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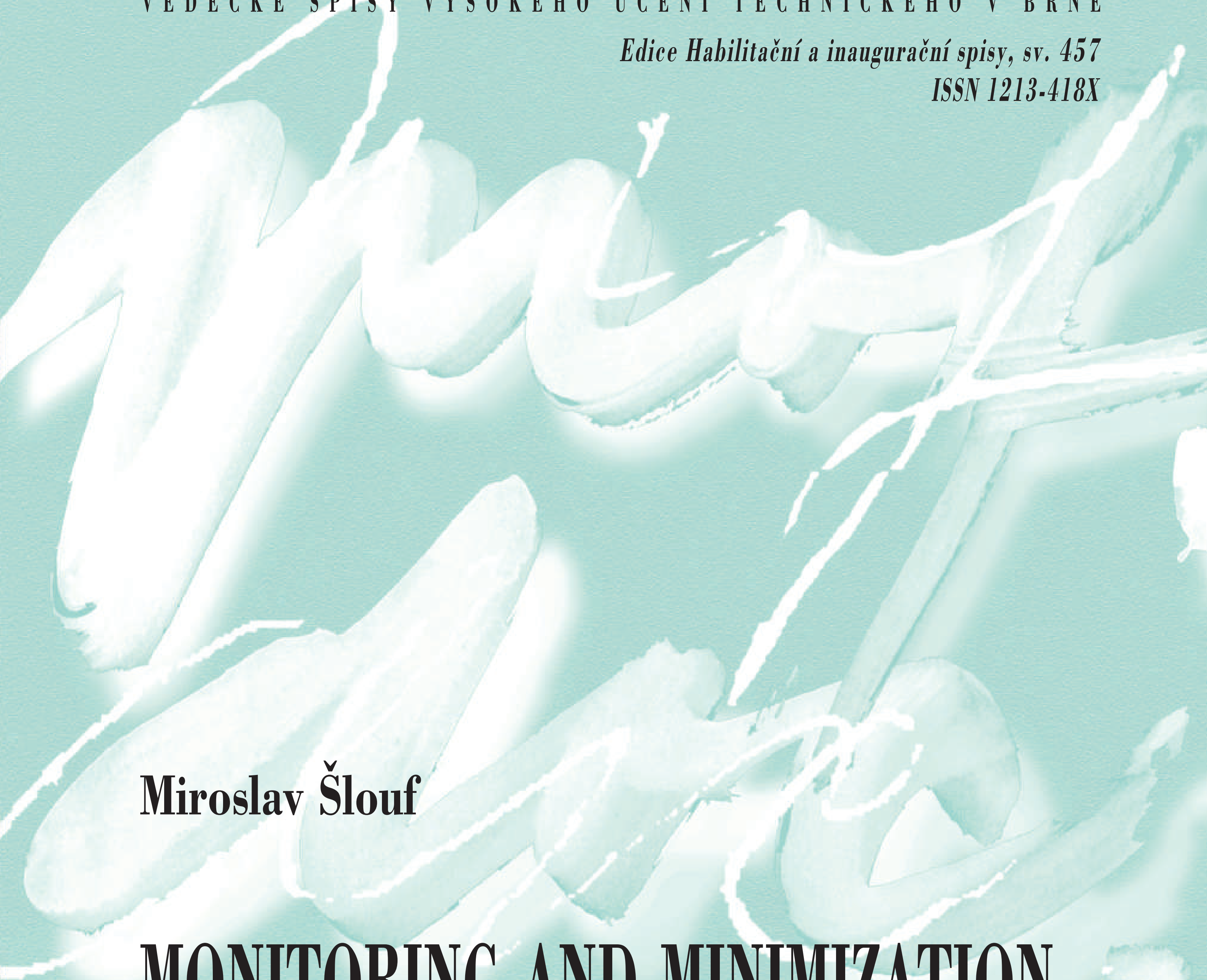

Miroslav Šlouf

MONITORING AND MINIMIZATION OF UHMWPE WEAR IN TOTAL JOINT REPLACEMENTS

BRNO UNIVERSITY OF TECHNOLOGY
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INSTITUTE OF MATERIALS SCIENCE

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MONITORING AND MINIMIZATION OF UHMWPE WEAR IN TOTAL JOINT REPLACEMENTS

SLEDOVÁNÍ A MINIMALIZACE OTĚRU UHMWPE V KLOUBNÍCH NÁHRADÁCH

SHORT VERSION OF HABILITATION THESIS

VUTIUM

BRNO 2013

KEYWORDS

UHMWPE for total joint replacement, lifetime, oxidative degradation, wear resistance

KLÍČOVÁ SLOVA

UHMWPE pro kloubní náhrady, životnost, oxidativní degradace, odolnost vůči otěru

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1 INTRODUCTION

1.1 TOTAL JOINT REPLACEMENTS

Replacements of big human joints (total joint replacement, arthroplasty) became a common orthopedic operation. Average life is longer, more active and, as a result, more surgical repairs of damaged joints are required. In more developed countries, the number of arthroplasties per one million of inhabitants is higher than 1000 [1]. The most frequently replaced joints are hips and knees (>90%), followed by all other joints such as shoulders, elbows etc. (<10 %). Majority of contemporary total joint replacements/arthroplasties (TJR/TJA) is based on ultra-high molecular weight polyethylene (UHMWPE) [2]. Fig. 1 shows the two most common TJR: total hip replacement (THR, THA) and total knee replacement (TKR, TKA). All TJRs are made from two articulating components: THR is composed of femoral head (metal or ceramics) and acetabular cup (usually UHMWPE, alternatively ceramics or metal), and TKR consists of femoral component (usually metal, occasionally ceramics) and tibial insert (almost exclusively UHMWPE).

