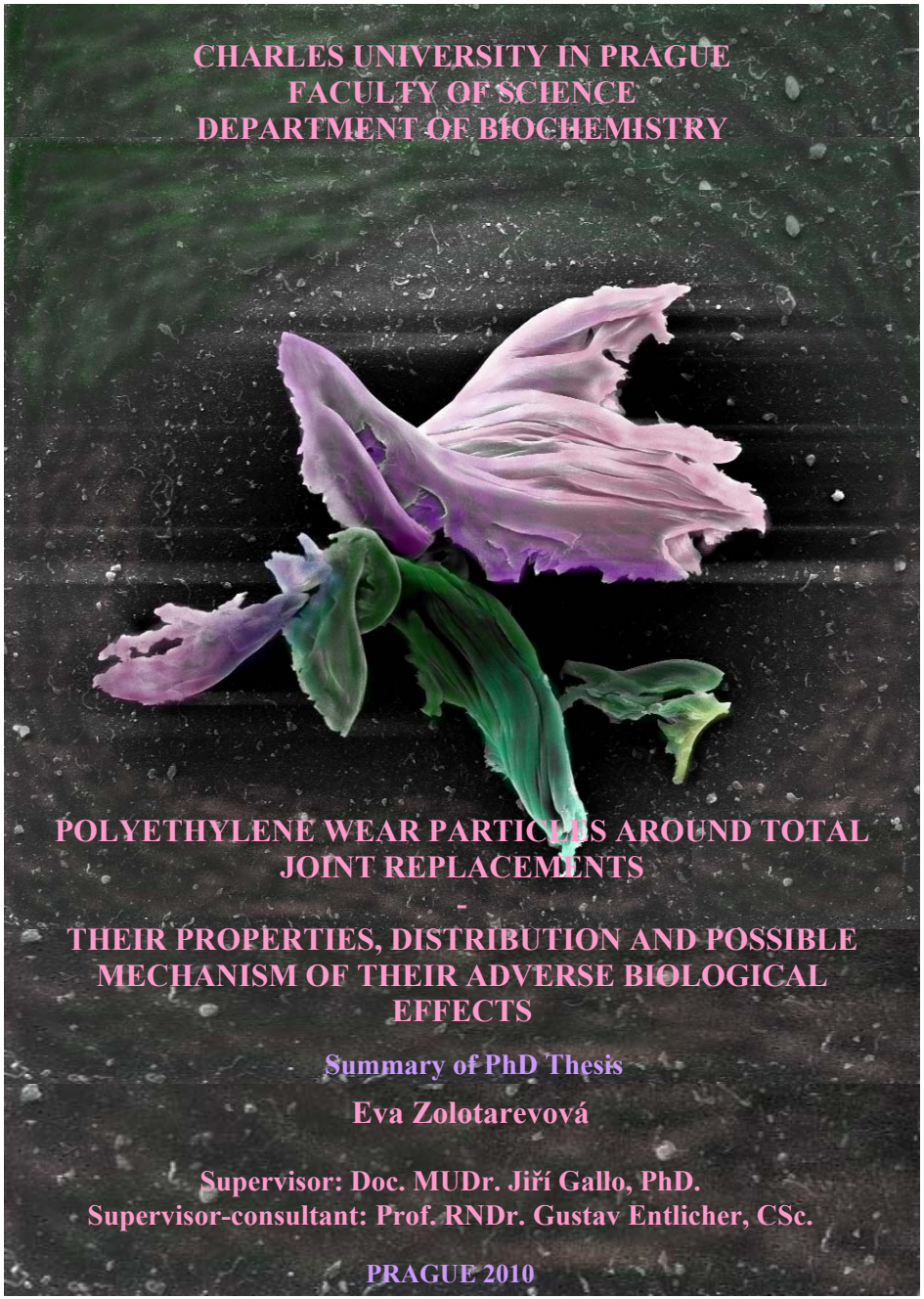
CHARLES UNIVERSITY IN PRAGUE
FACULTY OF SCIENCE
DEPARTMENT OF BIOCHEMISTRY
POLYETHYLENE WEAR PARTICLES AROUND TOTAL JOINT REPLACEMENTS
-
THEIR PROPERTIES, DISTRIBUTION AND POSSIBLE MECHANISM OF THEIR ADVERSE BIOLOGICAL EFFECTS
Summary of PhD Thesis
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Supervisor: Doc. MUDr. Jiří Gallo, PhD.
Supervisor-consultant: Prof. RNDr. Gustav Entlicher, CSc.
PRAGUE 2010

