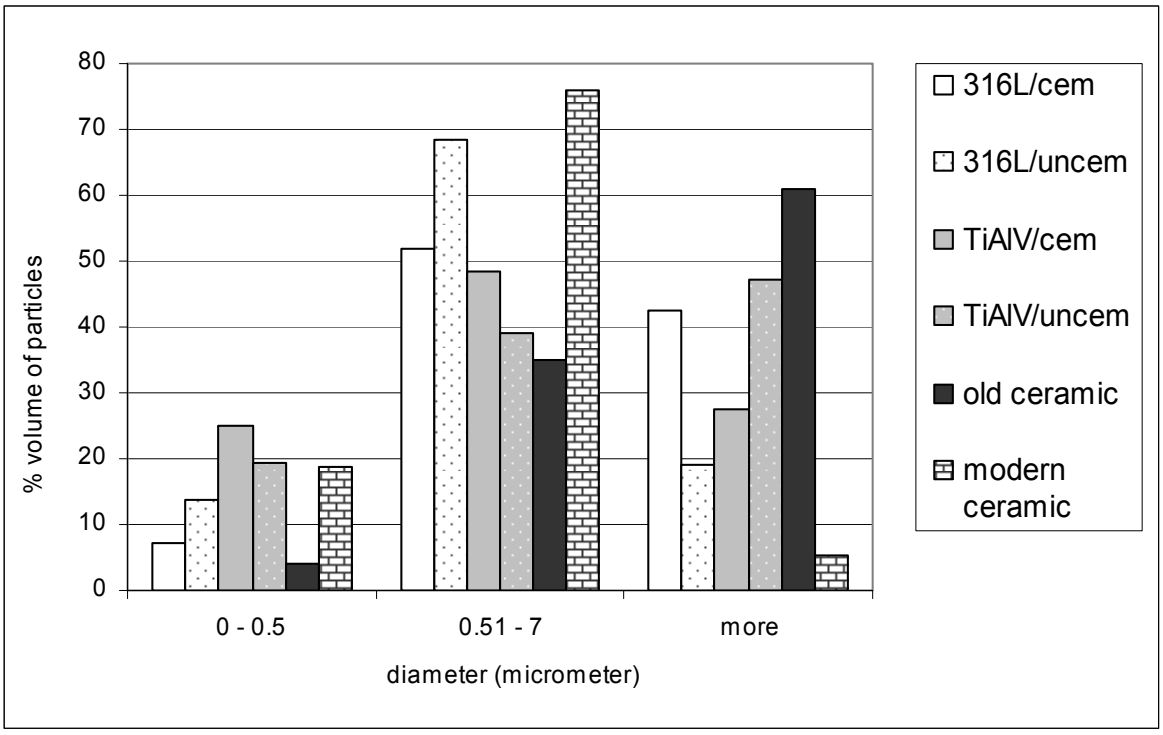


Fig. 4.2.10. Volume distribution of polyethylene particles
(316L/cem – stainless steel femoral head group with cemented cup, 316L/uncem – stainless steel femoral head group with uncemented cup, TiAlV/cem – titanium alloy femoral head group with cemented cup, TiAlV/uncem – titanium alloy femoral head group with uncemented cup).

The biggest particle volume concentration was in the 0.51-7 μm size range. Although the majority of the number of particles were in the 0-0.5 μm size range (Fig. 4.2.8.) this had little effects on the volumetric concentration distribution due to the extremely small volume of the individual particles below 0.5 μm. The biggest particle

volume concentration in the size bigger than 7 μm was in the group of old ceramic femoral heads while the smallest was in the group of modern ceramic femoral heads.

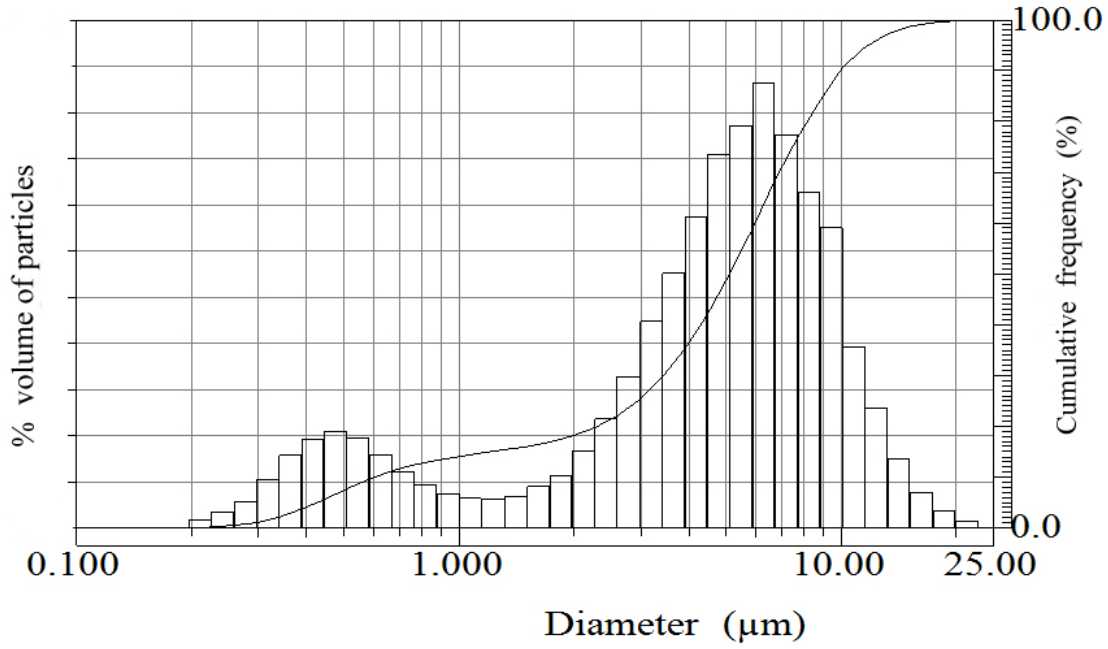


Fig. 4.2.11. The example of laser scattering polyethylene particle volumetric concentration distribution.

4.2.4 Discussion

Cement particles were isolated from periprosthetic tissues retrieved from all examined implant groups. Polymethylmethacrylate is very brittle material, which in conditions of repeating loading fracture releasing wear particles. The fracture of cement mantle did not depend on the femoral head material since in all examined implants bone cement particles were found.

Cement particles can intensify wear of femoral head material and acetabular component material [138, 170]. Hard acrylic particles can be entrapped between the articulating bearing surfaces and contribute to third-body wear [58]. Cement particles can also be embedded in the surface of polyethylene cup and lead to abrasive wear of femoral head [59]. Cement particles in all examined implants enhanced the wear process.

Organic particles were found in 94% of tissue samples. Calcium and phosphorus (Ca-P)-rich particles similar to bone minerals were also very common.

Metal particles were isolated only in 23% of examined tissue samples. Metal particles were expected to be found in all tissue samples coming from groups with metal femoral heads, because of many wear traces on the metal femoral heads surfaces (Fig. 4.1.2. and Fig. 4.1.3.).

Stainless steel particles coming from femoral head material were isolated from 12% of tissue samples in the group of stainless steel femoral head with uncemented cups. Titanium alloy particles could originate either from femoral stems or femoral heads. In the examined cases titanium particles originated from femoral stems, because they were found in the groups with old ceramic and stainless steel femoral heads. Titanium alloy particles were found in 12% of tissue samples.

The isolation method was focused on the polyethylene particles isolation. Probably this method was not sensitive enough to isolate metal particles. Maybe small metal particles could be lost during the isolation procedure. The application of strong alkali for tissue digestion may lead to dissolution of the very small metal particles [103]. It could be also possible that small metal particles do not descend to the bottom of the tube during the differential centrifugation and are mixed with polyethylene particles. During the SEM image procedure they are covered by gold layer, which precludes their identification by energy dispersive X-ray spectroscopy.

During isolation procedure no ceramic particles were found, although many erosive wear traces on the ceramic femoral heads. Ceramic particles are even smaller than metal particles [158] therefore they could not be identified by this method.

Polyethylene particles were isolated from all examined tissue samples. Polyethylene is very susceptible to wear. In normal usage of artificial joint, polyethylene wears out intensively producing numerous wear particles. Different sizes and morphologies of polyethylene particles were found in examined tissue samples (Fig. 4.2.5.). SEM images analysis revealed that majority of polyethylene particles was very small (Fig. 4.2.8.). Ninety percent of them were smaller than 0.5 μm (equivalent circle diameter) in all groups. The result obtained is in accordance with the literature data reporting that the majority of particles could be smaller than 0.5 μm in size [15, 19, 91, 103, 146, 157].

Size of polyethylene particles in this study was measured on the basis of representative 2D scanning electron microscopy (SEM) images. This method has many limitations: underestimation large size fraction of particles, time-consuming and examination of small fraction of the sample.

The SEM pictures for analysis were done in high magnification (10000 and 20000), because the isolated particles were mostly very small. The high magnification of analyzed SEM images can introduce errors in the amount of big particles that can be significantly reduced. The SEM images in the high magnification cannot enclose any of big particles, which would then occupy almost whole area of the picture. This could be the reason why only few percent of polyethylene particles in the SEM image analysis were bigger than 0.5 μm. The isolation revealed particles bigger than 20 μm (Fig. 4.2.5. a), but they were not taken into account during image analysis, because they were bigger than the area of analyzed images.

The image analysis was very time consuming procedure, every polyethylene particle should have been pointed manually. Even though more than fifty four thousands of particles were pointed out, it was still small sample, which could bring a certain error.

The average size of polyethylene particles in all groups was smaller than 0.3 μm (Fig. 4.2.8.). The significant difference was visible in the ceramic groups. In the modern ceramic group polyethylene particles were the smallest, while in the old ceramic group polyethylene particles were the biggest (Fig. 4.2.6.) (statistically significant $p<0.01$ in Kruskal-Wallis test). There was small difference in the average size of polyethylene

particles among metal femoral head groups (Fig. 4.2.7.). However, the differences were statistically significant $p<0.01$ in Kruskal-Wallis test, because the particle populations have different distribution for each metal femoral head group of implant.

The SEM images analysis have shown that many polyethylene particles were smaller than 0.1 μm (Fig. 4.2.9.). Wear debris of nanometer size is taken up by the immune cells in a different manner than phagocytosis, probably by pinocytosis [77]. Pinocytosis is an uptake of small particles with fluid into defence system cells; it is a routine cell activity. The pinocytosis does not activate the inflammatory reaction in the same way as phagocytosis does, so the particles in this size are not biologically active and do not endanger the stability of the implant [55, 57, 75]. Moreover intensive transport of small particles from periprosthetic tissue by lymphatic vessels to regional lymph nodes and occasionally other tissues [37, 165] limits the biological influence of very small polyethylene particles.

Several previous studies have employed filter with the 0.2 μm pore length and vacuum filtrating [1, 23, 91, 93, 111, 117] for wear particle isolation. Such a procedure could cause the loss of particle smaller than pore size. The polyethylene particles smaller than 0.1 μm were found by the transmission electron microscopy (TEM) analysis in the study of Benz and co-workers [15], by the field emission gun scanning electron microscopy (FEGSEM) by Galvin and co-workers [48] and by the scanning electron microscopy (SEM) in the study of Scott et al. [142]. We did not use the vacuum filtration of particles through the filter, so we obtained more than 60% of particles smaller than pores in the filter paper. It is important, because some authors neglect this fracture of wear particles due to inadequate method procedure [87, 96].

The biggest number of biological inert particles was observed in the modern ceramic group of implants, where more than forty percent (42%) of polyethylene particles were smaller than 0.1 μm. In the group of old ceramic femoral heads only 13% of all particles were smaller than 0.1 μm, which indicated that 87% of particles were biological active in this group (statistically significant $p<0.01$ in Kruskal-Wallis test).

The polyethylene particles of the phagocytosable size are the most important in the aseptic loosening [55]. Although the mononuclear histiocytes (macrophages) cannot digest polyethylene particles, they phagocytosed them. After phagocytosis macrophages become active and produce many inflammatory mediators (TNF-α, IL-1, IL-6) [129, 145], which evoke inflammation in the periprosthetic tissues. These mediators activate osteoclasts to

resorb bone tissue, decrease the activity of osteoblasts producing bone tissue, and activate matrix metalloproteinase's digesting the extracellular matrix of bone tissue. All these effects subsequently lead to bone resorption. The prosthesis becomes then unstable, causes a lot of pain and revision surgery is needed.

For all examined implant groups the predominant percentage number of the wear debris particles was in the size range 0.11-0.5 μm. Particles in this size range could be phagocytosed by the macrophages and evoke inflammatory cascade. It has been shown that particles within the critical range between 0.2 and 0.8 μm determine the biological response to the debris [75]. More particles in this size range were in the group with old ceramic femoral heads, which suggests more acute inflammatory reaction in the periprosthetic tissue (statistically significant $p<0.01$ in Kruskal-Wallis test).

The differences according to the large polyethylene particles were observed among examined groups. The biggest number of large particles was revealed in old ceramic group (statistically significant $p<0.01$ in Kruskal-Wallis test). Large polyethylene particles are surrounded by the giant cells in the periprosthetic tissues. Bigger number of large particle involves more giant cells to defence the organism and isolate them from the tissue. There are data confirming the osteolytic features of giant cells [83, 125, 132, 135, 136]. Large particles can also induce more intense rise in the white blood-cell count and in the production of prostaglandin E2 and metalloproteinases [104], which contribute to bone resorption. Additionally, big particles can have knife-like to needle-like shape and under the motion of the joints may caused increased soft tissue trauma with local small bleeding episodes and other increased fibrosis and periprosthetic joint restriction [37, 46]. All these data confirmed that large particles are a menace to implant stability.

Larger particles contribute little to the percentage frequency distribution of the particles size, especially in the SEM analysis but they significantly influence the total number of particles produced per unit volume of wear debris [17, 146]. In the laser particles analysis percentage volume of particles was examined. The laser examination confirmed that particles of big size significantly contribute to the volume of particles (Fig. 4.2.10.). Particles smaller than 0.5 μm (even though they comprise large number of particles showed in the SEM image analysis) had very small, negligible contribution in percentage volume distribution. On the other hand, particles larger than 0.5 μm, although fewer in number, had greater contribution of the overall percentage volume distribution due to greater volume of individual particle. It was demonstrated that a single 10-micron-

diameter particle contains the same volume as 8000 of the 0.5-micron particle [111], so the number of particles produced per unit of wear volume is greatly increased when the particles are very small.