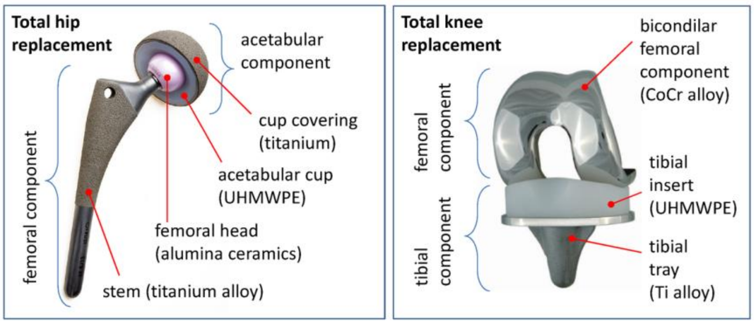


Figure 1. *Typical total joint replacements of two most frequently replaced joints: hip (left) and knee (right). Source of THR and TKR photographs: Beznoska Ltd.; www.beznoska.cz.*

1.2 UHMWPE IN TOTAL JOINT REPLACEMENTS

Since its clinical introduction in 1962, UHMWPE is the key component of both hip and knee joint replacements [3]. Considering rapid changes in the field of orthopedics, the long-term role of UHMWPE in joint arthroplasty is fairly remarkable. UHMWPE has been so successful due to the following properties: (i) *excellent biocompatibility* of the bulk polymer (ii) *favourable tribological properties*, namely high wear resistance and good friction properties, and finally (iii) *sufficient mechanical properties* for given application [3,4].

Nowadays, UHMWPE is officially called *gold standard* for TJRs [5]. Alternative bearing materials, such as ceramics and metals, achieved only limited success. The ceramic components exhibit very high wear resistance, but suffer from brittleness. The metallic components are not popular due to biocompatibility issues connected with dangerous metallic wear particles. Details associated with history and comparison of UHMWPE, ceramics and metallic bearing components can be found in the full version of this habilitation theses and elsewhere [2, B2].

However, the UHMWPE-based TJRs do not last forever. During the first decade of their life, UHMWPE joint replacements are usually highly successful and less than 1% of them have to be revised (re-operated, replaced). However, after 10 years from implantation the survivorship significantly decreases [6]. Orthopedics-related reasons of TJR failures, such as malpositioning, dislocation and septic loosening, are more-or-less the same for all types of TJRs. Two most important material-related reasons of the UHMWPE-based TJRs were identified as *wear* and *oxidative degradation* [4, 7, 8].

Wear of UHMWPE is a release of microscopic particles from the polymer surface due to mutual motion of polymer and metallic/ceramic parts of the artificial joint. From macroscopic point of view, severe wear leads to thinning of the UHMWPE component (Fig. 2a) followed by mechanical failures of the whole TJR. From microscopic point of view, the wear is even more dangerous: UHMWPE wear particles (Fig. 2b) are released into surrounding tissues, where they cause inflammatory reactions, formation of osteoaggressive granuloma (Fig. 2c) and bone resorption (Fig. 2d), which results in bone loosening and revision of TJR [4, 8].

Oxidation of UHMWPE is the second major reason of TJR failures. The oxidation (or oxidative degradation) occurs not only during the UHMWPE synthesis, consolidation, modification and storage due to outer atmosphere, but also after the implantation due to oxygen dissolved in body fluids (*in vivo* oxidation, refs. [8–10]). The basic scheme of UHMWPE oxidative degradation is called Bolland's cycle [11]. Briefly, the alkyl radicals present in the polymer (mostly residual radicals from UHMWPE modifications) react with O_2 forming peroxy radicals, which attack other polymer molecules forming hydroperoxides and renewing the original alkyl radicals. The decomposition of hydroperoxides leads to more stable oxidation products, namely ketones, secondary alcohols, carboxylic acids and esters [11,12]. In case of carboxylic acids and esters the reaction is associated with chain scissions. The chain scissions are connected with a decrease in mechanical performance of the polymer, including its key property for given application – wear resistance [8, 13]. In fact the oxidation and the structure of UHMWPE radicals are more complex [7–14], but the basic principle represented by Bolland's cycle holds.

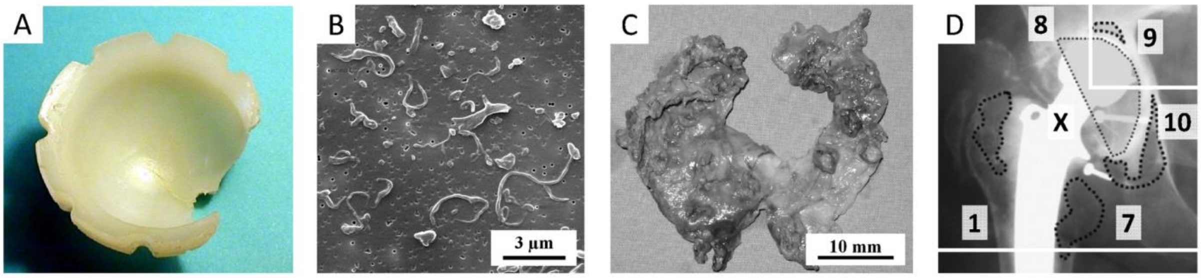


Figure 2. *Wear of UHMWPE at macroscopic (a) and microscopic level (b). Photograph (a) shows explanted, heavily worn polymer component of THR. SEM micrograph (b) visualizes in vivo microscopic UHMWPE wear particles. Photograph (c) shows explanted granulomatic tissue, which contains wear particles. Radiographic image of THR (d) documents damage of bones around failed THR before revision (damaged areas denoted by dotted lines; orthopedic zones, in which the damage is evaluated, are denoted by numbers according to Gruen [15] and DeLee [16]).*

1.3 NEW GENERATIONS OF UHMWPE FOR TJR

The first UHMWPE-based TJRs were made of virgin UHMWPE. The polymer was not further modified, but only sterilized, usually by gamma radiation (radiation dose ≤ 40 kGy). This material has been called standard or non-crosslinked or *0. generation UHMWPE*. The standard UHMWPE brought significant increase in TJR lifetime in comparison with previous designs based on poly(tetrafluoroethylene) and metals [2, B1, B2]. It was successfully used through decades and several manufacturers have been using it until now [B2]. However, in the course of time it has been realized that TJRs with 0. generation UHMWPE fail due to wear and oxidation (section 1.2).