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Front-page picture:

Polyethylene wear particle isolated from granuloma tissue surrounding total hip replacement;

Electron microscope microphotograph; particle size app. 90 x 80 μm; retouched

Introduction

For many people all over the world a replacement with an implant of irreversibly damaged joint is often the only way how to come back to normal life without pain and mobility limitation. Nowadays, greater and greater requirements are asked of quality of all replacement components, especially in terms of quality of used materials. Biomechanical properties of hip and knee replacements have been already solved. The most often used materials for manufacturing joint replacements are: metal alloys (Cr, Co, Mo, Ti), ceramics, polymers – especially polyethylene. With the increasing number of patients who use a long-term joint implant, problems related to its wear come forward. The problem is serious especially for young and active patients with big mobility demands. These patients need to have their endoprostheses replaced after a few years because of their loosening. Endoprosthesis life-cycle is influenced by so-called aseptic loosening, means an interaction process of the organism and the implant, where the binding between the implant and patient bone is loosened.

Only at the end of seventies – almost 20 years after the polyethylene implementation to the manufacturing processes of acetabular cups of total hip endoprostheses the reaction of organism to polyethylene wear particles was described. In the process of aseptic loosening the polyethylene wear particles, generated by head friction within the joint cup, play a major role. [1]

Polyethylene

Polyethylene is a key and hardly replaceable material for manufacture of acetabular cups of hip endoprosthesis and articulating insert of knee endoprosthesis. At present, the most used type is low-pressure ultra high molecular weight polyethylene (UHMWPE). It is a polymer of 71 000-214 000 groups of ethylene with total relative molecular weight of 2-6 millions. Its outstanding properties are: chemical inactivity, lubricity, stability and wear resistance. Sterilization process expressively influences the wear resistance. It can be done by three different ways: ionizing irradiation, ethylene oxide or plasma. Since 1999 polyethylene cups used in clinical practice have been sterilized by irradiation with accelerated electrons in combination with thermal treatment. The wear of this highly cross-linked polyethylene is very low. However, irradiation has to be applied so that cross-linking (creation of new cross C-C bonds far between parts of molecule) outweighs the chain cleavage and simultaneously the thermal treatment has to eliminate presence of free radicals. [1-3]

Polyethylene wear particles and granuloma formation

Despite of outstanding polyethylene properties a relatively large amount of polyethylene wear particles is generated by friction between the polyethylene and the metal or the ceramic component of the endoprosthesis. The polyethylene wear particles are one of the major

reasons for the implant failure. The organism reacts to these particles as to a foreign material and tries to enclose or eliminate them. The particles accumulate in the joint; the lymphatic or blood circulation also transport them to e.g. lymph nodes, liver or spleen. These biologically active particles are at the beginning of an aseptic inflammatory process in an organism. Depending on their size they are phagocytized by macrophages (particles size 0.1 – 10 μm are phagocytized most frequently), and activate lymphocytes and thus initiate the production of many pro-inflammatory mediators. Immunologicaly mediated processes are facilitated by interaction between macrophages and lymphocytes. The result is formation of inflammatory tissue rich in polyethylene granuloma around the bone. Increased granuloma formation leads to large bone resorption – osteolysis and the implant loosening follows. [4-6]

Isolation of polyethylene wear particles

In order to identify, characterize and quantify the polyethylene wear particles from granuloma tissue several methods have been developed and refined. There are essentially three techniques depending on the environment of the isolation process: hydrolysis under acidic conditions (most often nitric acid [7]), hydrolysis in alkaline environment (potassium hydroxide [8]), and hydrolysis by proteolytic enzymes [9].