Analysis based on the laser scattering is very quick and easy method, allowing many samples to be analyzed; nevertheless it can bring some errors in particle measurements. SEM image analysis confirmed that small polyethylene particles are always aggregated and were never found in the form of individual particles. In the laser analysis big conglomerate composed of many small particles could be counted as one large particle, which could lead to overestimation of large particles.

The accurate characterization of retrieved polyethylene particles is very important in prediction of the cellular response of the periprosthetic tissue. The detailed examination of wear particles also provides information on the wear mechanisms of implant components.

This study revealed the presence of bone cement particles in all examined periprosthetic tissue samples, which enhances the wear process of all implant components.

It has been also shown, that examined implant differed in the amount of biological inactive particles that do not evoke inflammation and do not lead to osteolysis and aseptic loosening.

4.3 The histological analysis of the periprosthetic tissue samples

4.3.1 Material

The subject of this study was periprosthetic fibrous tissue samples collected during revision surgery for aseptic loosening of total hip replacements at the Valdoltra Orthopaedic Hospital in Slovenia. The tissue samples were collected at the discretion of surgeon from different locations around the loosened implants.

4.3.2 Methods

The tissues samples collected during revision surgeries were fixed in a 10% buffered formalin (0.4 M, pH=7), embedded in paraffin and sectioned into 5 μm thick paraffin sections. For microscopic examination, histological slides were stained with haematoxylin and eosin. Oil-o-red staining was used for visualization of polyethylene particles in cytoplasm of macrophages and in giant cells. Histological slides were analyzed using light and polarized microscopy (Olympus BX51, Tokyo, Japan) equipped with a digital camera. The modified version of the Mirra classification [37] was used to grade the content of metal particles, number of mononuclear histiocytes, giant cells, acute and chronic inflammatory cells, as well as areas of necrosis and necrobiosis (Tab. 4.3.1.). Different types of cells were identified by considering the size of the cell, nuclear morphology and colour of cytoplasma. Every feature was graded on a scale of 0 to 3+, where (0) denoted the absence of the considered feature, and (1+) and (3+) denoted the lowest and highest content, respectively.

On every slide at least three representative fields were examined under a magnification of 400 (HPF – high power field) or 100 (LPF – low power field) times. The results were presented as a histogram showing the percentage of tissue samples with different grades of the Mirra classification from 0 to 3+.

The data distribution was checked by the Lilliefors test (STATISTICA, version 6, StatSoft, Inc.). Because the normality assumption was not met for the data, the non-parametric Kruskal-Wallis test was used. In all tests statistically significance was used judged based on a p value of less than 0.05.

Table 4.3.1 Modified Mirra classification of periprosthetic tissues after [37].

Modified Mirra Classification			
Histology	**+1**	**+2**	**+3**
Metal Particles	Slate blue histicytes (< 10 visible, black metal particles/histiocyte)	Dusty blcka histiocytes (10 to 100 visible, black metal particles/histiocyte)	Jet black histiocytes (> 100 visible black metal particles/histiocyte)
Mononuclear histiocytes	1-5 cells/HPF	6-49 cells/HPF	50 or more cells/HPF
Giant cells (multinucleated histicytes)	1 cell/HPF	2-4 cells/HPF	5 or more cells/HPF
Polymethylmethacrylate globules	1-3 globules/LPF	4-6 globules/LPF	7 or more globules/LPF
Acute inflammatory cells (neutrophils)	1-5 cells/HPF	6-49 cells/HPF	50 or more cells/HPF
Chronic inflammatory cells (lymphocytes, plasma cells)	1-9 cells/HPF	10-49 cells/HPF	50 or more cells/HPF
Necrosis	1-2 mm of necrosis/slide	3-9 mm of necrosis/slide	> 1 cm of necrosis/slide
Necrobiosis	1-2 mm of necrobiosis/slide	3-9 mm of necrobiosis/slide	> 1 cm of necrobiosis/slide

4.3.3 Results

All examined periprosthetic tissue samples showed an intensive collagenization and characteristics of granulomatous inflammatory tissue, which contained macrophages, giant cells and fibrocytes. Additionally, the areas of necrosis and necrobiosis were visible in almost all tissue samples.

Metal wear particles were identified as oval or rounded, dark particles, which did not transmitted the light. They were present in the histiocytes (Fig. 4.3.1.), giant cells or in the areas of necrobiosis.

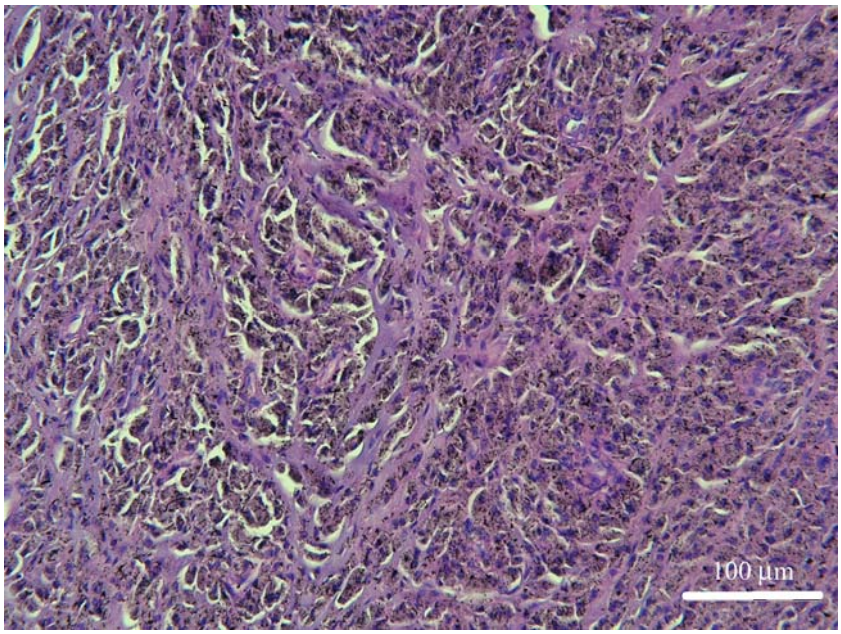


Fig. 4.3.1. Light micrograph of capsule tissue from patient who had a metal-polyethylene hip revised for aseptic loosening.
The tissue contains a high number of macrophages with metal particles.
Hematoxylin and eosin (x 200).

Metal particles were present in all examined tissue samples (Fig. 4.3.2.). Consequently, no sample was graded as 0 for metal particles. The highest intensity of metal particles was observed in the periprosthetic tissues surrounded loosened implants with stainless steel femoral heads and cemented cups (40% of tissue samples revealed grade 3+, Fig. 4.3.2.) (statistically significant in Kruskal-Wallis test $p<0.05$). The lowest amount of metal particles was observed in the tissues originated from titanium femoral heads implants with uncemented cups (100% of examined tissues with the grade 1+) and for modern ceramic group (87.5% of tissue samples with the grade of 1+).

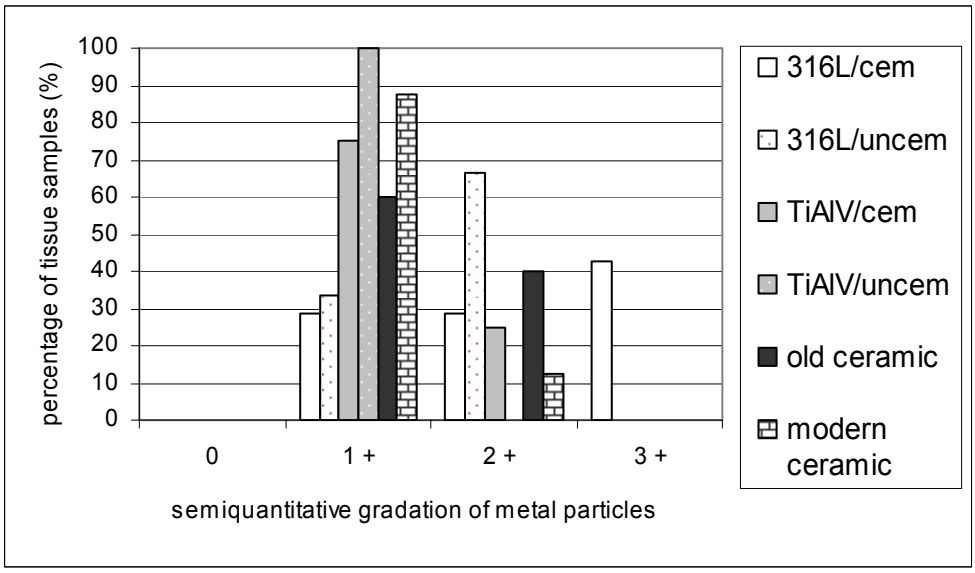


Fig. 4.3.2. Semiquantitative gradation of metal particles in periprosthetic tissue of different groups of implants.
Histogram showing the percentages of tissue samples for different grades (316L/cem – stainless steel femoral head group with cemented cup, 316L/uncem – stainless steel femoral head group with uncemented cup, TiAlV/cem – titanium alloy femoral head group with cemented cup, TiAlV/uncem – titanium alloy femoral head group with uncemented cup).

Polyethylene particles transmit the light and remain colourless in the slides stained with haematoxylin and eosin, whereas they become pink in the slides stained by the oil-o-red method (Fig. 4.3.3.). The polarized light was used to identify the polyethylene particles. Polyethylene particles, because of their birefringence, have typical shiny appearance under the polarized light (Fig. 4.3.4.).

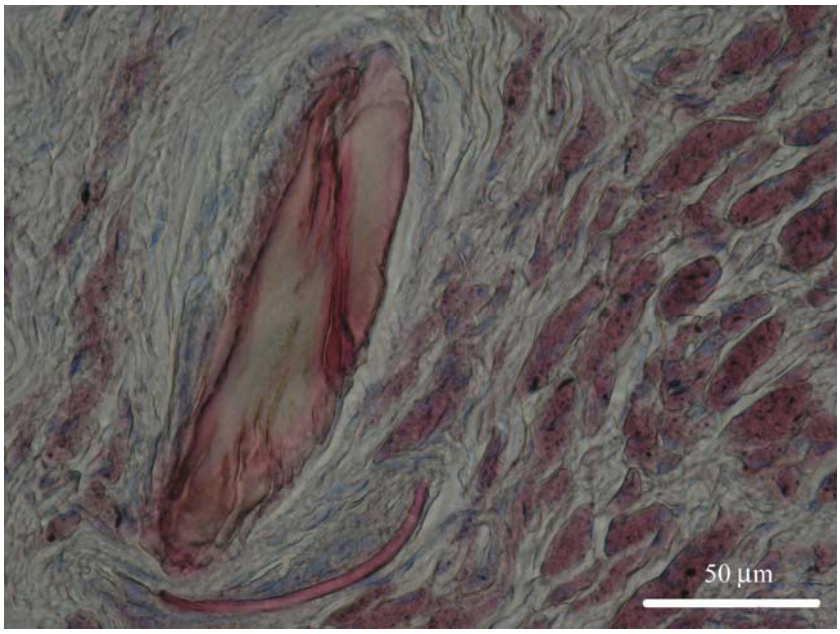


Fig. 4.3.3. Polyethylene wear particles stained by the Oil-o-red method (x 500).

Polyethylene particles were visible in macrophages (small polyethylene particles) (Fig. 4.3.4.) and in the giant cells (big polyethylene particles) (Fig. 4.3.3. and Fig. 4.3.4.).

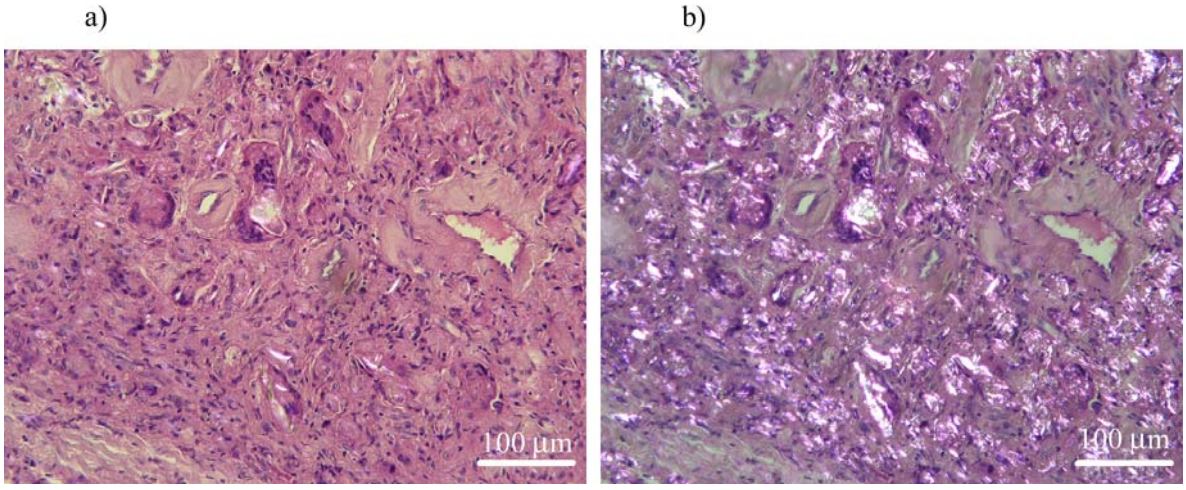


Fig. 4.3.4. Polyethylene wear particles in the periprosthetic tissue a) under light microscopy and b) polarized microscopy. The same field is shown on both pictures. Hematoxylin and eosin (x 200).

The histological analysis revealed differences among the numbers of macrophages in the periprosthetic tissues (Fig. 4.3.5.) (statistically significant in Kruskal-Wallis test $p<0.05$). Macrophages were present in all examined tissue samples. However, a higher number of macrophages was found in cemented and uncemented implants with stainless steel heads and for implants with femoral heads made of old ceramics. These three groups were graded 3+ denoting 50 or more macrophages per high power field (57%, 44% and 20% of tissue samples, respectively). The smallest number of macrophages was observed in the modern ceramic group and in the group of implants with titanium femoral head and uncemented cup.