In late 1990s, intensive research in the field of UHMWPE resulted in *1. generation UHMWPE*. All modifications of UHMWPE for TJR have to maintain its medical-grade purity. Therefore, the modifications must be based on physical, rather than chemical procedures. After unsuccessful experiments with carbon-fiber-reinforced UHMWPE [17, 18] and high-pressure crystallized UHMWPE [19–21], it showed that the right way how to prepare UHMWPE with increased wear and oxidation resistance was irradiation with ionizing radiation (gamma radiation, accelerated electrons) combined with thermal treatment (heating above/below T_m = remelting/annealing). Under carefully selected conditions, irradiation of UHMWPE results in formation of crosslinks among UHMWPE chains (crosslinking), which is associated with increase in wear resistance [1, 12, 22, 23], while thermal treatment removes residual radicals from the irradiation that cause long-term oxidative degradation [11–14]. In the last, sterilization step, traditional gamma irradiation is being replaced with better methods (ethylenoxide or gas plasma sterilization), which do not produce further radicals [8, 9, 22]. As the conditions during irradiation, thermal treatment, and sterilization (radiation dose, temperature, atmosphere…) strongly impact on the final structure and properties of UHMWPE, every manufacturer has its own proprietary procedure and huge amount of commercial 1st generation UHMWPEs exist [24]. In general, the 1st generation UHMWPEs exhibit higher wear and oxidation resistance at the cost of certain decrease in mechanical properties in comparison with 0. generation polymers [C7, V2–V5, 1,2]. Short- and middle-term clinical results of 1st generation UHMWPEs are encouraging [24].

In the first decade of the 21st century, several groups of researchers prepared *2nd generation UHMWPEs*. Their motivation was to maintain high wear resistance of the 1st generation UHMWPEs, further improve oxidation resistance, and achieve mechanical properties comparable to those of unmodified polymer. As of 2009, there were at least three commercially available 2nd generation UHMWPEs: ArCom XL polyethylene (clinically introduced in 2005, product of Biomet, USA) is highly-crosslinked, mechanically annealed polymer [24, 25]. E-Poly HXLPE (clinically introduced in 2007, product of Biomet, USA) is highly-crosslinked polymer doped with vitamin E [24, 26]. X3 polyethylene (clinically introduced in 2005, product of Stryker Orthopedic, USA) is sequentially-crosslinked polymer (three irradiations followed by annealing instead of single irradiation used by other manufacturers; refs. [24, 27]). Common features of all above-listed 2nd generation UHMWPEs are: (i) no remelting step, which was shown to decrease mechanical performance more than annealing, (ii) detectable amount of residual radicals from irradiation, (iii) improved mechanical properties according to a few studies, and (iv) patented modification process. The improvement of mechanical properties 2nd generation UHMWPEs is controversial in certain cases and measurable amount of residual radicals is a potential risk from the point of view of long-term oxidative degradation [28]. Therefore, only long-term clinical data will show if 2nd generation UHMWPEs bring measurable prolongation of TJR lifetime.

1.4 SUBJECT OF THIS HABILITATION THESIS

Shortly after year 2000, a group of Czech researchers, surgeons and manufacturers founded an *UHMWPE project*. The project consisted of research workers at the Institute of Macromolecular Chemistry (IMC) and the Charles University (UK), orthopedic surgeons at Motol Faculty hospital, Praha (FN Motol), and TJR manufacturers from company Beznoska s.r.o., Kladno (Beznoska Ltd). *Orthopedics-related part of the project* was focused on **monitoring of UHMWPE wear**, oxidative degradation and clinical performance, so that it was possible to evaluate and compare quality of contemporary types of the polymer for TJR on the Czech market. *Materials-science-related part* was focused on **minimization of UHMWPE wear** and optimization other relevant properties of the material, aiming at production of highly-crosslinked UHMWPE, which would be suitable for production of original Czech TJRs with increased lifetime.

Author of this habilitation thesis joined UHMWPE project in 2002. Two years later he became a leader of the project in the sense that he led series of grants of the whole team joining IMC, UK, FN Motol and Beznoska Ltd. This habilitation thesis comments the most important results achieved during the project, which have been published within period 2004–2012. *In the orthopedic part of the project*, new, fast and reproducible techniques of *in vivo* UHMWPE wear particles monitoring and quantification were introduced [J1–J4, J10]. The established methods have been employed in analyses of wear debris, comparison of various UHMWPEs and analysis of TJR failures [J5–J9, C1–C3, C6]. *In the materials part of the project,* we developed and patented highly-crosslinked UHMWPE [P1], which has been used by Beznoska Ltd. for production of TJR since 2007. We proved that the quality of our highly-crosslinked UHMWPE was fully comparable with foreign products on the market [V2, V7]. The established methods of UHMWPE characterization were published [J11–J14] and employed in comparison of UHMWPE types available on the Czech market [J15, C8, C9].