Aim of study

- To optimize the isolation method for obtaining polyethylene wear particles from granuloma tissue around hip endoprostheses
- To adapt the isolation method for large-scale preparation of polyethylene wear particles
- To plot the calibration curve for conversion of the relative amount particles to the absolute one
- To observe the physico-chemical properties of particles
- To determine a possible effect of centrifugation on particles morphology
- To determine, if besides wear particles of usual size much smaller particles are also generated
- To describe the polyethylene wear particles distribution in granuloma tissue or more precisely, if more particles are present in granules or surrounding tissue, respectively?
- To observe binding of serum proteins to isolated “native” polyethylene wear particles and the consequences of this bond

3. **Zolotarevova, E.,** Entlicher, G., Lapcikova, M., Slouf, M., Pokorny, D., Sosna, A.: Určení počtu a hmotnosti polyethylenových otěrových částic izolovaných z periprotetické tkáně z okolí endoprotéz kyčelních a kolenních kloubů. XIII. Národní kongres ČSOT, Plzeň, Česká Republika, Kniha abstrakt (2009) p. 31
4. Entlicher, G., **Zolotarevova, E.,** Lapcikova, M., Slouf, M., Pokorny, D., Sosna, A.: Determination of amount and number of polyethylene wear debris isolated from periprosthetic tissues around total joint replacements. 10th EFORT Congress, Vienna, Austria, (2009) P1945, p.325

Other publications

1. Pokorny, D., Slouf, M., Dybal, J., **Zolotarevova, E.,** Vesely, F., Jahoda, D., Vavrik, P., Landor, I., Entlicher, G., Sosna, A.: New method for quantification of UHMWPE wear particles around total joint replacements. Acta Chir Orthop Traum Čech. 76, 374-381 (2009) **IF_{2010} 1.628**
2. Pokorny, D., Slouf, M., Vesely, F., Fulin, P., Jahoda, D., Sosna, A., Belacek, J., Landor, I., **Zolotarevova, E.,** Popelka, S.: Distribution of UHMWPE wear particles in periprosthetic tissues of total hip replacements. Acta Chir Orthop Traum Čech. 77, 87-92 (2010) **IF_{2010} 1.628**

Abstracts

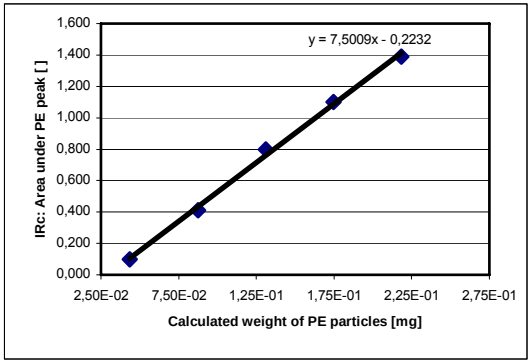
1. Lapcikova, M., Slouf, M., Fejfarkova, Z., **Zolotarevova, E**., Entlicher, G., Pokorny, D., Sosna, A.: Influence of centrifugation on morphology of UHMWPE wear particles. UHMWPE 2007, Madrid, Spain, 14.-15.09.2007 Abstract O-12, p. 26
2. **Zolotarevova, E**., Lapcikova, M., Slouf, M., Entlicher, G., Pokorny, D., Vesely, F., Sosna, A.: Distribution of polyethylene wear debris and bone particles in granuloma tissue around total hip joint replacements. Hip International 18 (2) 173-174 (2008)

Results

Some of physico-chemical properties of polyethylene wear particles have been described. These particles are hydrophobic and have marked electrostatic charge. It is not possible to separate them by weighing but in the form of suspension of known concentration only. In aqueous medium they flotate very quickly; the suitable medium is 50% propan-2-ol solution. When suspended, particles aggregate very quickly; therefore they need to be disaggregated by sonication before any filtration or any other step. [10-14]