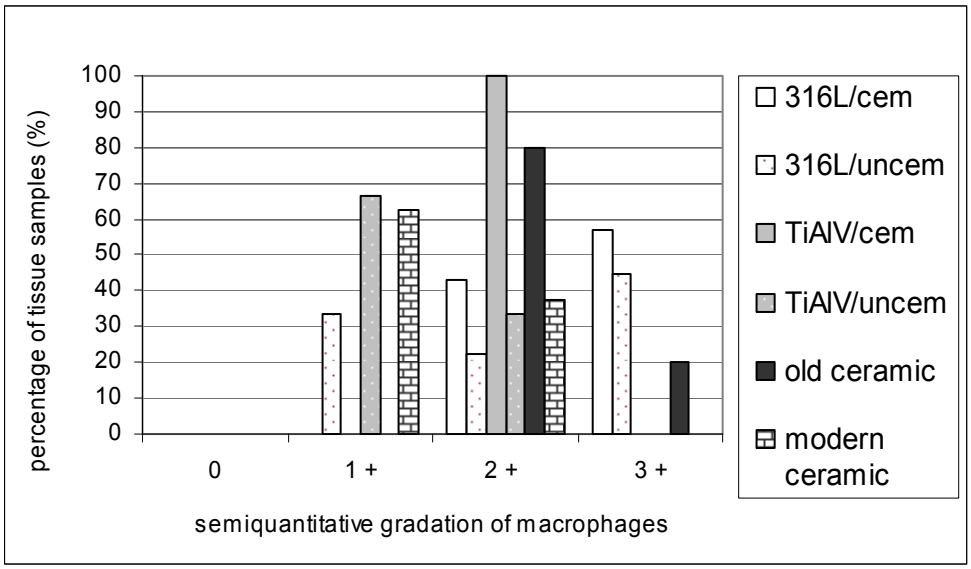


Fig. 4.3.5. Comparison of the histological grades of macrophages for different groups of implants.
Tissues were graded as described in Table 4.3.1. (316L/cem – stainless steel femoral head group with cemented cup, 316L/uncem – stainless steel femoral head group with uncemented cup, TiAlV/cem – titanium alloy femoral head group with cemented cup, TiAlV/uncem – titanium alloy femoral head group with uncemented cup).

The differences were also visible in the amount of giant cells (Fig. 4.3.6.) in the periprosthetic tissues from different implants groups (Fig. 4.3.7.) (statistically significant in Kruskal-Wallis test $p<0.05$). The lowest amount of giant cells was observed in the group with femoral heads made of modern ceramics. All other groups exhibited giant cells in the grade 3+, which refers to 5 or more giant cells per high power field.

For the group with femoral heads made of old ceramics, 60% of all tissue samples showed the grade 3+ for giant cells.

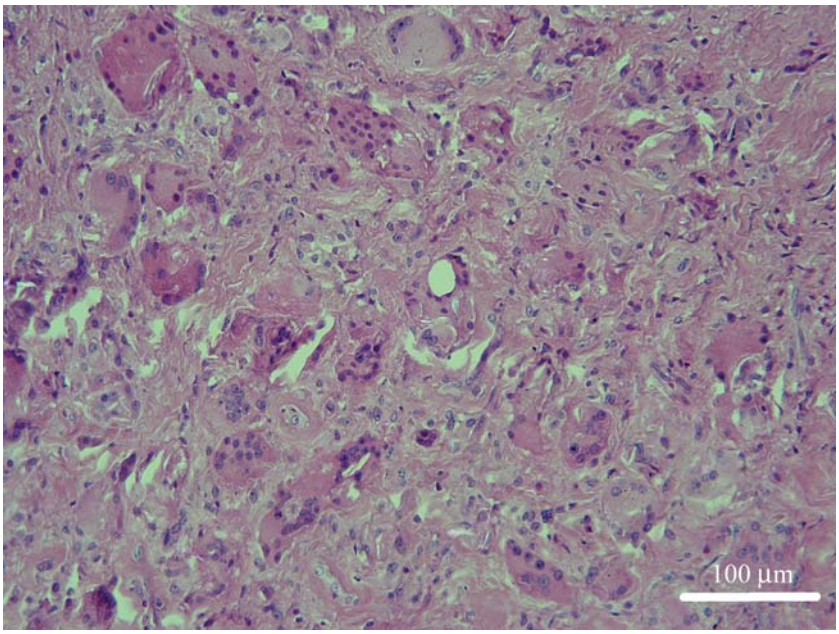


Fig. 4.3.6. Numerous giant cells in the periprosthetic tissue surrounded loosened implant with stainless steel femoral head and cemented cup. Hematoxylin and eosin (x 200).

Bone cement particles were visible in the histological slides as empty spaces (Fig. 4.3.8.). During the slide preparation procedure, polymethylmethacrylate is dissolved by xylene solvent. The size of remaining spaces in the tissues is usually in range of 100 µm. Granules of barium sulphate or zirconium dioxide, added as agents for radiological contrast, are visible as black spots at the periphery of empty space (Fig. 4.3.8.).

Semiquantitative gradation showed that more cement particles were visible in the tissues surrounded implants with metal femoral heads combined with cemented polyethylene cups (Fig. 4.3.9.), but the difference was statistically insignificant.

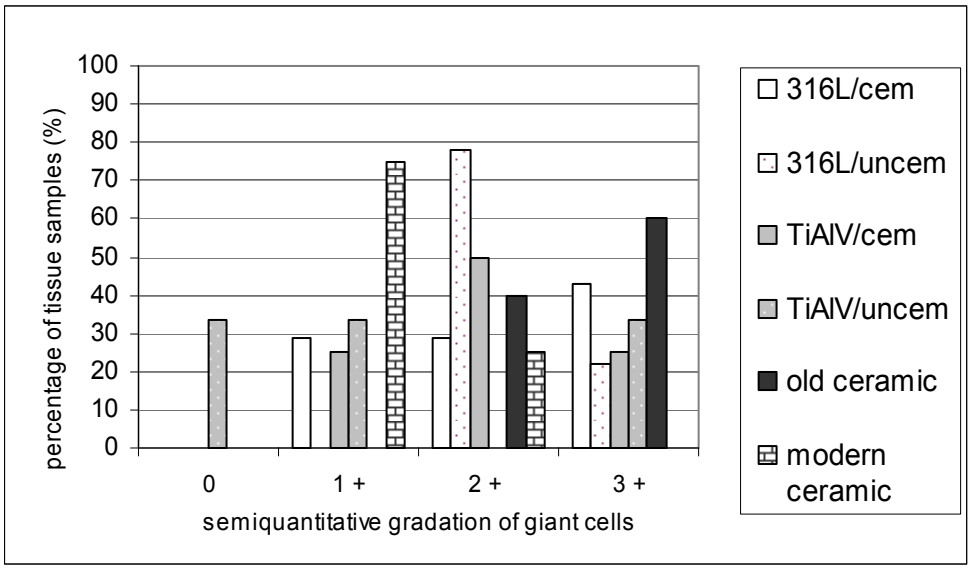


Fig. 4.3.7. Semiquantitative gradation of giant cells in periprosthetic tissue of different groups of implants.
Histogram showing the percentages of tissue samples for different grades (316L/cem – stainless steel femoral head group with cemented cup, 316L/uncem – stainless steel femoral head group with uncemented cup, TiAlV/cem – titanium alloy femoral head group with cemented cup, TiAlV/uncem – titanium alloy femoral head group with uncemented cup).

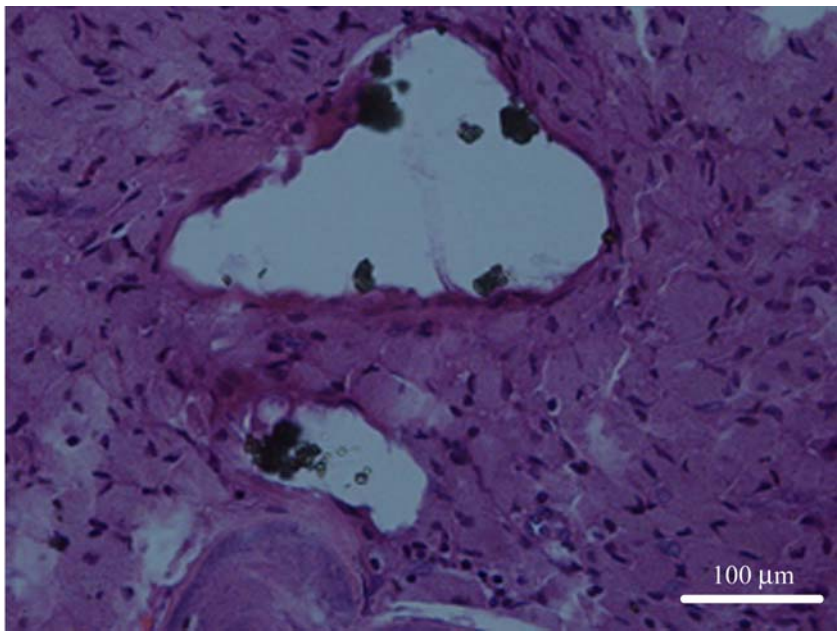


Fig. 4.3.8. Large empty spaces in the periprosthetic tissue representing masses of bone cement particles (PMMA) surrounded by the giant cells.
The bone cement was dissolved during slides preparation, only crystals of ZrO_2 or $BaSO_4$ remained Hematoxylin and eosin (x 200).

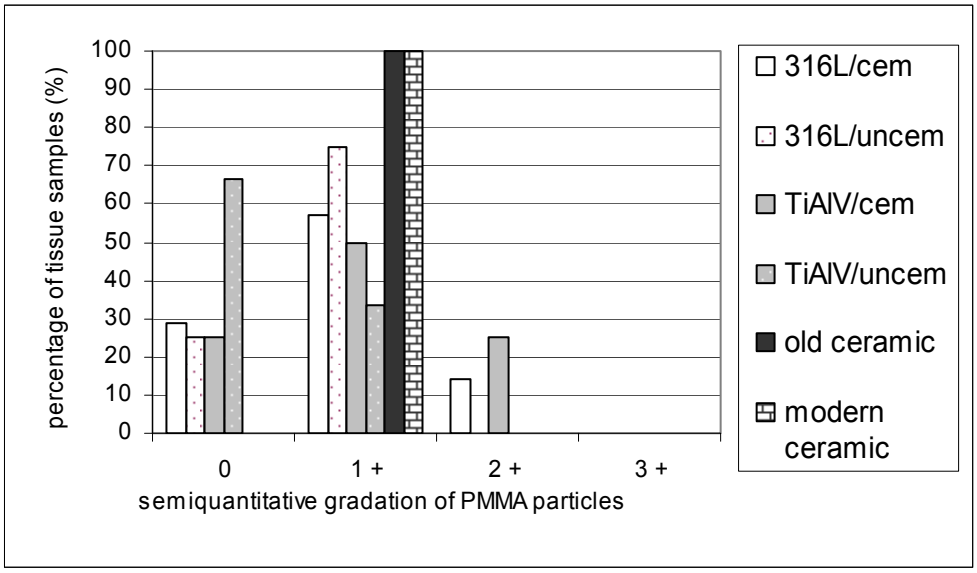


Fig. 4.3.9. Semiquantitative gradation of PMMA particles (bone cement) in periprosthetic tissue of different groups of implants.
Histogram showing the percentages of tissue samples for different grades (316L/cem – stainless steel femoral head group with cemented cup, 316L/uncem – stainless steel femoral head group with uncemented cup, TiAlV/cem – titanium alloy femoral head group with cemented cup, TiAlV/uncem – titanium alloy femoral head group with uncemented cup).

Number of acute inflammatory cells (neutrophils) was very small for all groups and can be neglected (Fig. 4.3.10.).

Chronic inflammatory cells (lymphocytes and plasma cells) were visible in higher concentration (grade 2+) in groups with metal heads (except titanium femoral heads combined with uncemented cups) and old ceramics (Fig. 4.3.11.), but the difference was statistically insignificant.

The biggest areas of necrosis (Fig. 4.3.12.) with the grade 3+ (Fig. 4.3.13.) were found in the both groups with stainless steel femoral heads.

Semiquantitative gradation showed that the biggest areas of necrobiosis were found in the tissues surrounded metal implants combined with uncemented polyethylene cups and in the group with femoral heads made of old ceramics (Fig. 4.3.15.). However the difference was statistically insignificant.

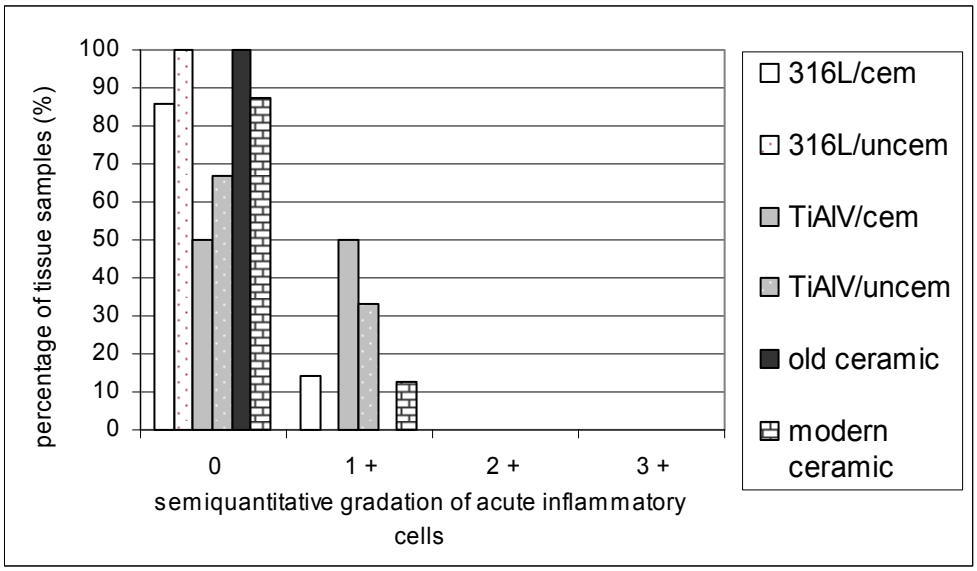


Fig. 4.3.10. Semiquantitative gradation of acute inflammatory cells in periprosthetic tissue of different groups of implants.
Histogram showing the percentages of tissue samples for different grades (316L/cem – stainless steel femoral head group with cemented cup, 316L/uncem – stainless steel femoral head group with uncemented cup, TiAlV/cem – titanium alloy femoral head group with cemented cup, TiAlV/uncem – titanium alloy femoral head group with uncemented cup).