2 MONITORING OF UHMWPE WEAR

2.1 QUANTIFICATION OF UHMWPE WEAR PARTICLES

The quantification of UHMWPE wear particles around TJR is of imprortance for orthopedic analyses of TJR failures. Our particular objective was to correlate amounts of in vivo UHMWPE wear particles with extent of tissue damage in specific zones around TJR (Fig. 2d). Therefore, we needed an efficient method for quantification of UHMWPE particles in specific locations around TJR. It is worth noting that here the term *quantification* means determination of *both* particle *morphology* (size and shape distributions) and *concentration* (number of particles per unit weight of damaged tissue). Determination of particle morphology is relatively straightforward with electron microscopes, whereas determination of particle concentration is demanding from the point of view of sampling, accuracy, reproducibility, and high throughput in order to achieve statistically significant results within reasonable time.

2.1.1 Existing quantification methods

Quantification of *in vivo* UHMWPE wear particles (Fig. 2b) requires collaboration of orthopedic surgeons (precise and reproducible sampling during TJR revisions), biochemists (isolation of in vivo UHMWPE wear particles from the tissues sampled during the revisions), and analytical/polymer scientists (accurate quantification of isolated polymer microparticles in the solution). In the case of our UHMWPE project (section 1.4) the sampling was performed at FN Motol, isolation of particles at UK, and quantification of isolated particles at IMC.

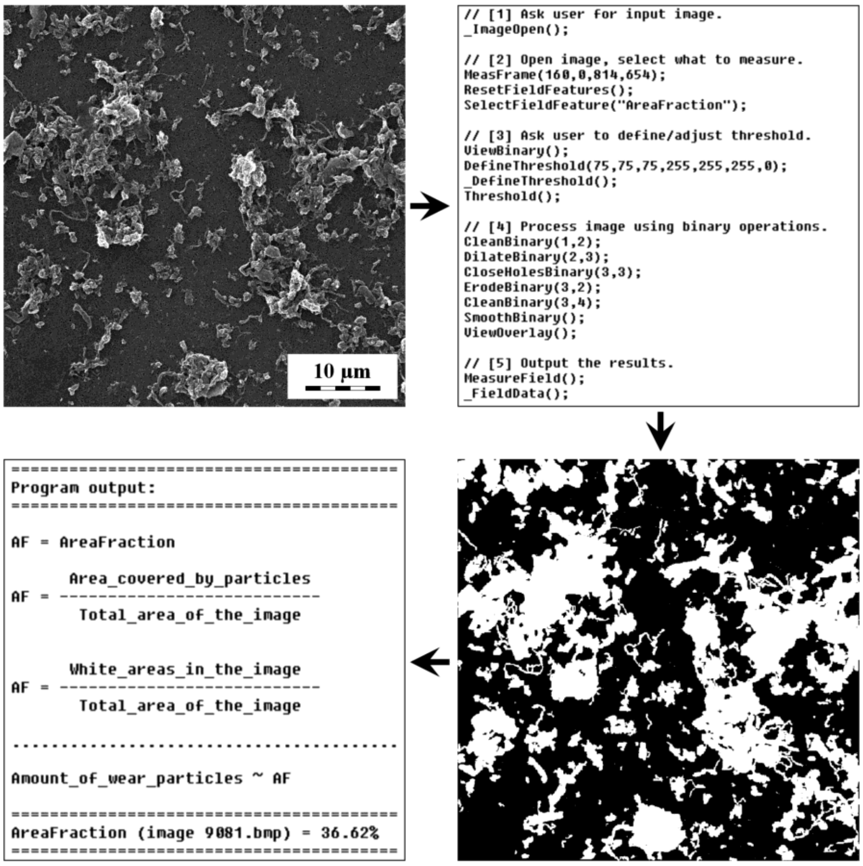
At the beginning of UHMWPE project, the quantification of *in vivo* UHMWPE wear particles in tissues around TJR was not fully resolved in the literature. Techniques based on weighing [29] suffered from lower accuracy due to the negligible mass of particles from *in vivo* samples. Light scattering based techniques had intrinsic problems with particle agglomeration and conversions among intensity, volume and number distributions [30]. Electric resistance particle size analyzers were not widely available and did not catch particles below 0.58 μm, although the most biologically active particles are in the range 0.1–1 μm [31, 32]. Estimation of particle amount from SEM micrographs of isolated particles on microfilters was time consuming and imprecise [33, 34].

2.1.2 Development of new and more efficient methods

The first method of quantification of UHMWPE wear particles we developed [J1] was called LSc (light scattering with calibration spheres). It was based on precise and reproducible orthopedic sampling from individual zones around THR (Fig. 2d; harvesting of granulomatic tissues with minimum contamination, developed at FN Motol), simple isolation of particles (HNO_3 digestion resulting in UHMWPE particle suspensions; developed in collaboration of UK and IMC) and elastic light scattering of *unknown* amount of wear particles with *known* added mass of 500 μm glass calibration spheres (developed at IMC). The LSc measurement of each sample (suspension of UHMWPE wear particles isolated from one particular zone) included two light scattering experiments (1st: suspension of particles only; 2nd: the same suspension *with* calibration spheres). The measurement was followed by a set of calculations (corrections for background, unit weight of calibration spheres, unit volume of UHMWPE particle suspension, etc.). The method could yield both relative and absolute numbers of UHMWPE wear particles, depending on the complexity of the calibration process. The reliability of LSc method was confirmed by the fact that the highest numbers of wear particles were repeatedly found in zone 10 (Fig. 2d; ref. [J1]), which accorded with the orthopedic observations of high tissue and bone damage in this zone (more details in section 2.2 and refs. [J1, J9, C1]). Although LSc method suffered from lower accuracy due to instability/agglomeration of the suspensions, we were able to confirm our assumption that the distribution of wear particles around TJR had been rather inhomogeneous. The experience gathered with LSc was employed in development of more efficient methods [J3, J4, J10].