Calibration curve for polyethylene wear particles

Polyethylene wear particles (17.5 mg) were isolated from granuloma tissues by modified nitric acid hydrolysis. The calibration curve (Graph 1) was constructed from the average IRc value (method of particles quantification by infrared spectroscopy) and calculated weight of PE particles. The results of IRc used so far in relative values were successfully converted to the absolute ones. [10]



Graph 1: Relationship between IRc and calculated weight of PE particles

Effect of centrifugation on particles morphology

Polycarbonate membranes with captured PE particles size 0.1-10 μm underwent image analysis. No qualitative difference was found between the particles separated by spontaneous flotation and those separated by centrifugation at 500, 16 000 a 105 000 x g. Particles morphology was characterized by equivalent diameter, circularity and elongation. Histograms (Fig. 1, page 7) demonstrate that particles sizes and shapes remained constant and did not change with varying *g*-force during centrifugation. Thus centrifugation does not influence UHMWPE wear particles morphology. [14]

Nanometer size wear particles

Thanks to the effective isolation method, polyethylene wear particles size 15-25 nm were isolated from granuloma samples of two patients. Particles purity was confirmed by IRc method and EDAX spectra (energy X-rays dispersion analysis). The size of nanoparticles was determined by image analysis. The complete histogram (Fig. 2, page 8) proved that in both cases the wear particles were quite small, with relatively narrow size distribution. Complete results of image analysis are summarized in Tab. 1, page 8. [13]

List of publications included in the PhD thesis

1. **Zolotarevova, E.,** Fejfarkova, Z., Entlicher, G., Lapcikova, M., Slouf, M., Pokorny, D., Sosna, A.: Can centrifugation affect the morpholoy of polyethylene wear debris? Wear 265, 1914-1917 (2008) **IF_{2010} 1.771**
2. Lapcikova, M., Slouf, M., Dybal, J., **Zolotarevova, E.,** Entlicher, G., Pokorny, D., Gallo, J., Sosna, A.: Nanometer size wear debris generated from ultra high molecular weight polyethylene *in vivo*. Wear 266, 349-355 (2009) **IF_{2010} 1.771**
3. **Zolotarevova, E.,** Entlicher, G., Pavlova, E., Slouf, M., Pokorny, D., Vesely, F., Gallo, J., Sosna, A.: Distribution of polyethylene wear particles and bone fragments in periprosthetic tissue around total hip joint replacements. Acta Biomaterialia 6, 3595-3600 (2010) **IF_{2010} 3.975**
4. **Zolotarevova, E.,** Hudecek, J., Spundova, M., Entlicher, G.: Binding of proteins to ultra high molecular weight polyethylene wear particles as a possible mechanism of macrophage and lymphocyte activation. J Biomed Mater Res. Part A 95A, 950-955 (2010) **IF_{2010} 3.318**

13. Lapcikova, M., Slouf, M., Dybal, J., Zolotarevova, E., Entlicher, G., Pokorny, D., Gallo, J., Sosna, A.: Nanometer size wear debris generated from ultra high molecular weight polyethylene in vivo. Wear 266, 249-355 (2009)
14. Zolotarevova, E., Fejfarkova, Z., Entlicher, G., Lapcikova, M., Slouf, M., Pokorny, D., Sosna, A.: Can centrifugation affect the morpholoy of polyethylene wear debris? Wear 265, 1914-1917 (2008)
15. Slouf, M., Eklova, S., Kumstatova J., Berger, S., Synkova, H., Sosna, A., Pokorny, D., Spundova, M., Entlicher, G.: Izolation, characterization and quantification of polyethylene wear debris from periprosthetic tissues around total joint replacements. Wear 262, 1171-1181 (2007)
16. Pokorny, D., Slouf, M., Vesely, F., Fulin, P., Jahoda, D., Sosna, A., Belacek, J., Landor, I., Zolotarevova, E., Popelka, S.: Distribution of UHMWPE wear particles in periprosthetic tissues of total hip replacements. Acta Chir Orthop Traum Čech. 77, 87-92 (2010)