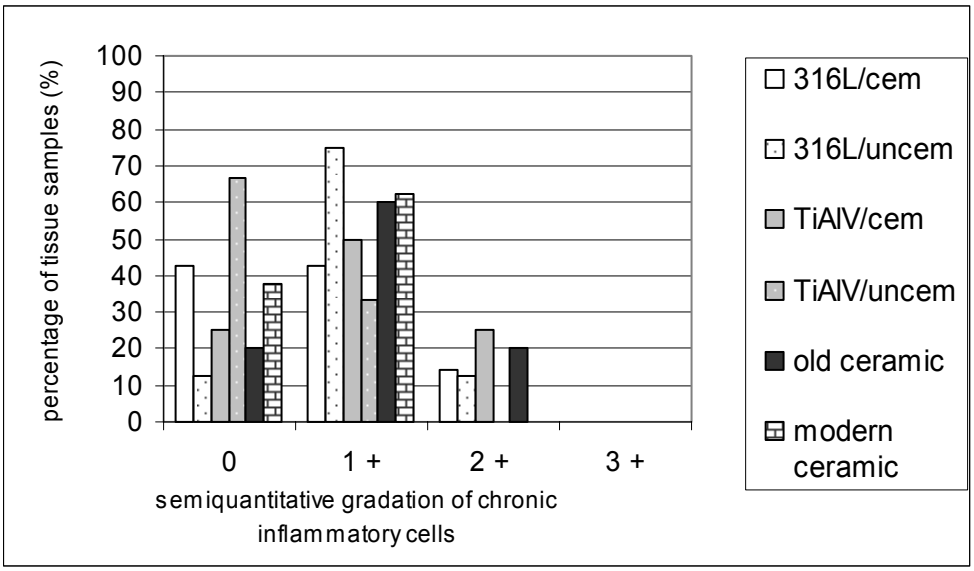


Fig. 4.3.11. Semiquantitative gradation of chronic inflammatory cells in periprosthetic tissue of different groups of implants.
Histogram showing the percentages of tissue samples for different grades (316L/cem – stainless steel femoral head group with cemented cup, 316L/uncem – stainless steel femoral head group with uncemented cup, TiAlV/cem – titanium alloy femoral head group with cemented cup, TiAlV/uncem – titanium alloy femoral head group with uncemented cup).

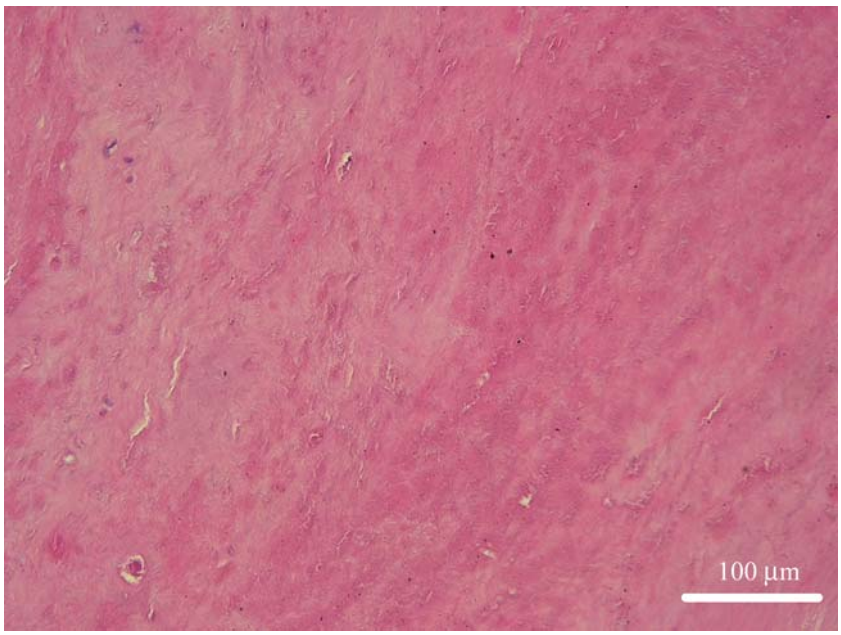


Fig. 4.3.12. Photomicrograph showing necrosis in the periprosthetic tissue.
Hematoxylin and eosin (x 200).

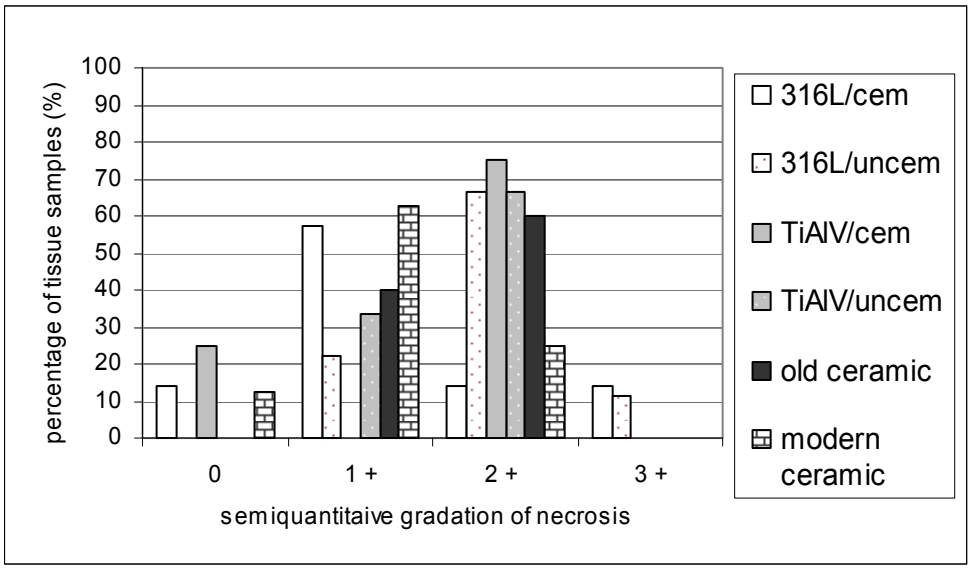


Fig. 4.3.13. Semiquantitative gradation of area of necrosis in periprosthetic tissue of different groups of implants.
Histogram showing the percentages of tissue samples for different grades (316L/cem – stainless steel femoral head group with cemented cup, 316L/uncem – stainless steel femoral head group with uncemented cup, TiAlV/cem – titanium alloy femoral head group with cemented cup, TiAlV/uncem – titanium alloy femoral head group with uncemented cup).

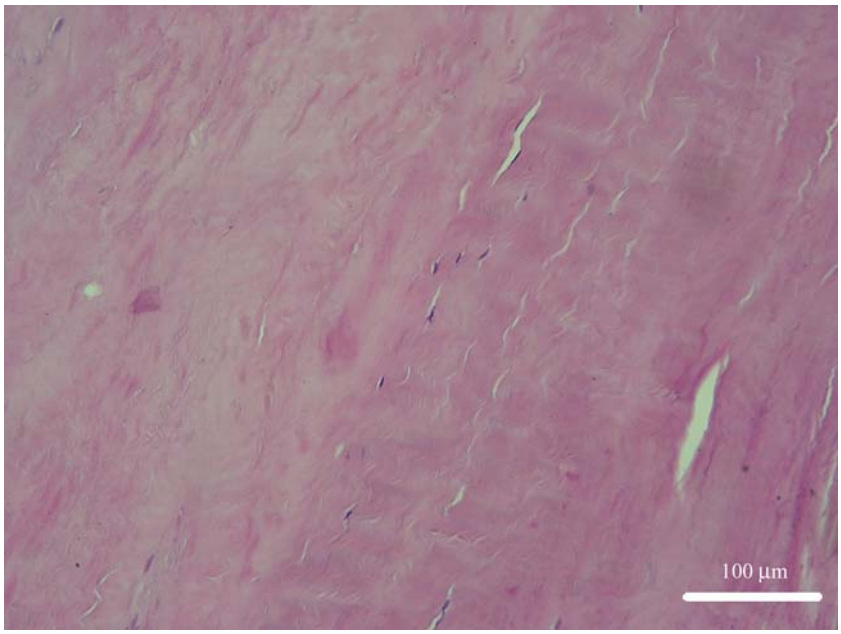


Fig. 4.3.14. Photomicrograph showing necrobiosis in the periprosthetic tissue.
Hematoxylin and eosin (x 200).

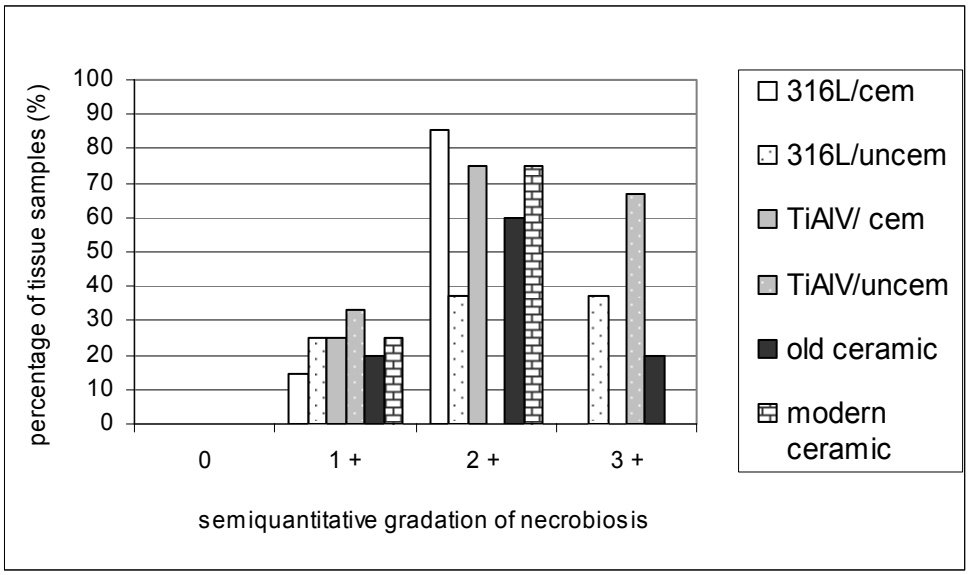


Fig. 4.3.15. Semiquantitative gradation of areas of necrobiosis in periprosthetic tissue of different groups of implants.
Histogram showing the percentages of tissue samples for different grades (316L/cem – stainless steel femoral head group with cemented cup, 316L/uncem – stainless steel femoral head group with uncemented cup, TiAlV/cem – titanium alloy femoral head group with cemented cup, TiAlV/uncem – titanium alloy femoral head group with uncemented cup).

4.3.4 Discussion

At the beginning of the use of total hip replacements the process of loosening was regarded to be related mainly to the mechanical factors. It was believed that if the mechanical stress is properly transferred to the bone present around implants, they can support the joint function without loosening and without causing periprosthetic osteolysis. However, the osteolysis and fibrous tissue on the bone-implant interface appeared even in technically well-performed total joint replacements [27]. The histopathology of the periprosthetic tissue of the retrieved hip prosthesis showed that a macrophagical reaction was evident even in the presence of a stable bone-cement interface [144]. This suggests that besides mechanical factors, also biological factors participate in the mechanism of aseptic loosening. The tissue reaction to wear particles therefore determines the long-term success of an implant. Enhanced inflammatory reaction contributes to the osteolysis and subsequently leads to the loosening of the implant. It is thus important to carry out the histological analysis of periprosthetic tissues to gain information about the intensity of inflammatory response as a function of the type of implant.

The histological analysis of periprosthetic tissues performed in this study confirmed that the inflammatory fibrous tissues were present in all examined tissue samples.

Metal particles were graded by the appearance of the macrophages and number of metal particles within them. Metal particles originate from the corrosion or wear process of metal components of the prostheses: stems and femoral heads. When present in a large amount, metal particles may cause intensive coloration, black or various shades of grey, of the joint tissue. This process is known as metallosis. Metal particles are mainly stored in macrophages that are cells responsible for the exclusion of foreign bodies. Moreover, the presence of physiological fluids increases the release of ions from the surface of metal particles by a corrosion reaction. Although metal particles are small, their total surface area is enormously big. Consequently, high concentrations of metal ions can be released due to dissolution of metal particles [108]. Released metal ions may be toxic for human body and lead to necrosis of periprosthetic tissues [75, 108, 114], evoke immunological response, and also induce neoplasia. Metal particles and ions are toxic for osteoblasts [4], and they decrease osteoblasts activity in bone tissue formation around implant, which precludes osteointegration of implant.

The highest number of metal particles was observed in tissues surrounded loosened implants with stainless steel femoral heads and cemented polyethylene cups. In these implants, metal wear particles originated from the wear of femoral heads and femoral stems. Increased dissolution of metal particles can lead to increased metal concentrations in serum and blood [115]. Therefore, it was recommended that these patients are carefully monitored.

In the groups of patients with metal femoral heads, more metal particles were visible in the groups with cemented cups than in the groups with uncemented acetabular components (statistically significant in Kruskal-Wallis test $p<0.05$). It seems that cement particles originating from the fixation of polyethylene cup intensify the wear process of metal components and increase the generation of metal particles. However, no significant difference in the roughness of metal femoral heads combined with cemented or uncemented polyethylene cups was observed (Fig. 4.1.12.), so probably the metal particles come from worn stems.

Less metal particles were found in the group with titanium alloy femoral heads than in the group with stainless steel femoral heads. There was no significant difference in the roughness between titanium and stainless steel femoral heads (Fig. 4.1.12.), which could suggest that the same amount of metal particles would be found in both groups, which was not the case. A possible explanation would be that particles of titanium alloy were smaller than stainless steel particles. Smaller metal particles may be more easily transported away from the joint tissue and migrate with body fluids to lymph nodes [37].