In the next step, we tried to employ quasi-elastic light scattering (QELS) instead of static light scattering (LS) to quantify UHMWPE wear particles. For QELS experiments, we developed a method of reproducible preparation of small gold calibration particles with tunable size [J2] (size of nanoparticles in the range 5–200 nm; upper detection limit of the QELS method is 6 μm). Although QELS showed to be unsuitable for quantification of UHMWPE particles due to severe problems with agglomeration [35], subsequent research of metallic nanoparticles with various sizes, shapes and/or chemical composition resulted in a different application: the particles were employed in multiple immunolabeling of biological specimens [P2, 36, 37].

The second successful method of UHMWPE quantification we developed was called SEMq (semi-automated quantitative analysis of SEM micrographs with isolated particles) [J3]. The sampling during TJR revision was like in LSc method, with two improvements: (i) isolation protocols were perfected so that the suspension of UHMWPE wear particles did not contain any detectable impurities and (ii) the isolated UHMWPE wear particles were caught on microporous polycarbonate (PC) membranes in dried state and visualized by SEM (Fig. 3).



```
// [1] Ask user for input image.
_ImageOpen();

// [2] Open image, select what to measure.
MeasFrame(160,0,814,654);
ResetFieldFeatures();
SelectFieldFeature("AreaFraction");

// [3] Ask user to define/adjust threshold.
ViewBinary();
DefineThreshold(75,75,75,255,255,255,0);
_DefineThreshold();
Threshold();

// [4] Process image using binary operations.
CleanBinary(1,2);
DilateBinary(2,3);
CloseHolesBinary(3,3);
ErodeBinary(3,2);
CleanBinary(3,4);
SmoothBinary();
ViewOverlay();

// [5] Output the results.
MeasureField();
_FieldData();
```

```
=======================================
Program output:
=======================================

AF = AreaFraction

       Area_covered_by_particles
AF = ---------------------------
        Total_area_of_the_image


       White_areas_in_the_image
AF = ---------------------------
        Total_area_of_the_image

.......................................

Amount_of_wear_particles ~ AF

=======================================
AreaFraction (image 9081.bmp) = 36.62%
=======================================
```

Figure3. *Principle of SEMq method: the isolated UHMWPE wear particles on a microporous polycarbonate filter are visualized on a SEM micrograph, the micrographs are processed by an image-analysis script that semi-automatically converts the (grayscale) micrograph on the (black-and-white) binary image; although the single particles cannot be reliably counted, their number is quite precisely proportional to the area they occupy.*

As for the *improved isolation of the UHMWPE wear particles* from the freeze-dried samples, we tested three methods: alkaline hydrolysis (digestion with KOH), acid hydrolysis (digestion with HNO_3), and enzymatic hydrolysis (digestion with pronase and collagenase). In the first step, all three methods included delipidation with chloroform:methanol mixture, in the next steps all methods employed digestions, repeated aspirations, washing, decantation and/or centrifugation, and in the last step they all used filtration through microporous polycarbonate (PC) membranes. The pre-filtration (10μm PC membrane) removed the biggest particles. Consequently, the SEMq method quantifies the most biologically active particles <10 μm [38–40]. The purity of the isolated wear particles was verified by SEM, EDX and FTIR. Considering all aspects (time, cost of chemicals, final purity), the HNO_3 digestion method was found the most convenient. As for the *semi-automated image analysis of the SEM micrographs* (Fig. 3), it was shown that the area occupied by the wear particles (morphological descriptor *AreaFraction*, AF) could be determined efficiently and reproducibly, by means of an image analysis script (Fig. 3, upper right image). We proved that the AF is (after normalization described in detail in [J3]) proportional to the number of UHMWPE wear particles on the membrane. In order to achieve sufficient accuracy, it was necessary to analyze 8 micrographs from random locations on the membrane per each sample. Nevertheless, this was not such a big obstacle once the image analysis was successfully automated. The SEMq method was more reproducible than LSc and confirmed that the distribution of the numbers of wear particles around TJR was quite non-homogeneous. The numbers of wear particles in various locations around TJR frequently differed by as much as one order of magnitude.

The third method for quantification *in vivo* UHMWPE wear particles was called IRc (IR spectroscopy with internal calibration) [J4]. The IRc combined the advantages of both previous techniques: it was fast like LSc [J1] and reliable like SEMq [J3]. The samples were harvested and the particles isolated like in SEMq [J3]; final sample after isolation were pure UHMWPE wear particles on 10μm PC membrane. The amount of the UHMWPE wear particles was shown to be proportional to the intensity of the IR peak at 2850 cm^{-1}, which was normalized to the same intensity of the peaks of PC. Therefore, the PC membrane acted not only as a filter during isolation, but also as an internal standard during IR-based quantifications, as illustrated in Fig. 4.