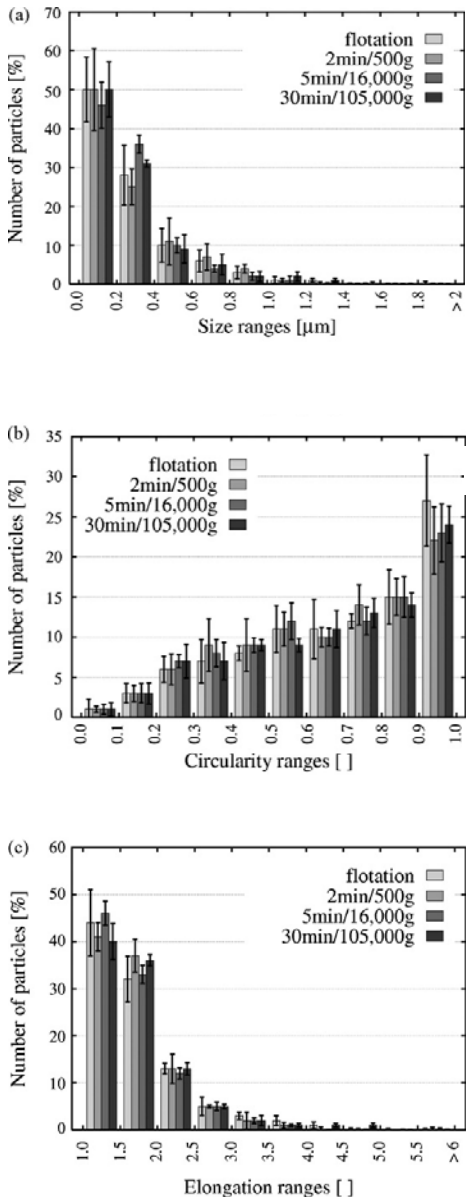


Fig. 1: Distribution of particle sizes (a) and shapes (b, c) flotating in different conditions [14]

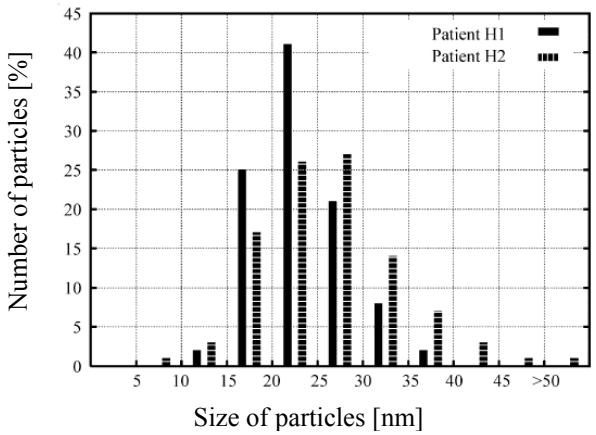


Fig. 2: Histogram of size distribution of UHMWPE wear particles isolated from patients no.1 (H1) and patient no.2 (H2) [13]

Tab. 1: Results of image analysis of UHMWPE nanoparticles [13]

Patient	Equivalent diameter (nm)	Circularity ( )	Elongation ( )
No.1 (H1)	18.47	0.97	1.29
No.2 (H2)	21.20	0.93	1.35

Distribution of PE particles in granuloma tissue

Sequential degradation of native samples of granuloma tissues by proteolytic enzymes (protease from *Streptomyces griseus* and collagenase) was used to study the distribution of UHMWPE wear particles. Polyethylene wear particles were isolated from acquired lyzates and granules by the nitric acid method. The scheme on Fig. 3, page 9 summarizes the isolating process. [12]