Mononuclear histiocytes (macrophages) are considered to be the most important cells in the aseptic loosening of orthopaedic implants. They belong to the immunological system. Mononuclear histiocytes are stationary, immobile macrophages responsible for isolation and elimination of foreign bodies from the human tissues. Macrophages phagocytose foreign bodies and thus isolate them from other cells. Organic foreign bodies, such as bacteria or dead cells, can be digested by enzymes present in the macrophages. In the periprosthetic tissues, wear particles are treated as foreign bodies by macrophages. They become phagocytosed, but they cannot be digested, because they are inorganic materials (polyethylene, metal or ceramic). Indigestible wear particles are eliminated from the macrophages without any changes in the composition and continuously activate other macrophages. Phagocytosis of particles that cannot be digested causes many changes in the macrophages: increased metabolism, enhanced mobility and irreversible activity to

produce proinflammatory mediators (cytokines, interleukins, for example TNF-α, IL-1, IL-6), which evoke inflammation in the periprosthetic tissues [129, 145, 174]. These mediators have potential osteolytic features, activate osteoclasts to resorb bone tissue, decrease the activity of the osteoblasts producing bone tissue, and activate matrix metalloproteinases to digesting the extracellular matrix of bone tissue. All these effects subsequently lead to bone resorption. Under these conditions the implant is surrounded by the inflamed, soft tissue, which does not provide a sufficient support and anchorage. The prosthesis becomes unstable causing a lot of pain and a decreased mobility leading to the need for a revision surgery.

Moreover, macrophages that have phagocytosed wear particles are able to provoke directly the bone resorption and differentiation to osteoclasts [10, 58, 119, 128, 135, 136]. The number of macrophages in the periprosthetic tissue determines the malignancy of the inflammatory reaction.

The highest amount of macrophages was found in the tissues surrounded loosened cemented and uncemented implants with stainless steel femoral heads and with femoral heads made of old ceramics (Fig. 4.3.5.) (statistically significant in Kruskal-Wallis test $p<0.05$). This was indicated by an intensive inflammatory reaction to huge amount of wear particles. Macrophages could contain metal particles (Fig. 4.3.1.). Since in these three groups the highest amount of metal particles was observed (Fig. 4.3.2.), many macrophages were recruited to eliminate them. Besides metal particles, macrophages contained small polyethylene particles abundantly present in the periprosthetic tissues (Fig. 4.3.4.). Ohashi et al. showed that the presence of polyethylene particles stimulate the production of fibrous tissue around loosened implant [127].

The smallest number of macrophages was observed in the tissues with femoral heads made of modern ceramics and in the group of titanium alloy femoral heads combined with uncemented polyethylene cups. At the same time, the smallest intensity of metal particles was observed in these groups (Fig. 4.3.2.), so the macrophages in these groups were mostly saturated by polyethylene particles. Smaller number of macrophages release smaller amount of inflammatory mediators. The periprosthetic tissues surrounded these implants exhibited a milder histological reaction, so the probability of implant loosening is smaller than in other groups.

Multinucleated giant cells (MNGC) are characteristic for inflammatory reaction around loosened implants. They arise during fusion of many macrophages and are engaged

in the removal of large foreign objects. In the periprosthetic tissues, giant cells are responsible for the elimination big polyethylene and bone cement particles. There is no agreement in the literature concerning the function of the giant cells in the aseptic loosening; the osteolytic potential of these cells is still unknown. Some literature data reported that giant cells are passive cells, aiming only to isolate big particles from other cells [10]. However, many authors suggest that giant cells share certain morphologic, phenotypic, and functional characteristics with osteoclasts responsible for digestion of bone tissue [83, 125, 128, 132, 135, 136]. This suggests that giant cells can rather directly digest bone tissue thus contributing to the osteolysis around loosened implants. Therefore, they play a significant role in the aseptic loosening of orthopaedic implants. In favour of the latter hypothesis, similar cytokine profiles were observed for macrophages, foreign body giant cells and mature osteoclasts, thus indicating that osteoclasts and foreign body giant cells are of macrophage origin [125] and all of them can take part in bone tissue resorption. Kadoya et al. [85] showed by electron microscopy that giant cells have an osteoclast-like ruffled border, which suggests an ability of these cells to resorb bone.

Therefore, increased number of giant cells in periprosthetic tissue is a menace to bone tissue around orthopaedic implant and decreases the ability of an implant for osteointegration.

The biggest amount of giant cells was observed in the tissues surrounded loosened implants with femoral heads made of old ceramics (Fig. 4.3.7.) (statistically significant in Kruskal-Wallis test $p<0.05$). Semiquantitative gradation showed that sixty percent of tissue samples exhibited grade 3+ denoted by five or more giant cells per high power field. A huge number of large wear particles, made of polyethylene or bone cement, were isolated from the tissue by giant cells. This would suggest that patients with a high volume of giant cells in the periprosthetic tissues are more susceptible for bone resorption.

The smallest amount of giant cells was found in the group of implants with femoral heads made of modern ceramics (no tissue sample exhibited 3+ grade), thus indicating a small amount of big particles in these tissues. Patient with prostheses made of modern ceramics are potentially less subjected to osteolysis, because a lesser amount of giant cells take part in bone resorption.

Polymethylmethacrylate (bone cement) particles were dissolved during slide processing (Fig. 4.3.8.). Remaining empty spaces were quite big, ca. 100 μm, and contained radiological contrast granules within them. Bone cement particles were

surrounded by giant cells. More bone cement particles were visible in the groups of implant combined with cemented polyethylene cups (Fig. 4.3.9.). In these implants, cement particles originated from the brittle bone cement mantle around femoral stem and acetabular component. However the difference was statistically insignificant.

Number of neutrophilis, acute inflammatory cells, was very small in all examined groups. The presence of these cells in the periprosthetic tissue samples can be an indication of infection [30], but bacteriological assessment negated the development of infection in all examined tissue samples.

Lymphocytes are one of the characteristics of a chronic inflammation. Lymphocytic reaction can indicate hypersensitiveness to wear debris. Mirra et al. [118] suggested that lymphocytes can indicate an infection proceeding around implant or chronic inflammation evoked by many wear particles. Because no signs of infection were observed, the lymphocytes were connected to the tissue response to wear particles. However, the number of lymphocytes was not so high as to indicate that they were involved in a specific response. Lymphocytes may have an immuno-modulatory effect on the foreign body reaction [52]. The difference in amount of chronic inflammation cells was insignificant.

Conventional necrosis is characterized by acellular debris without evidence of a collagenous background (Fig. 4.3.12.). The term necrobiosis associated with periprosthetic tissue was introduced by Doorn and co-workers [37]. Necrobiosis is characterized by the maintenance of organization of collagen fibres between which wear particles can be found, usually without fibroblast nuclei and outlines of cells [37] (Fig. 4.3.14.). Necrobiosis is characteristic for the initial stage of tissues damage, whereas the appearance of conventional necrosis is more likely in the later stage.

It has been reported that metal particles can lead to the necrosis of periprosthetic tissues [108, 114]. The release of ions from small ceramic and metal wear debris can be toxic and induce tissue necrobiosis [64]. There are also other hypotheses about the origin of necrosis and necrobiosis. Damage of the tissue due the micro-movements of prosthetic components can contribute to the necrosis and necrobiosis of periprosthetic tissue. A disturbance in the blood supply may also cause necrosis and necrobiosis of tissues [37]. Henssge et al. [68] suggested that necrosis of periprosthetic tissue is caused by the cement particles. In this study no significant difference was visible among areas of necrosis and necrobiosis.

The histological analysis of periprosthetic tissues showed that aseptic loosening of orthopaedic implants is influenced by the tissue response to implant material and wear particles. Different intensity of inflammatory reaction was found in tissues surrounding different kinds of implants. This study evidenced that examined implants differed in amount of macrophages and giant cells and thus differed in potential of osteolysis. The group of implants made of old ceramics had the greatest biological potential accelerating the process of loosening, because of the highest amount of wear particles and cells responsible for aseptic loosening in the tissues surrounding these implants.

It was also proved that more metal particles that are toxic are present in the tissues surrounding implants with metal femoral heads and old ceramic femoral heads. The toxicity of metal particles was confirmed by the large areas of necrosis in these groups. Necrotic tissues cannot give any support for prosthesis which than develop loosening.

4.4 The characterization of the biological reaction proceeding at the bone-implant interface

The study of the effect of wear particles on the cell cultures was carried out at the Biostructure Centrum of Warsaw Medical University, Division of Histology and Embryology under the supervision of Professor Jacek Malejczyk. The in vitro experiments offer the opportunity to mimic the biological reactions proceeding at the bone-implant interface in the artificial joint and to predict the influence of wear particles on periprosthetic tissues in vivo. In the experiments, synovial cells and peripheral blood leukocytes were exposed to polyethylene particles generated using the pin-on-disc apparatus.

4.4.1 Material

The study was carried out using cell cultures of synovial cells and peripheral blood leukocytes. Fragments of synovial membranes were obtained from patients during synovectomy surgery (excision of the synovial membrane of the joint) at the Institute of Rheumatology in Warsaw. Synovial cells were examined because they are intensively exposed to wear particles in vivo. Human synovial cells were isolated from synovial membrane samples by enzymatic digestion using collagenase (Sigma-Aldrich) at 37ºC for 2 hours.

The experiments were also performed on peripheral blood leukocytes obtained from peripheral blood drawn from the author of this work. Peripheral blood leukocytes were isolated by Ficoll-Hypaque density gradient centrifugation (Histopaque 1077, Sigma-Aldrich). Peripheral blood leukocytes can differentiate into macrophages, which play an important role in periprosthetic tissues.

The obtained cells were cultured in RPMI 1640 medium (Sigma-Aldrich) containing 0.5% antibiotics under the standard conditions (90% humidity, 5% CO_2, 37ºC).

The examined cells were stimulated by wear particles of ultra high molecular weight polyethylene (UHMWPE). The study was focused on polyethylene particles

because they are the most abundant wear particles in artificial joint containing polyethylene components.

Polyethylene wear particles (GUR 1050) used in this study were generated in vitro under sterile conditions on a commercial pin-on-disc apparatus (Perplas Medical. Lancashire, UK). The considered wear particles were smaller than 1 μm and were free of endotoxins. Morphology of the particles was examined by scanning electron microscopy (SEM, Hitachi S-2600) (Fig. 4.4.1.). Polyethylene particles were sterilized in ethylene oxide.

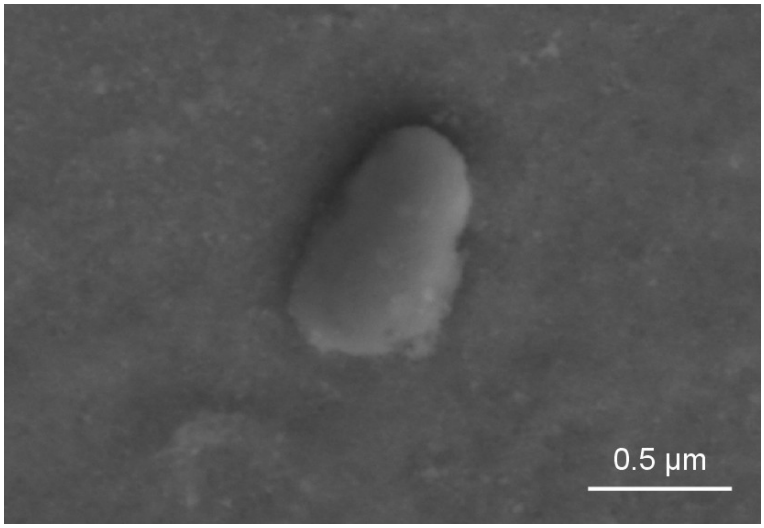


Fig. 4.4.1. Polyethylene particle used for in vitro studies (SEM, x 50000)

4.4.2 Methods

Stimulation of cell cultures by wear particles

Cells were cultivated in 24-well plastic microplates. The confluent cultures of human synovial cells from 2-4 passages and peripheral blood leukocytes after isolation were treated with polyethylene wear particles in the concentration of $6x10^5$ particles per one million of cells. Polyethylene particles were added directly to the culture medium (RPMI medium with antibiotics, without fetal calf serum). It has been shown that fetal calf serum modulated the inflammatory response of examined cell cultures because of endotoxin content (unpublished data Malejczyk et al.); therefore, the cells were cultivated without fetal calf serum.

After 24 or 48 hours treatment, cell culture media were collected and frozen, and cells were counted. Cell cultures incubated with lipopolysaccharide from the walls of gram-negative bacteria (LPS, Sigma-Aldrich) were used as a positive control. All experiments were performed according to the protocols approved by the ethics committee.

The quantitative determination of cytokines (IL-1β, TNF-α) and precursor of metaloproteinase 1 (pro-MMP-1)

The concentration of IL-1β, TNF-α and pro-MMP-1 in conditioned media were determined by commercially available double sandwich quantitative enzyme-linked immunosorbent assay (ELISA) (Biosource or R&D Systems) according to the manufacturer's protocol. The optical densities of examined samples were read using microplate spectrophotometry reader capable of measuring the absorbance at 450 nm. For all tests, the standard curves were drawn based on the absorbance of known amount of proteins.

Measurement of metaloproteinases activity by zymography

Zymography method was used to analyze gelatinases (MMP-2 and MMP-9) activity in the conditioned media of synovial cells and peripheral blood leukocytes cultures. Zymography is an electrophoretic technique that involves the determination of the ability of culture supernatants to digest gelatin in sodium dodecyl sulphate polyacrylamide gels (SDS-PAGE).

The samples were loaded on polyacrylamide gels containing gelatin and electrophoresed. Following the electrophoresis, gels were incubated for 1 hour in incubation buffer (Tris-HCl, $CaCl_2$, $ZnCl_2$ and Triton X-100) to remove SDS (dissociating agent used to denature native proteins), and then in the same buffer without Triton X-100 at 37ºC for 3 hours. Gels were stained with Coomassie brilliant blue and distained in a solution of methanol. Enzyme activity was detected as a clear zone in a dark stained background where the substrate has been degraded by enzymes.