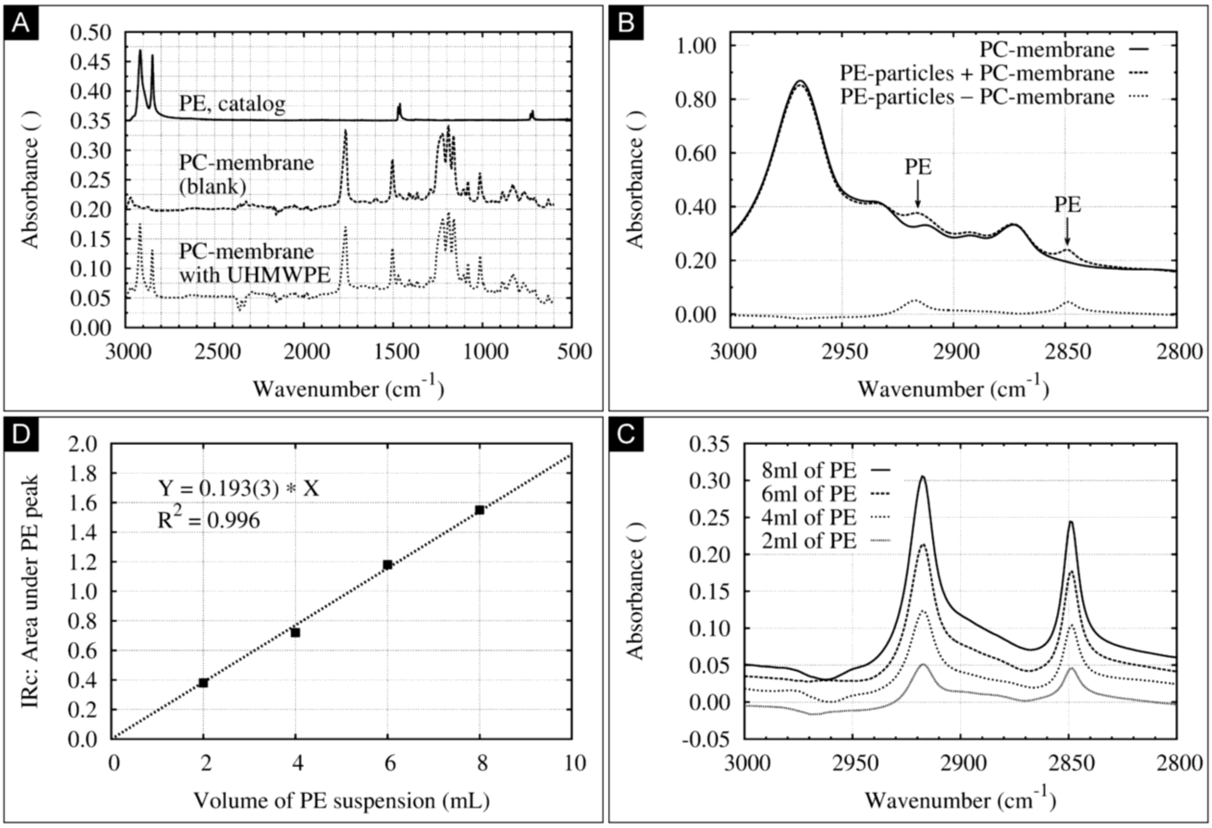


Figure 4. *Quantification of the UHMWPE wear particles by IRc method: (a) IR/ATR spectra of pure PE, clean PC membrane, and PC membrane with PE particles; (b) IR spectra in transmission mode, showing small but measurable PE peaks, whose intensity is evaluated after normalization and subtraction of PC background; (c) IR spectra of a testing sample in the form of suspension containing the isolated PE wear particles, from which 2, 4, 6 and 8 mL were filtered through four PC membranes – the IRc signal increased linearly as documented in (d).*

The IRc method was our final technique, optimized for quantification of *in vivo* UHMWPE wear particles. Great majority of the results discussed in the following text were achieved with IRc. Our initial publication about IRc [J4] demonstrated several other advantages of the method, which just listed below for the sake of brevity: (i) the isolated particles were without impurities, as evidenced by both IR spectra (Fig. 4a) and EDX microanalysis (Fig. 5a), (ii) the IRc-determined amounts of wear particles correlate very well with those from SEMq (Fig. 5b), which mutually confirms the accuracy and the precision of both methods, (iii) the IRc method can be combined with SEM analysis of single particles in order to obtain morphological description and/or absolute amounts of wear debris and, (iv) the IRc results correspond to orthopedic evaluation of tissue damage in zones around TJR (section 2.2; see also refs. [C3, C6]).

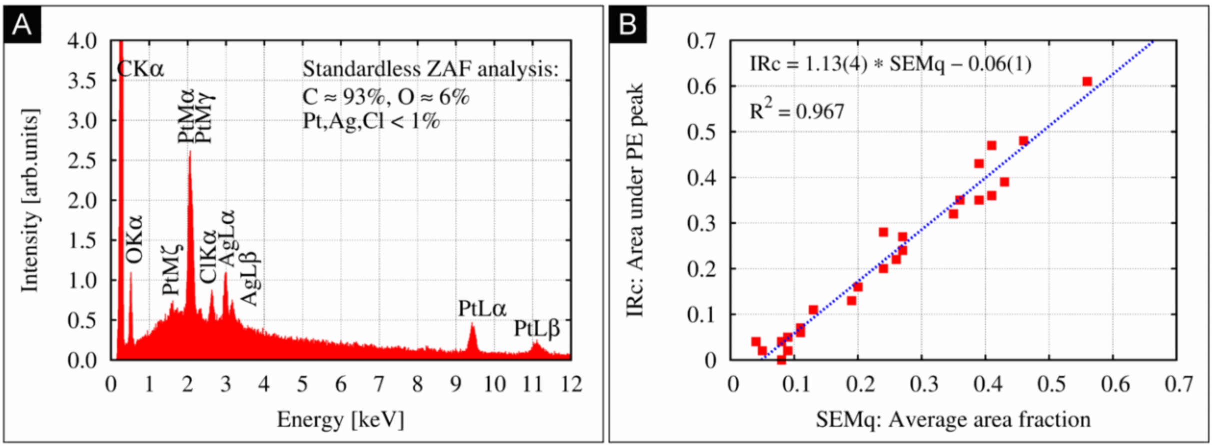


Figure 5. *SEMq method: (a) EDX spectrum of isolated UHMWPE wear particles on PC membrane; the peaks of C and O come from the PE particles and PC membrane, the traces of Pt, Ag and Cl result from the fact that sample was fixed with conductive silver paste and sputter-coated with a 4nm Pt layer in order to eliminate charging in the SEM microscope. (b) Correlation between SEMq and IRc methods; SEMq estimates the amount of PE particles from the morphological descriptior* AreaFraction, *i.e. from the relative area covered by the PE particles on PC membrane (Fig. 3), while IRc estimates the total amount of PE particles from the area of PE peak on normalized IR spectrum (Fig.4).*

2.2 ORTHOPEDIC EVALUATION

2.2.1 Correlation between UHMWPE wear and tissue damage

The searching for the correlation between concentration of UHMWPE wear particles and extent of tissue/bone damage in specific zones around TJR was the main objective of the orthopedic part of the project. Positive proof of the correlation for particular joint replacement confirmed that the UHMWPE wear particles were the real reason of its failure, whereas non-existence of the correlation indicated other problems. Therefore, the UHMWPE wear particle quantification contributes to orthopedic analyses of TJR failures and quality.