8. Shanbhag, A.S., Jacobs, J.J., Glant, T.T., Gilbert, J.L., Black, J., Galante, J.O.: Composition and morphology of wear debris in failed uncemented total hip replacements. J Bone Joint Surg. 76-B, 60-67 (1994)
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10. Slouf, M., Pokorny, D., Entlicher, G., Dybal, J., Synkova, H., Lapcikova, M., Fejfarkova, Z., Spundova, M., Vesely, F., Sosna, A.: Quantification of UHMWPE wear debris in periprosthetic tissues of hip arthroplasty: description of a new method based on IR and comparison with radiographic appearance. Wear 265, 674-684 (2008)
11. Zolotarevova, E., Hudecek, J., Spundova, M., Entlicher, G.: Binding of proteins to ultra high molecular weight polyethylene wear particles as a possible mechanism of macrophage and lymphocyte activation. J Biomed Mater Res. Part A 95A, 950-955 (2010)
12. Zolotarevova, E., Entlicher, G., Pavlova, E., Slouf, M., Pokorny, D., Vesely, F., Gallo, J., Sosna, A.: Distribution of polyethylene wear particles and bone fragments in periprosthetic tissue around total hip joint replacements. Acta Biomaterialia 6, 3595-3600 (2010)

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5. Green, T.R., Fisher, J., Stone, M., Wroblewski, B.M., Ingham, E.: Polyethylene particles of a „critical size" are necessary for the induction of cytokines by macrophages in vitro. Biomaterials. 19, 2297-2302 (1998)
6. Ingham, E., Fisher, J.: The role of macropahges in osteolysis of total joint replacement. Biomaterials. 26, 1271-1286 (2005)
7. Margevicius, K.J., Bauer, T.W., McMahon, J.T., Brown, S.A., Merritt, K.: Isolation and characterization of debris in membranes around total joint prostheses. J Bone Joint Surg. 76-A, 1664-1675 (1994)

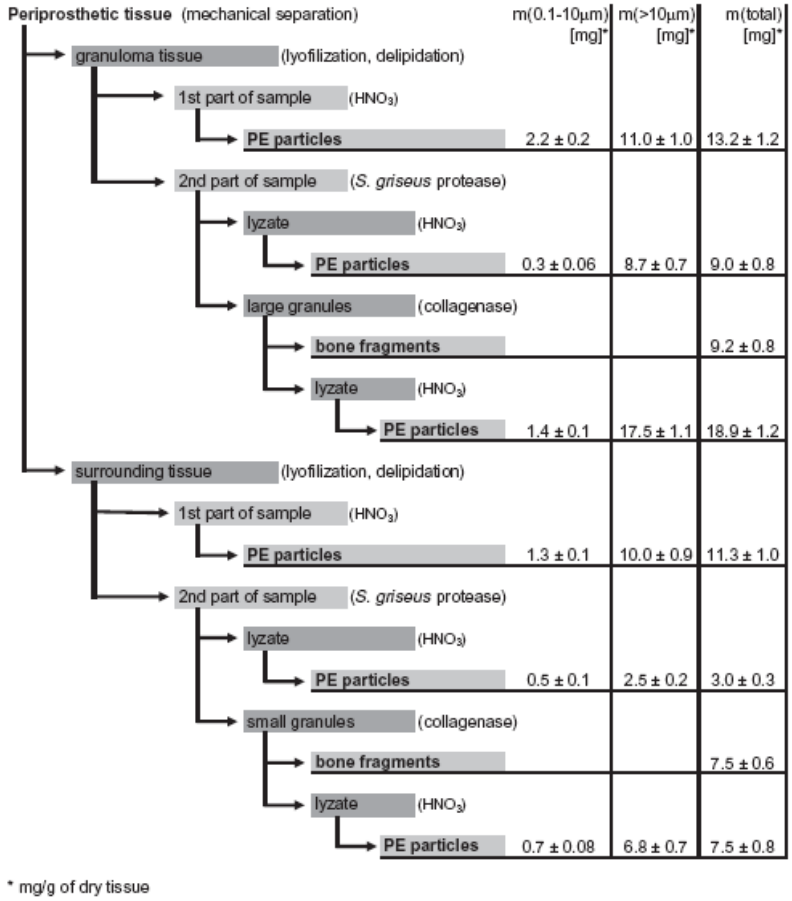


Fig. 3: An overall scheme of wear particle isolation and quantification [12]