Transmission electron microscopy

Cell cultures of synovial cells incubated with polyethylene wear particles were fixed overnight in a solution of glutaraldehyde. Thereafter, specimens were washed in phosphate buffer, fixed with 1% osmium tetroxide, dehydrated in ethanol and embedded in epoxy resin (eponate). Thin sections were cut on ultramicrotome (Reichert) with diamond knife and viewed with transmission electron microscope (TEM, Hitachi).

Statistics

Data was analyzed by unpaired Student t-test, because the variables were normally distributed (Shapiro-Wilk test) (STATISTICA, version 6, StatSoft, Inc.). P values smaller than 0.05 were considered significant.

4.4.3 Results

Cells exposed to the polyethylene wear particles released proinflammatory mediators and enzymes to culture media. The activity of cytokines and enzymes was measured in conditioned media of investigated cell cultures.

Examined synovial cells were retrieved from three different patients diagnosed for arthrosis. The inflammatory response significantly differed among patients, but the trend was similar. In this work for simplification the results for one patient were presented. The results were shown as a mean value of three repetitive tests with standard deviation.

The synovial cells cultures after 24 hours incubation with polyethylene particles revealed increased activity of interleukin-1 β (Fig. 4.4.2.), one of the most important proinflammatory cytokine. The stimulation was statistically significant ($p<0.05$ in Student t-test).

The incubation of synovial cells with wear particles caused decline of inactive form of metaloproteinase 1 (pro-MMP-1) (Fig. 4.4.3.) [44]. The difference was statistically significant ($p<0.01$ in Student t-test).

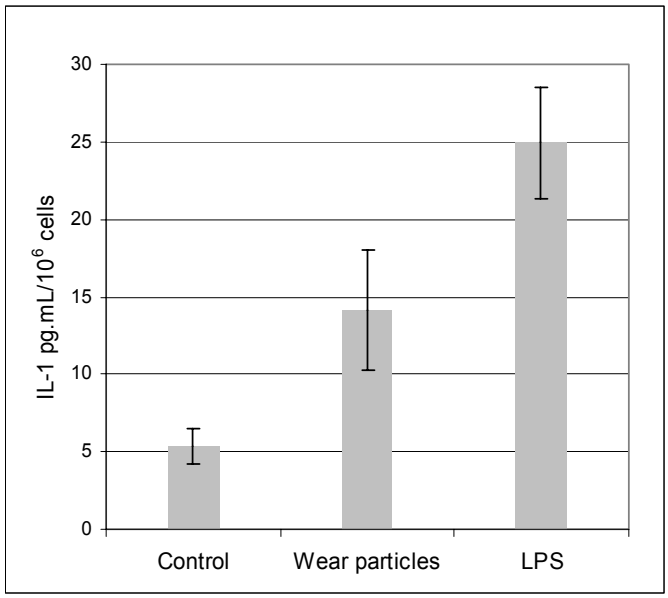


Fig. 4.4.2. The quantity of interleukin-1 (IL-1 β) in the supernatants from the synovial cells cultures exposed to polyethylene wear particles in comparison to control (not stimulated cell cultures) and cell cultures stimulated by LPS (results with standard deviation).

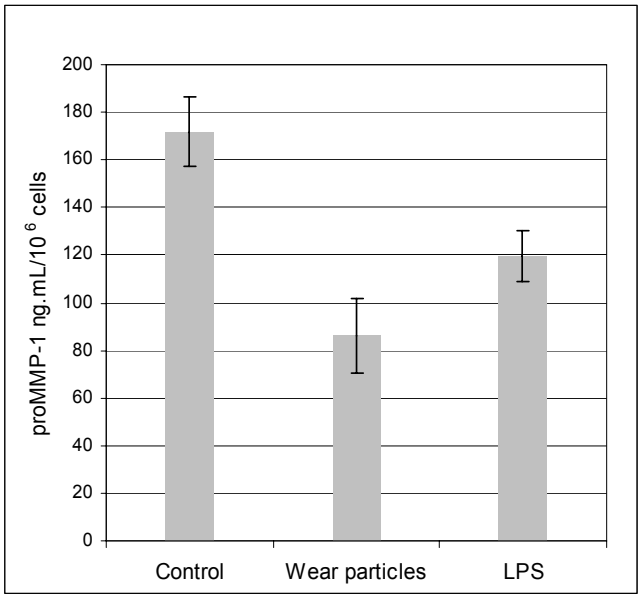


Fig. 4.4.3. The quantity of pro-MMP-1 in culture media from synovial cell cultures exposed to polyethylene particles in comparison to control (not stimulated cell cultures) and cell cultures stimulated by LPS (results with standard deviation).

The zymography show no differences in the activity of metaloproteinases MMP-2 and MMP-9 between the control and cells stimulated by polyethylene particles both in cases of synovial cells and peripheral blood leukocytes (Fig. 4.4.4.).

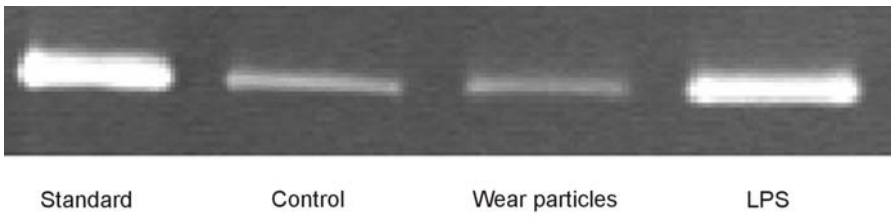


Fig. 4.4.4. Detection of MMP-9 in the conditioned media collected from cultured peripheral blood leukocytes, by gelatin zymography.

This study has shown an enhanced production of tumor necrosis factor (TNF-α) in culture media from peripheral blood leukocytes exposed to polyethylene wear particles. After 24 hours of incubation with wear particles, the TNF-α activity was 10 times bigger than in control culture ($p<0.01$ in Student t-test). The incubation of peripheral blood leukocytes with wear particles for 48 hours caused the decline in the TNF-α activity (Fig.4.4.5.) ($p<0.01$ in Student t-test). The difference between activation of TNF-α in conditioned media after 24 and 48 hours was statistically significant ($p<0.05$ in Student t-test).

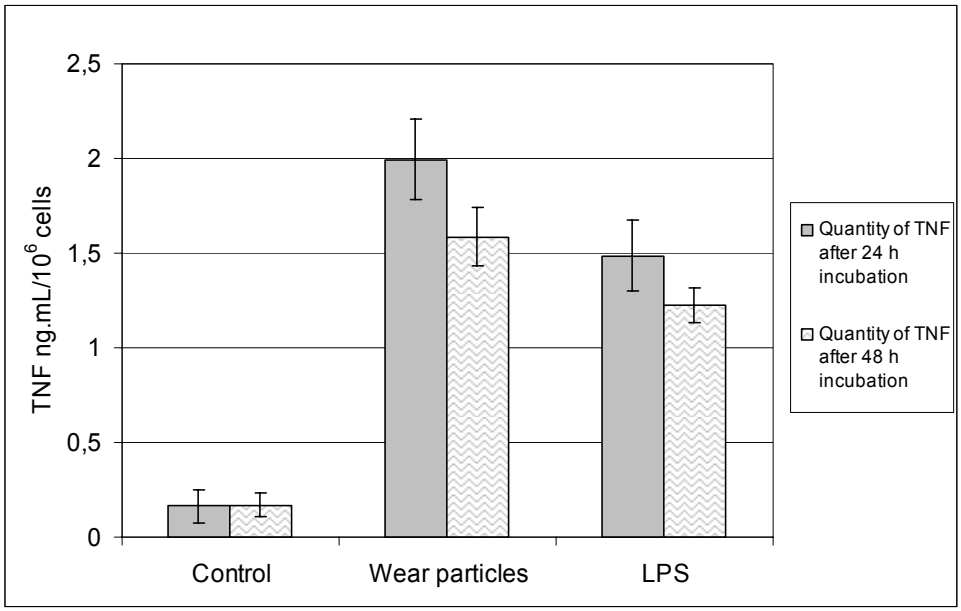


Fig. 4.4.5. TNF-α released by peripheral blood leukocytes incubated for 24 and 48 hours with wear particles in comparison to control and cell cultures stimulated by LPS (results with standard deviation).

The images of transmission electron microscope have shown the phagocytosis of polyethylene wear particles by the synovial cells after 24 hours incubation in vitro (Fig. 4.4.6.).

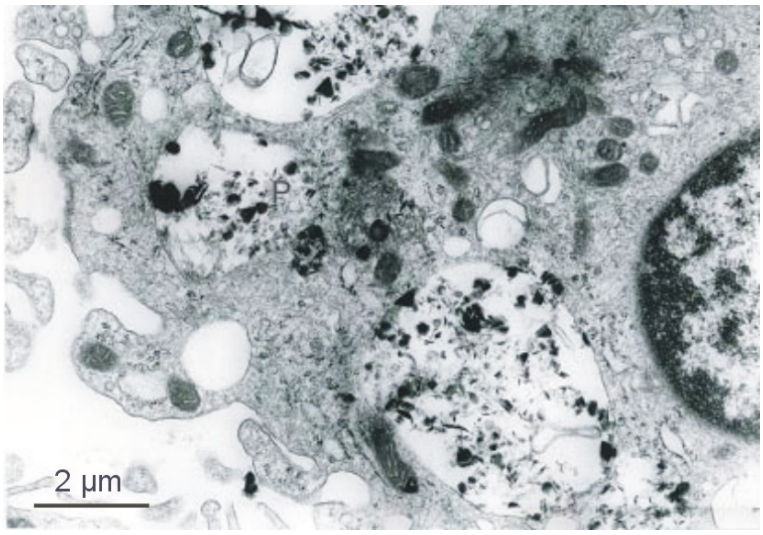


Fig. 4.4.6. The synovial cell that was incubated with polyethylene particles for 24 hours. In vacuole small foreign bodies are visible (TEM, x 10000).

4.4.4 Discussion

In vitro experiments were conducted on the synovial cells and peripheral blood leukocytes. Synovial cells are intensively exposed to wear particles in vivo, so it was reasonable to examine their response to wear particles under laboratory conditions. Peripheral blood leukocytes are included in the process of phagocytosis. After reaching the inflamed tissue, they differentiate into macrophages, which can phagocytose polyethylene wear particles, so it was important to in vitro investigate the effect of wear particles on these cells.

In this work, many different approaches of polyethylene wear particles exposure to cell cultures were examined. Due to their low density (0.94g/cm^2), polyethylene particles may float on top of culture medium limiting the contact between the cells and wear particles. Wear particles were anchored in collagen or albumin according to the method by Ingram and co-workers [77] to maximize the contact of cells with wear particles. However, none of these techniques enhanced the inflammatory effect of polyethylene particles on examined cells. The TEM images of synovial cell treated with polyethylene particles added to the culture medium have shown the phagocytosis of wear particles (particles were

visible inside the vacuoles of synovial cells) (Fig. 4.4.6.). Therefore, the particles were added directly to the culture medium of examined cells.

The biological response of examined cells to polyethylene wear particles was evaluated on the basis of activity of proinflammatory mediators and enzymes activity in the culture media.

The interleukin-1 (IL-1) is one of the most important proinflammatory cytokine and can be treated as an inflammation marker. This cytokine exhibits a number of redundant and peliotropic effects that contribute to the severity of the inflammatory response. The increased activity of interleukin-1 (IL-1) was revealed in synovial cell cultures incubated with polyethylene particles (statistically significant $p<0.05$ in Student t-test). The escalation of this cytokine activity confirmed the initial phase of inflammation evoked by wear particles. Interleukin-1 (IL-1) is an osteoclasts-activating factor and therefore causes the proliferation of osteoclast that break down bone tissue [90] and contributes to osteolysis around orthopaedic implants. Interleukin-1 has also an inhibitory effect on osteoblast function that precludes balance of the bone tissue loss. There is no consensus in the literature about the influence of wear particles on the enhanced production of interleukin-1. Wooley and co-workers [177] revealed an increased activity of interleukin-1 in response to wear particles, while Catelas and co-workers [28] have shown no enhancement in activity of this cytokine in cell cultures cultivated with wear particles. This study confirmed the influence of wear particles on interleukin-1 activity and therefore the inflammatory effect of wear particles on examined cells.

In the periprosthetic tissue matrix metaloproteinase (MMP) are induced [153], so it was worthwhile to check whether they are stimulated by polyethylene wear particles in vitro. MMPs are important for remodelling of the extracellular matrix in both normal and pathological conditions. Connective tissue metabolism is normally characterized by equilibrium between degradation and synthesis of extracellular matrix that is maintained by a balanced activation and inhibition of MMPs [123]. Deviation from the equilibrium may lead to the replacement of extracellular matrix by inflammatory and fibrous tissue [123] observed around loosened orthopaedic implants.

Matrix metalloproteinases are involved in bone tissue resorption [95]. MMPs are responsible for degradation of the nonmineralized osteoid layer covering calcific bone surfaces, which is essential for exposure of the mineralized matrix to osteoclasts. Matrix

metaloproteinases show one of the first signs of inflammatory response to wear particles [151].

Most matrix metalloproteinases are secreted as inactive pro-enzymes and are activated in extracellular environment. The decline in the level of proenzyme of metaloproteinase 1 (proMMP-1) in the supernatants collected from cell cultures treated with wear debris, indicated activation of this enzyme (Fig. 4.4.3.) (statistically significant $p<0.05$ in Student t-test). The zymogene was converted into active form, therefore smaller amount of precursor of MMP-1 (proMMP-1) was observed. In normal tissues, the level of collagenase-1 is usually low. By contrast, its expression is elevated when the system faces a disturbance, such as wound healing, repair, or remodelling process [140], all of them appear around orthopaedic implants.

Secretion of MMPs can be enhanced by proinflammatory cytokines that are secreted in response to wear particles. There are many studies that examine the influence of elevated level of IL-1 on collagenase-1 (MMP-1) secretion [33, 88, 141, 161]. Experiments with addition of recombinant IL-1 to synovial fibroblast cultures have shown induction in collagenases activity [33, 100]. Konttinen and co-workers, on the other hand, revealed that the correlation between IL-1 and MMP-1 is not significant [89]. It could suggest that the MMP-1 activity is influenced by many factors. In this study the correlation between IL-1 and MMP-1 was observed. However, synovial cells exposed to polyethylene particles most probably exhibited elevated levels of different cytokines that can together with interleukin-1 activate MMP-1.