During the development of UHMWPE wear particle quantification methods (section 2.1.2; refs. [J1–J4]), we had already proved and/or verified our original assumptions: (i) By means of LSc method [J1], it was confirmed that the distribution of particles in various zones around TJR was inhomogeneous, despite some other studies claimed that the numbers of particles vary little [30]. (ii) SEMq method [J3] was more reliable than LSc; it showed that the numbers of UHMWPE wear particles in various zones may differ by more than one order of magnitude. (iii) IRc method yielded larger sets of results that confirmed findings from LSc and SEMq [J1–J4].

All three methods (LSc, SEMq, IRc) were also applied on several real cases of revised THRs during their testing. In general, very good agreement between number of UHMWPE wear particles and tissue damage in individual zones around TJR was found. The complete results are given the original papers [J1, J3, J4]. One illustrative example showing the comparison of IRc results with the orthopedic evaluation is shown here (copied from ref. [J4]). Patient #18 with total hip arthroplasty on the right (Fig. 6), was an 83-years-old woman, whose fractured acetabulum had to be fixed with screws before the first implant was inserted; the screws are clearly visible on the radiographic image (Fig. 6a). Before the revision, the acetabulum was very irregular and defective and the UHMWPE cup was displaced, worn and completely broken. The maximum of osteolysis and damaged tissue, called (osteoagressive) granuloma (Fig. 2c), was found, in the following order, in zones III > II > I. A smaller amount of granuloma was taken from femoral zone 7; the amount of granuloma in zone 1 was too small to be processed. The results of IRc (Fig. 6b) fit these surgical data almost perfectly: by far the highest volume of wear debris was found in zone III, which exhibited the highest extent of tissue damage, less wear debris was localized in zone II and the lowest volume of wear particles was detected in zones I and 7.

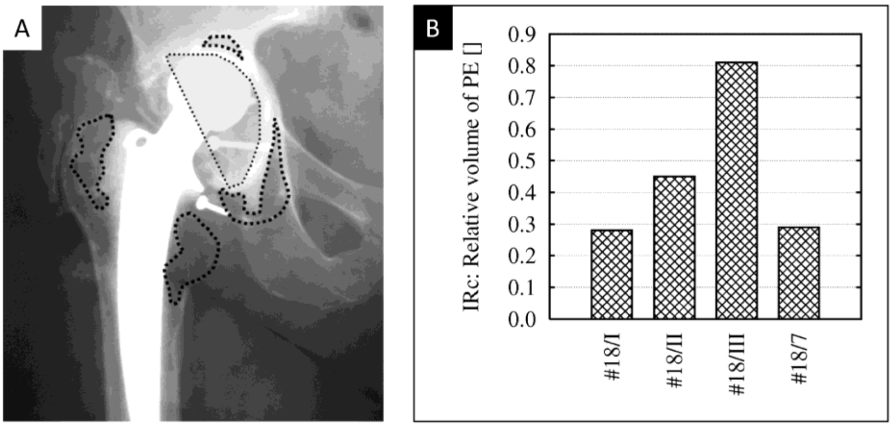


Figure 6. *Correlation between IRc and radiographic results for patient #18. The thin dotted line in radiographic image marks UHMWPE cup, the thick dotted line denotes damaged tissue in zones around THR, from which the granuloma was taken. Radiographic image (a) shows the highest tissue damage in zones III, II and 7. During the revision, the highest volumes of granuloma were found in acetabular zones (III > II > I), the amount of granuloma in femoral zones was smaller (7 > 1). IRc results (a) show the decrease of PE volume in zones in the following order: III > II > I = 7 (damaged tissue from zone 1 was too small for isolation and further processing).*

Further results associated with *in vivo* wear particles quantification around TJRs appeared in several orthopedic studies in the local Czechoslovak orthopedic journal [C1–C3, C6]. The studies are not a part of this habilitation thesis as they are concentrated on orthopedic problems, but the conclusions associated with wear particle analysis are summarized in this paragraph. In [C1] we introduced LSc method to the Czech orthopedic community (it was the first method of wear particle quantification available in the Czech Republic that time) and commented its results from the clinical point of view. In [C2] we described the newer and more reliable method IRc, demonstrated its application to another 3 selected cases of THR and concluded that the correlation between amount of wear debris and tissue damage was observed in all studied cases so far [J1, C1, J3, J4], which implied that UHMWPE wear is the real cause of all investigated TJR failures. In [C3] we applied the IRc method together with the morphological analysis of UHMWPE wear particles by means of SEM on a specific orthopedic problem: comparison of TKRs with different femoral components; we concluded that a ceramic femoral component *in vivo* failed to demonstrate any advantage in comparison with a standard metal component. In [C6] we described in more detail our method of morphological evaluation of the wear particles, which was called MORF; the method uses the same isolation protocol as IRc and the morphological analysis of the wear particles on SEM micrographs is performed by means of “mathematical filtering”, which simplifies the experimental part and makes the results more accurate.