There were more wear particles in the granuloma tissue than in the surrounding tissue, and more wear particles in the granules than in the tissue closely surrounding the granules. There were more small particles in granules than larger ones. Also more small particles than large ones were present in the granules. Moreover, small particles were

distributed in periprosthetic tissue more evenly than large ones. In addition to the wear particles, bone fragments were also found in the granules. The existence and purity of bone fragments was confirmed by EDAX spectra (Fig. 4, page 10). Tab. 2, page 10 shows overall granules: lyzates ratios. A similar amount of wear particles was obtained by both, the sequential enzymatic hydrolysis with the aid of proteolytic enzymes followed by acid hydrolysis, and by direct acid hydrolysis by nitric acid. [12]

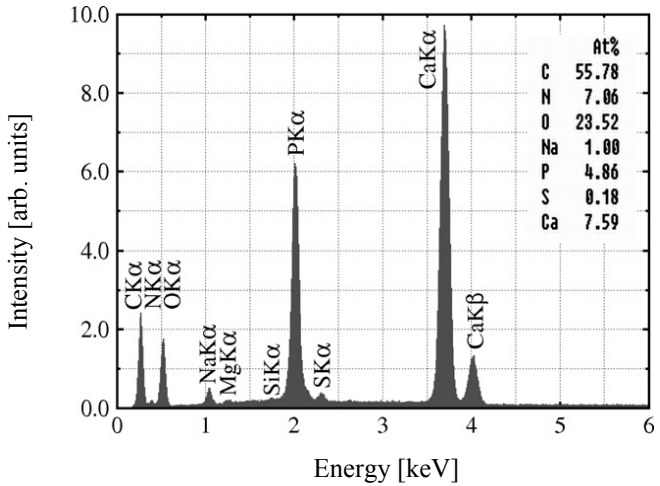


Fig. 4: EDAX spectrum of isolated bone fragments on 0,1 μm polycarbonate membrane [12]

Tab. 2: Distribution of wear particles inside and outside of granules [12]

	Granules (large and small) (%)	Lyzates (%)
PE particles (0,1-10 μm)	72	29
PE particles (> 10 μm)	68	32
PE particles (all)	69	31

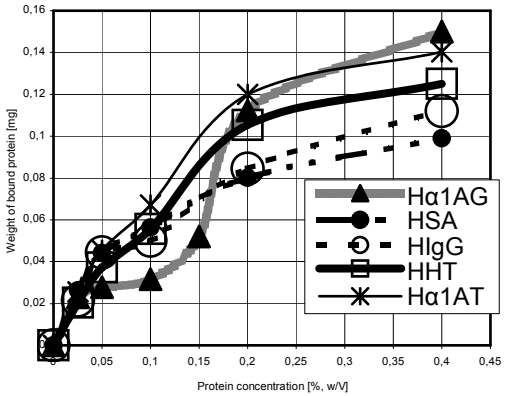
Since the very beginning it has been exciting to find out if wear particles can present some antigens themselves (as fragments of polyethylene chains) or vicariously. Both alternatives remain possible; however, it seems that the vicarious activity is the one more likely. There proteins adsorb on particles and become denaturated. Because of this fact adsorbed and denaturated endogenous proteins may be recognized as foreign ones [11].

Discussion

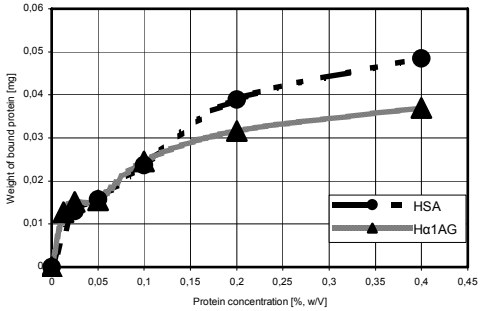
This PhD thesis in essence rounds off the range of queries asked several years ago when the task was set by Doc. RNDr. Zdenek Horak, PhD. The original isolation procedure for polyethylene wear particles [15], identification of some of their physico-chemical properties and manipulation accordingly has been successful in the process of this thesis accomplishment [12]. It became possible to quantify the particles at relative as well as absolute scale [10]. The thesis managed to disprove the published opinion that centrifugation can affect the morphology of isolated particles [14]. The issue of distribution of polyethylene wear particles around hip endoprothesis has been solved at two levels: "macroscopically", the distribution of wear particles in zones around endoprosthesis was showed (this thesis does not include these data) [16]. Furthermore, at the „microscopic" level, the distribution of wear particles in granuloma tissue from the zones surrounding endoprostheses with most particles has been determined. The granuloma tissue formed in zones with most particles because of that. It was observed that the common term - osteoagressive granuloma - used by orthopedist is rather misguided. Granuloma is not aggressive to bone tissue. On the contrary, the granuloma formation reduces the effective amount of wear particles with negative biological properties [12]. The whole project has been sealed by possible explanations of biological activity wear particles.