Wear particles used in the performed in vitro experiments did not enhance the activity of gelatinases (MMP-2 and MMP-9). These enzymes can take part in connective tissue remodeling. A probable explanation would be that in the examined cell cultures the specific tissue inhibitors of MMPs (TIMPs) that prevent an excessive degradation of normal tissue were activated. Perhaps also the 24-hours incubation time with wear particles was too short to activate the gelatinases.

The activity of tumor necrosis factor (TNF) in the culture media from peripheral blood leukocytes incubated with wear particles was increased (Fig. 4.4.5.) (statistically significant $p<0.05$ in Student t-test). After 24 hours incubation time the quantity of TNF was ten times bigger than in the control media ($p<0.01$ in Student t-test). Longer treatment of cell cultures with wear particles caused a decline in the activity of TNF. This could suggest that longer time of exposure of peripheral blood leukocytes to wear particles

caused the activation of defence mechanisms. In the culture media of synovial cells no stimulation of TNF was observed in response to wear particles because tumor necrosis factor is mainly liberated by monocytes and macrophages [130] not by synovial fibroblasts.

TNF causes the proliferation of osteoclast progenitors and activates osteoclasts to resorb bone [90]. Besides interleukin-1, TNF is the most important cytokine for promoting the osteolysis around orthopaedic implants and evoking aseptic loosening [55, 76, 90, 113].

In vitro experiments on the cell cultures incubated with wear particles give the opportunity to simulate the situation present in the artificial joint and to separate the impact of wear particles from other factors influencing the aseptic loosening. Although cell-culture studies cannot directly duplicate the conditions that exist in vivo, the effects of specific types of particles on cellular metabolism can lead to better understanding of mechanisms engaged in aseptic loosening.

The performed in vitro experiments confirmed the inflammatory effect of polyethylene particles on synovial cells and peripheral blood leukocytes and the importance of their role in the process of aseptic loosening. It has been shown that examined particles evoke the increased levels of proinflammatory and potentially osteolytic cytokines (IL-1 and TNF), as well as increased activity of enzymes which degrade extracellular matrix (matrix metaloproteinase-1).

4.5 Hypothetical sequences of the loosening process of the artificial joint

On the basis of literature review and performed studies the hypothetical scheme of events leading to aseptic loosening was created (Fig. 4.5.1.). It has been shown that wear process of implant biomaterials and biological response to generated wear particles are among the most important causes for the failure of orthopaedic implants.

It has been proposed that the change in the mechanical loads in the bone tissue after total joint replacement plays the fundamental role in the aseptic loosening. The implant insertion alerts dramatically the magnitude of stresses and strains in the joint [10, 11, 14, 40]. Mechanical characteristics of biomaterials used for implantation significantly differ from those of bone tissue (e.g. Young modulus). Bone surrounding the implant is subjected to smaller loads, because rigid implant takes over the loads.. The implant thus "stress-shields" the bone tissue. According to Wolff's law the unloaded bone tissue will become less dense because there is no mechanical stimulus, which maintained the bone remodeling. The change in the density of bone tissue (osteopenia) due to redistribution of loads may contribute to implant loosening. Mechanical conditions evoke micromovements that lead to intensified friction and wear of implant materials. The change of strains around joint implant may also evoke inflammation and granulation of periprosthetic tissue.

It has been suggested that the tissue damage caused by the surgical treatment is the crucial factor that determines the stability and survivorship of an implant. The implantation of total joint replacement includes drilling and cutting that may lead to intensive bone tissue damage. The components of natural joint have to be removed and the femoral canal have to be extended and cleaned. These activities may lead to necrosis of periprosthetic tissue, inflammation and tissue granulation.

The usage of bone cement during implantation intensifies the tissue damages. Bone cement is a polymer that stabilizes joint prosthesis during polymerization. Since the polymerization is an exothermic reaction, high temperature leads to tissue damages. The results of this study showed that area of necrosis and necrobiosis in all examined periprosthetic tissue samples are present (Fig. 4.3.12., Fig. 4.3.14.). The necrotic and inflamed tissue cannot give enough support for the endoprosthesis and contributes to implant loosening.

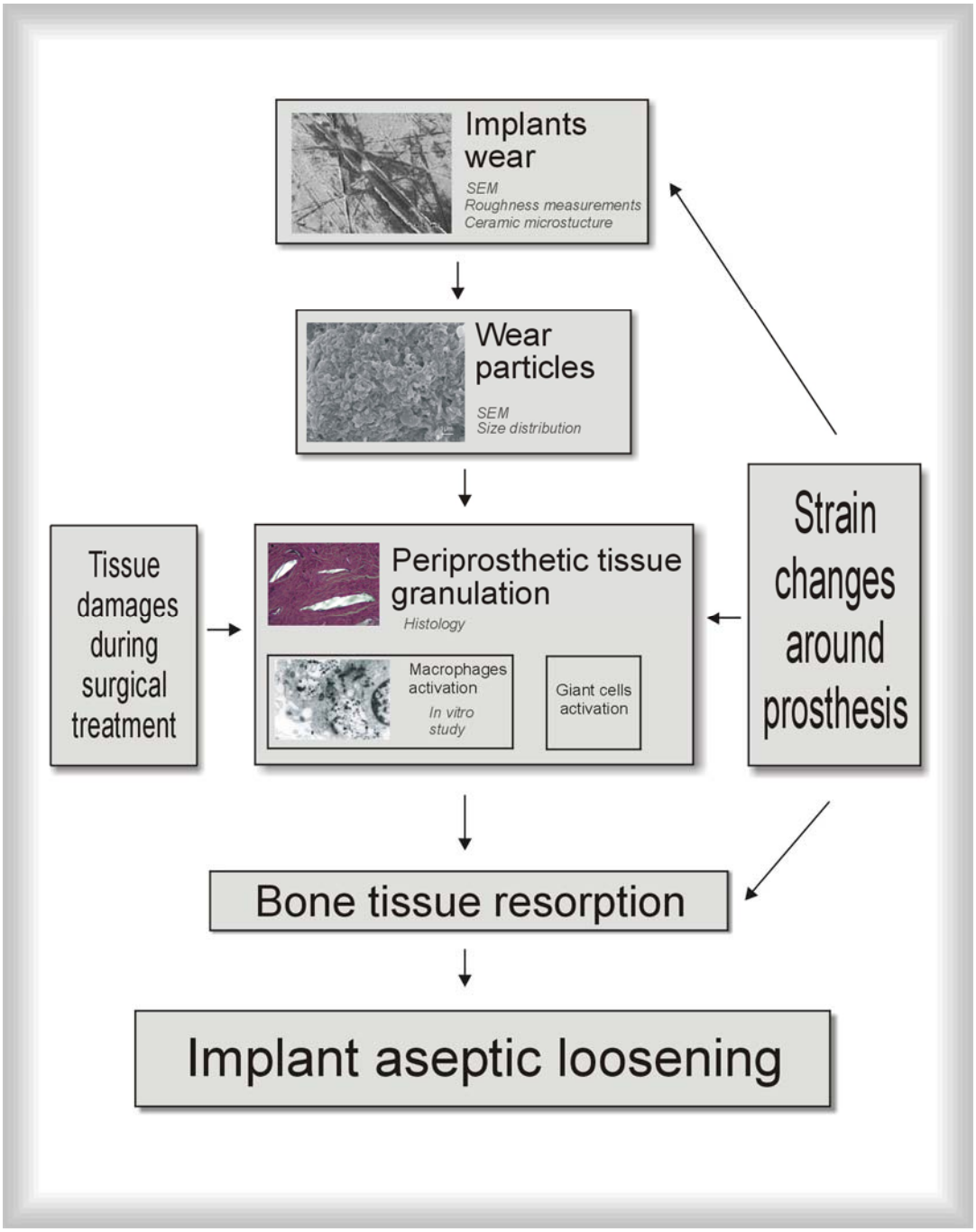


Fig. 4.5.1. Hypothetical scheme of aseptic loosening of orthopaedic implants.

The wear process and its consequences are considered one of the most important factors contributing to implant loosening. The wear traces were observed on the bearing surfaces of all forty-three examined loosened hip implants. The mechanical conditions, high loads and friction taking place in artificial joints determine the wear of orthopaedic implants. In the analyzed cases the abrasive, fatigue and adhesive wear were observed (Fig. 4.1.2., Fig. 4.1.3., Fig. 4.1.4.). All implant components – polyethylene cup (Fig. 4.1.7., Fig. 4.1.8.), metal (Fig. 4.1.2.) and ceramic femoral heads (Fig. 4.1.5) – were subjected to wear process. Bone cement mantle fractured and was subjected to degradation since the particles of bone cement were found in all analyzed cases – chapter 4.2.

The measurements have shown significant differences in the roughness parameters among examined biomaterials (Fig. 4.1.12. – Fig. 4.1.15.). These parameters were dependent on the wear susceptibility and the surface finish of the particular material. All performed experiments indicated that wear process in artificial joints is very intensive and cannot be neglected when considering the reasons of aseptic loosening of the orthopaedic implants.

During the wear process wear particles were generated. In this work a detailed analysis of wear particles was carried out. The effect of wear particles on the quality of periprosthetic tissue was also confirmed. Wear particles were mainly generated between articulating surfaces of artificial joints. They were suspended in the pseudosynovial fluid and could be transported with this fluid into periprosthetic tissues. Such a huge amount of wear particles induced the biological response in the periprosthetic tissues. The histological analysis indicated that wear particles in all examined tissue samples evoked inflammation and tissue granulation (Fig. 4.3.1., Fig. 4.3.4., Fig. 4.3.6., Fig. 4.3.8. and Fig. 4.3.12.). The presence of wear particles of polyethylene, metal and bone cement in periprosthetic tissue samples was confirmed (chapter 4.2). The most abundant particles in all considered cases were made of polyethylene. It was assumed that they play one of the most important roles in the development of implant loosening. The conducted experiments pointed out that not only material but also the size of wear particles determines the tissue response. Therefore, the size distribution of polyethylene wear particles was performed. The analysis of polyethylene wear particles showed that 90% of wear particles was smaller than 1 μm (Fig. 4.2.9.).

Big polyethylene wear particles in the periprosthetic tissue were surrounded by giant cells (Fig. 4.3.6.). These cells can take part in bone tissue resorption [83, 125, 128,

132, 135, 136]. Small polyethylene particles were phagocytosed, which was evidenced in the in vitro studies (Fig. 4.4.6.). The phagocytosis activated secretion of proinflammatory mediators by the immune system cells. Even short incubation time of human cells with wear particles in the laboratory conditions caused an increased activity of proinflammatory cytokines (Interleukin-1, Fig. 4.4.2., Tumor Necrosis Factor, Fig. 4.4.5.).

The proinflammatory cytokines exhibit a wide spectrum of activity. They can affect directly the activity of osteoclasts (cells responsible for bone tissue loss) or can favour the differentiation of osteoclast precursor into mature osteoclast. The cytokines secreted by immune system cells can decrease activity of osteoblast (cells responsible for bone tissue production), which subsequently precludes the compensation of bone tissue resorption. Proinflammatory cytokines can also activate the secretion of enzymes – metaloproteinases that can digest the bone tissue. Increased activity of metaloproteinases in response to wear particles was evidenced in the in vitro studies (Fig. 4.4.3.). All these processes lead to differences in strain and to the loss of bone tissue mass around joint implant. The prosthesis is deprived of mechanical support and can develop loosening.

The resorption of bone tissue significantly changes the mechanical conditions around the implant. The aim of the implantation is osteointegration of the implant with surrounding bone tissue. When the osteointegration occurs, the implant is stable and transfers the loads in a proper way. Lack of the bone support contributes to the development of fibrous, inflamed tissue. The connective tissue weakens the contact between bone and implant. The fibrous tissue cannot withstand the loading and aseptic loosening is possible.

Although many scientific studies have contributed to the understanding of the biological and mechanical processes leading to osteolysis, it still remains only partially understood.

The proposed scheme of implant loosening clearly identifies that aseptic loosening is attributed to a combination of mechanical and biological factors.

5 Conclusions

The complex examination study carried out on the retrieved hip implants and periprosthetic tissues surrounded these loosened implants gave the opportunity to explain the process of aseptic loosening..

In this work homogenous group of forty-three loosened implants (Műller type) with cemented titanium alloy stems and metal (stainless steel or titanium alloy) or ceramic femoral heads articulating against polyethylene cups fixed with or without bone cement were examined (Tab. 4.1.1.).

The analysis was aimed to characterize the surfaces of retrieved femoral heads and polyethylene cups (chapter 4.1), to study the morphology and size of wear particles (chapter 4.2) and histological response of periprosthetic tissue (chapter 4.3). It has been shown that all considered features are strongly connected to each other and may significantly affect the survivorship of orthopaedic implants.

The properties of implant surfaces determine the type of friction and wear of implant biomaterials that further influence the quality of generated wear particles. This study confirmed that implants with higher roughness of femoral heads lead to a more intense generation of large polyethylene wear particles. Numerous asperities present on the rough femoral head induce the formation of shreds of polyethylene on the counterpart cup surface. On the other hand, femoral heads with smooth surfaces generate huge amounts of very small particles.

The composition and size of wear particles have an impact on the type of periposthetic tissue response. Wear particles affect the intensity of inflammatory reaction and the potential of bone resorption that in consequence can lead to the aseptic loosening.

In the light of examined aspects, the least advisable for total joint replacements are femoral heads made of old ceramics. The bearing surfaces of old ceramic implants were several times more rough than other implants (Fig. 4.1.13.), which contributed to intensive wear of polyethylene cups. Almost ninety per cent (87%) of wear particles isolated from tissue surrounded loosened old ceramic implants were biological active and were able to evoke inflammation (particles bigger than 0.1 μm) (Fig. 4.2.9.). The histological analysis has shown high intensity of cells that contribute to bone resorption (macrophages and giant cell Fig. 4.3.5., Fig. 4.3.7.).

In 1980s, when the implants made of old ceramics were used (Tab. 4.1.2.), the demands for surface finish and level of impurities in ceramic material were not rigorous. In 1990s, these demands became more restrictive and ceramic implants of new generation exhibited much better features. SEM analysis revealed significant differences in the microstructure (grains and pores size) between ceramic implants of modern and old generations (Fig. 4.1.10., Fig. 4.1.11.). These differences determine the mode of friction, wear, size and morphology of wear particles and subsequently the biological response of periprosthetic tissues.

The conducted experiments have shown that the fixation of acetabular component (cementless or cemented) has no influence on the friction conditions, wear particles size and biological response of periprosthetic tissues. In all examined implants, stems were fixed using bone cement, which significantly affected all above-mentioned features.

The analysis of bearing surface of metal femoral heads revealed differences in the wear modes. On the stainless steel femoral heads many deep scratches were observed (Fig. 4.1.2., Fig. 4.1.19.), while on the surface of titanium alloy femoral heads shallow scratches were dominant (Fig. 4.1.2.). However, this difference did influence neither the roughness parameters the size of wear particles nor the biological response of periprosthetic tissue. All the examined metal femoral heads exhibited worse features than femoral heads made of modern ceramics.

The performed examinations indicated that the most beneficial for total joint replacements are femoral heads made of modern ceramics articulating against polyethylene acetabular cups. The measurements have shown that modern ceramic implants exhibited the smallest roughness of the bearing surfaces (Fig. 4.1.13.), and consequently the lowest level of wear of polyethylene cups. These implants attained the best mechanical feature concerning the friction (3D parameter, Tab. 4.1.4.). It was demonstrated that femoral heads made of modern ceramics contributed to the generation of many wear particles that are biologically inert. Forty per cent of polyethylene wear particles isolated from tissue samples surrounded these implants were smaller than 0.1 μm (Fig. 4.2.9.). Particles of this size do not evoke inflammatory reaction in the periprosthetic tissue; therefore, they do not prevent osteointegration. The histological analysis confirmed the smallest intensity of inflammatory reaction. Small amount of macrophages (Fig. 4.3.5.) and giant cells (Fig. 4.3.7.) were observed. These cells can be activated by wear particles and then they contribute to intensive bone tissue resorption and further to implant loosening.

Further scientific work aimed to improve the performance of total joint replacements should concentrate on the increase of the quality of modern ceramic material, which exhibit significantly better characteristics than implants made of old ceramics.

The modern ceramics used nowadays is very fracture resistant. The in vivo cracks of ceramic implants are very rare, although they could happen. The benefit of a ceramic bearing surface without the risk of head fracture can be obtained by fusing the ceramic layer onto a metal femoral head. It gives the opportunity to avoid fracture risk, simultaneously gaining very hard and smooth surface, which would decrease wear of polyethylene acetabular components. However, the long-term results concerning the survivorship of these implants are still required to contribute their in vivo behaviour.

The analyses performed on the retrieved implants are important for understanding their clinical performance since they contribute much valuable information about the processes taking place in the artificial joint in vivo.

The aseptic loosening of artificial joints is a complicated problem that requires the coordinated expertise of diverse fields including orthopaedic surgery, cell and molecular biology, clinical pharmacology and bioengineering. A successful solution of the clinical problem of aseptic loosening would have an enormous impact on the quality of life of patients with total joints replacements. In this work, a multidisciplinary approach to explain the process of aseptic loosening was presented. Besides mechanical and histological analysis, in vitro experiments were also performed (chapter 4.4).

The in vitro experiments are very useful tool for the examination of wear particles. They mimic the conditions in an artificial joint. The performed in vitro study revealed the influence of polyethylene wear particles on the cultures of synovial cells and peripheral blood leukocytes. It has been shown that incubation of these cells with wear particles leads to secretion of proinflammatory cytokines (TNF, Fig. 4.4.5. and IL-1, Fig. 4.4.2.) and proteolytic enzymes (metaloproteinases, Fig. 4.4.3.), which contribute to bone tissue resorption and, consequently, to implant loosening. The in vitro experiments are very precise but give only fragmentarily characteristics of the inflammatory reaction proceeding in the artificial joint. Nevertheless, the understanding of inflammatory reaction to wear particles can deliver information on how to modulate this process chemically, pharmacologically or using molecular biology in order to eliminate aseptic loosening.

Based on the performed studies and literature review the hypothetical sequence of events leading to the loosening of an orthopaedic implant was proposed (chapter 4.5,

Fig. 4.5.1.). This sequence is hypothetical because still many aspects of the aseptic loosening have not been explained. It was emphasized that both mechanical and biological aspects play a significant role in the aseptic loosening. Redistribution of load and consequently stress on the bone tissue due to implant insertion contribute to stress shielding and subsequently to osteolysis. Moreover wear particles responsible for evoking the inflammatory reaction lead to resorption of bone tissue around implant, which afterwards preclude implant osteointegration and lead to implant loosening.

6 Final conclusions

On the basis of conducted experiments it was assumed that wear particles strongly affect the process of aseptic loosening of orthopaedic implant. In this study the influence of wear mechanisms on wear particles quality was presented.

The detailed analysis of wear particles gave the opportunity to draw conclusions about periposthetic tissue degradation. It has been shown that size of wear particles strongly affects the character of inflammation in the tissues surrounding loosened implant. It has been revealed that quantity of biologically inert particles determines the osteolytic potential around endoprosthesis.

The in vitro experiments demonstrated that polyethylene particles can evoke strong inflammation leading to osteolysis and subsequently to aseptic loosening.

The performed study enabled to observe the implant failures on different levels and create the scheme of implant aseptic loosening.

This study has revealed that generation of wear particles still represents an important problem in modern implantology and is still a significant limitation for long-term survivorship of total joint replacements. Therefore all the attempts to extend the knowledge about biological reaction to wear particles are very important. They can deliver information how to limit the inflammation in periprosthetic tissue and consequently to prolong the survivorship of an implant.

Although this work has concentrated to wear and aseptic loosening of implants, it is known that most of total joint replacements function extremely well and have improved the quality of life to hundreds of thousands of patients. However, it is important to examine all the cases of implant failures in order to eliminate them in the future.

7 Abstract in English

The total joint replacement substitutes the damaged natural joint by the artificial materials. Millions of total joint replacement surgeries are performed worldwide annually to improve the quality of life of the patients by relieving pain and increasing mobility. Although the long-term performance of the majority of total hip replacements is excellent, some of them develop loosening that causes pain and reduces the patient's mobility. Eventually this process leads to the need of revision surgery. Aseptic loosening is the most common reason for revision. This process is very complex and still many aspects involved in it are not fully understood.

The goal of this study was to identify mechanical and biological factors responsible for aseptic loosening of hip implants based on the retrieved implants and tissue samples, as well as in vitro experiments. This study aimed to estimate the effect of wear process, wear particles and their biological impact on aseptic loosening.

The subject of the study were forty-three loosened hip prostheses with cemented self-locking (SL) stem made of titanium alloy (Ti6Al4V), and the acetabular component made of polyethylene (UHMWPE). Implants were divided into groups on the basis of the material of femoral head, which was either metal (stainless steel, AISI 316L, or titanium alloy, Ti6Al4V) or ceramic (Al_2O_3 of old or modern generation). Acetabular components were fixed with or without bone cement.

The characterization of the mechanical properties of the implant materials has revealed wear traces on all examined implants. The character of wear process on the femoral head determined the wear of polyethylene acetabular component.

The analysis of wear particles has shown the differences in the size distribution of wear particles depending on femoral head materials and their roughness. The experiments has revealed that femoral heads with smooth articulating surfaces contribute to the generation of wear particles that are mostly biologically inert and do not evoke an inflammatory reaction. On the other hand, higher roughness of the femoral heads resulted in the generation of biologically active wear particles.

The histological analysis has revealed the differences in the intensity of inflammation reaction among different groups of implants. The significant differences

have been noted in number of cells contributing to osteolysis, i.e. macrophages and giant cells.

The characterization of the biological reaction proceeded on the bone-implant interface was performed in in vitro experiments. It was shown that wear particles added into culture media evoked the secretion of proinflammatory cytokines and increased the amount of enzymes in the cell cultures. This study has indicated that polyethylene wear particles had a great biological potential to accelerate the inflammatory reaction and subsequently to promote process of implant loosening.

Based on the performed study and literature review the scheme of aseptic loosening was created. It was emphasized that both mechanical and biological aspects determine the process of implant loosening.

This study has indicated that polyethylene cups articulating against femoral heads made of modern ceramics were the most beneficial combination for total joint replacements. They have revealed the smallest roughness of articulating surfaces resulting in the generation mostly biologically inert particles. In the periprosthetic tissues surrounding these implants the smallest intensity of inflammatory reaction has been evidenced.

The conducted study revealed that wear particles play significant role in aseptic loosening. It has been shown that size of wear particles determine the quality of periprosthetic tissue and potential to implant failure.

8 Abstract in Polish

W całkowitej alloplastyce stawów ortopedycznych elementy naturalnego stawu zastąpione zostają sztucznymi materiałami. Rocznie na całym świecie przeprowadza się miliony zabiegów wymiany stawów, które przynoszą ulgę pacjentom i przywracają im zdolność normalnego, bezbolesnego poruszania się.

Mimo że większość wszczepianych endoprotez wspaniale spełnia swoją funkcję przenoszenia obciążeń, to niektóre z nich obluzowują się powodując ból i ograniczając mobilność pacjentom. W konsekwencji konieczne jest przeprowadzenie operacji rewizyjnej. Aseptyczne obluzowanie jest najczęstszą przyczyną niepowodzeń w implantacji endoprotez stawów ortopedycznych. Proces aseptycznego obluzowania endoprotez jest bardzo złożony, a wiele aspektów tego procesu wciąż pozostaje niewyjaśnionych.

Celem tej pracy było zidentyfikowanie mechanicznych i biologicznych czynników odpowiedzialnych za wywołanie aseptycznego obluzowania. W pracy oceniano wpływ zużycia biomateriałów, cząsteczek zużycia i ich biologicznej reakcji na proces aseptycznego obluzowania endoprotez stawów biodrowych.

Przedmiotem badań były czterdzieści trzy obluzowane endoprotezy stawu biodrowego z trzpieniami typu self-locking zbudowanymi ze stopu tytanu (Ti6Al4V) osadzonymi przy pomocy cementu kostnego. Panewki we wszystkich rozpatrywanych przypadkach były zbudowane z polietylenu o ultra wysokiej masie cząsteczkowej (UHMWPE), a osadzane były przy użyciu cementu kostnego lub bezcementowo. Implanty podzielono na grupy ze względu na materiał budujący głowy implantów, które były metaliczne (stal austenityczna, AISI 316L, lub stop tytanu, Ti6Al4V) lub ceramiczne (Al_2O_3 starej lub nowej generacji).

Charakterystyka mechanicznych właściwości materiałów budujących implanty wykazała zużycie wszystkich badanych endoprotez. Wykazano, że rodzaj procesu zużycia głów implantów stawów biodrowych determinuje rodzaj procesu zużycia polietylenowych panewek.

Analiza cząsteczek zużycia wykazała znaczące różnice w rozkładzie ich wielkości w zależności od materiału budującego głowę implantu stawu biodrowego oraz jego chropowatości. Badania eksperymentalne ujawniły, że głowy implantów stawów

biodrowych z gładkimi powierzchniami trącymi sprzyjają powstawaniu dużej ilości nieaktywnych biologicznie cząstek zużycia niewywołujących reakcji zapalnej. Z drugiej strony udokumentowano, że duża chropowatość głów implantów stawów biodrowych prowadzi do powstawania biologicznie aktywnych cząstek zużycia.

Analiza histologiczna wykazała różnice w intensywności reakcji zapalnej w tkankach otaczających obluzowane endoprotezy w zależności od materiałów budujących implanty. Znaczące różnice zanotowano w ilości komórek sprzyjających osteolizie takich jak makrofagi oraz komórki olbrzymie.

Ocenę biologicznej reakcji występującej na granicy kość-implant przeprowadzono na podstawie badań in vitro. Wykazano, że cząsteczki zużycia dodane do medium inkubującego hodowli komórkowych wywołują wzmożone wydzielanie cytokin prozapalnych i enzymów proteolitycznych. Przeprowadzone doświadczenia potwierdziły, że cząsteczki zużycia polietylenu mają duży potencjał biologiczny do wzmożenia reakcji zapalnej i w konsekwencji do wywołania aseptycznego obluzowania endoprotezy.

Na podstawie przeprowadzonych doświadczeń i przeglądu literatury stworzono schemat procesu aseptycznego obluzowania stawów ortopedycznych. Podkreślono, że zarówno mechaniczne jak i biologiczne czynniki determinują proces aseptycznego obluzowania.

W niniejszej pracy wykazano, że polietylenowe panewki współpracujące z ceramicznymi głowami implantów nowej generacji są jedną z najlepszych kombinacji dla całkowitej alloplastyki stawów biodrowych. Ceramiczne implanty nowej generacji charakteryzowały się najmniejszą chropowatością powierzchni ciernych, która sprzyjała powstawaniu nieaktywnych biologicznie cząsteczek zużycia, a w tkankach otaczających te implanty zaobserwowano najmniejszą natężenie reakcji zapalnej.

Przeprowadzone badania wykazały, że cząsteczki zużycia ciernego odgrywają istotna rolę w aseptycznym obluzowaniu implantów ortopedycznych. Dowiedziono, że wielkość cząsteczek zużycia determinuje jakość tkanek okołoimplantowych oraz osteolityczny potencjał.

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10 List of figures

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12 List of abbreviations

316L	stainless steel alloy
$BaSO_4$	barium sulphate
ECD	equivalent circle diameter
EDS	energy dispersive X-ray spectroscopy
HPF	high power field (x 400)
IL	interleukin
LPF	low power field (x 100)
LPS	lipopolysaccharide
MMP	matrix metalloproteinase
MNGC	multinucleated giant cells
NADPH	nicotinamide adenine dinuclotide phosphate oxidase
PE	polyethylene
PMMA	polymethylmethacrylate
ROS	reactive oxygen species
SD	standard deviations
SEM	scanning electron microscope
Sdc	section height difference between 20 and 80%
TEM	transmission electron microscope
THA	total hip arthroplasty
THR	total hip replacement
Ti6Al4V	titanium-aluminium-vanadium alloy
TNF	tumor necrosis factor
UHMWPE	ultra-high molecular weight polyethylene