Binding of serum proteins to PE particles

A method for measuring of protein binding to UHMWPE wear particles was developed and optimized. This method was also suitable for the testing of possible elution of bound proteins (binding reversibility). Results of the experiments demonstrating adsorption of proteins to the wear particles arising *in vivo* and isolated from granuloma tissues are summarized in Graph 2, page 12. The data presented in Graph 2 were obtained when a constant amount of the wear particles (0.5 mg) was used for binding. All five pure proteins tested showed similar dependence of the bound protein amount on the initial protein concentration. Binding curves similar to those obtained for wear particles and shown in Graph 2 were also obtained for commercial UHMWPE powder GUR 4120 (see Graph 3, page 12). The experiments also showed that binding of these proteins to the UHMWPE wear was neither significantly affected by the buffers pH range between 6.0-8.0, nor by the buffer composition (Tris, acetate, phosphate, NaCl). Due to the irreversibility of binding of proteins to UHMWPE wear particles, the determination of dissociation constants and the number of binding sites from, for example Scatchard plot, turned out meaningless. However, the number of bound protein molecules per an average wear particle could be estimated (Tab. 3, page 13), although the counts of the protein molecules bound to one UHMWPE particle are rather of an illustrative than exact value due to many variables and many presumptions used for this calculation. [11]



Graph 2: Binding of serum proteins to isolated UHMWPE wear particles in saline. Protein concentration varied, amount of wear particles maintained constant (0.5 mg) [11]
(human serum albumin (HSA), human immunoglobulin G (HIgG), human α_1-acid glycoprotein (Hα_1AG), human holo-transferrin (HHT), human α_1-antitrypsin (Hα_1AT))



Graph 3: Binding of serum proteins to GUR 4120 powder in saline. Protein concentration varies, amount of GUR 4120 particles maintained constant [11]
(human serum albumin (HSA), human α_1-acid glycoprotein (Hα_1AG))

Tab. 3: Binding of human serum proteins to UHMWPE wear particles [11]
(human serum albumin (HSA), human immunoglobulin G (HIgG), human α_1-acid glycoprotein (Hα_1AG), human holo-transferrin (HHT), human α_1-antitrypsin (Hα_1AT))

	Hα_1AG	HSA	HIgG	HHT	Hα_1AT
Protein relative molecular weight [ ]	45000	68000	156000	79550	53000
Protein concentration at relative saturation of UHMWPE first binding site [%, w/v]	0.05	0.1	0.075	0.075	0.075
Weight of protein bound to the first binding site [g] per 0.5 mg of wear particles	2.79×10^{-5}	5.64×10^{-5}	4.72×10^{-5}	4.65×10^{-5}	5.62×10^{-5}
Protein concentration at relative saturation of UHMWPE second binding site [%, w/v]	0.3	0.3	0.3	0.3	0.3
Weight of protein bound to the second binding site [g] per 0.5 mg of wear particles	10.33×10^{-5}	3.32×10^{-5}	5.11×10^{-5}	6.88×10^{-5}	7.40×10^{-5}
Number of protein molecules bound to the first binding site per one average UHMWPE particle	9800	13100	4800	9300	16800
Number of protein molecules bound to the second binding site per one average UHMWPE particle	36300	7700	5200	13700	22100