The most important results concerning the correlation between the numbers of wear particles and the extent of tissue damage in specific zones around TJR appeared in our study of 45 patients [J9]. In the first step, the TJR revisions were performed and UHMWPE wear particles were quantified by IRc. In the second step, the orthopedic data and IRc results of all patients were evaluated and compared. The main problem at this stage consisted in the fact that IRc results were *quantitative* (numbers giving amounts of UHMWPE wear particles in various zones around TJR), whereas orthopedic evaluation was *qualitative* (verbal orthopedic evaluation based on pre-operation radiographs and operation protocol describing the amount of tissue damage). In order to overcome this discrepancy, we converted both IRc and orthopedic evaluation to *statements*. Data from IRc were transformed to measurement statement (MS), which was a sequence of zones, ordered according to decreasing amount of UHMWPE wear particles. Orthopedic evaluation was transformed to orthopedic statement (OS), which was another sequence of zones, ordered according extent of observed bone and tissue damage. Once the data were represented by analogous structures, they could be evaluated by statistical analysis, which was performed as the last step of the study. We tested all pairs of orthopedic statements (OS) and measurement statements (MS) by means of multiple Friedman tests using SPSS 16.0 package. Briefly, we tested two statistical hypotheses:

- Hypothesis #1: Damage and amount of particles in zones around THR is homogeneous.
- Hypothesis #2: Orthopedic statements and measurement statements are not different.

The final result of statistical testing is confirmation or rejection of a hypothesis. The hypothesis is confirmed/rejected at a certain significance level α, which is usually/conventionally set at 0.05 (or 5%). The numeric result of statistical testing is *p*-value, which is a number in the interval 0–1 (or 0–100%). If the *p*-value exceeds the significance level α, the hypothesis is rejected (at given significance level α). In fact the *p*-value gives the probability that the results would be as extraordinary as observed, given the null hypothesis is true. In our case, the results of statistical testing were:

- Hypothesis #1 was rejected at the level of significance 0.001 = 0.1%. In other words, the probability that we would get such differences among the amounts of wear particles in different zones around THR just by coincidence was lower than 0.1%.
- Hypothesis #2 was *not* rejected at the level of significance 0.05 = 5%. In other words, the probability that we would get such a good correlation among the orthopedic evaluation (OS) and the IRc measurements (MS) just by coincidence was lower than 5%.

Details concerning the statistical evaluation and comparison of the results with the literature are given in the discussed study [J9]. It is worth noting that according to available literature, this was the first study that directly confirmed the correlation between the extent of tissue damage in zones around THR and the amount of UHMWPE wear debris in these zones. The study brought several conclusions important for clinical practice, which could be summarized as follows:

- The distribution of the UHMWPE wear particles around TJR is non-homogeneous.
- The highest tissue damage and the highest amounts of the UHMWPE wear particles were frequently found in zones III and 7, which correspond to experience of orthopedic surgeons in the sense that the damage in these zones is usually critical.
- The Extent of tissue damage in specific zones around THR correlates with the amount of 0.1–10μm UHMWPE wear particles quantified by IRc method.
- The whole set of data [J1, J3, J4, J9] confirms that wear particles are the real cause of most **THR** failures, observed in the collaborating hospitals. Consequently, UHMWPE with improved wear resistance is needed to increase lifetime of TJRs used in the Czech Republic hospitals. This is the most important result for materials-science-part of the project.
- Last but not the least, in the case of **TKR**s such a strong correlation between the wear debris and the tissue damage was not observed. This confirmed that wear resistance is more important for THR than for TKR. Consequently, the improvement of wear resistance at the cost of decrease in other mechanical properties may not be relevant for TKRs, which has been a subject of ongoing discussions in the orthopedic community [41].

2.2.2 Further problems associated with wear particles

During our work on the monitoring UHMWPE wear particles, we had to solve a few supplementary questions and/or problems. The problems associated with sampling, reproducibility and accuracy of our quantification methods were published within their development and [J1, J3, J4, J9] as discussed above. The problems connected with metal nanoparticles [J2] lead to different applications in the field of molecular biology [P3]. The remaining issues, such as impact of centrifugations on the wear particle morphology during isolations, problems with extremely small wear nanoparticles, encapsulation of particles inside granulomatic tissues etc., were published in a parallel series of papers [J5–J8, J10], which are briefly described in this section.

In [J5] we investigated the effect of centrifugation on morphology of polyethylene wear debris. Centrifugation at very high speeds (ultracentrifugation) is used during UHMWPE wear particle isolation techniques [42, 43] including our own [J3, J4]. However, it was suggested [44] that centrifugation or ultracentrifugation may change isolated particle morphology. As this assumption was mentioned without any experimental proof, we decided to analyze systematically the possible effect of centrifugation on morphology of *in vivo* UHMWPE wear particles. We selected four samples of periprosthetic tissues, in which the particles exhibited broad size distribution and elongated shapes. The isolations were made essentially as described in our previous work [J4], the only difference consisted in that all centrifugations were substituted by spontaneous flotation for at least 24 h. Then each of the UHMWPE wear particle suspensions was divided into four parts: (i) the first part was left as it was, (ii) the second part was centrifuged for 2 min at 500 × *g*, (iii) the third for 5 min at 16,000 × *g*, and (iv) the fourth for 30 min at 105,000 × *g*. The purity of isolated particles was verified by SEM, EDX and IR as described in our previous studies [J3, J4]. The morphology of isolated UHMWPE wear particles was assessed by image analysis of SEM micrographs (not shown here, see ref. [J5]). Image analyses (IMA) were performed for each of the patients/micrographs separately and evaluated both separated and averaged. On the whole we analyzed 320 SEM micrographs (4 patients × 4 centrifugation speeds × 20 micrographs per sample) with IMA program Lucia (Laboratory Imaging). The IMA outputs were morphological descriptors such as *EquivalentDiameter* and *Circularity*, whose distributions are shown in Fig. 7.

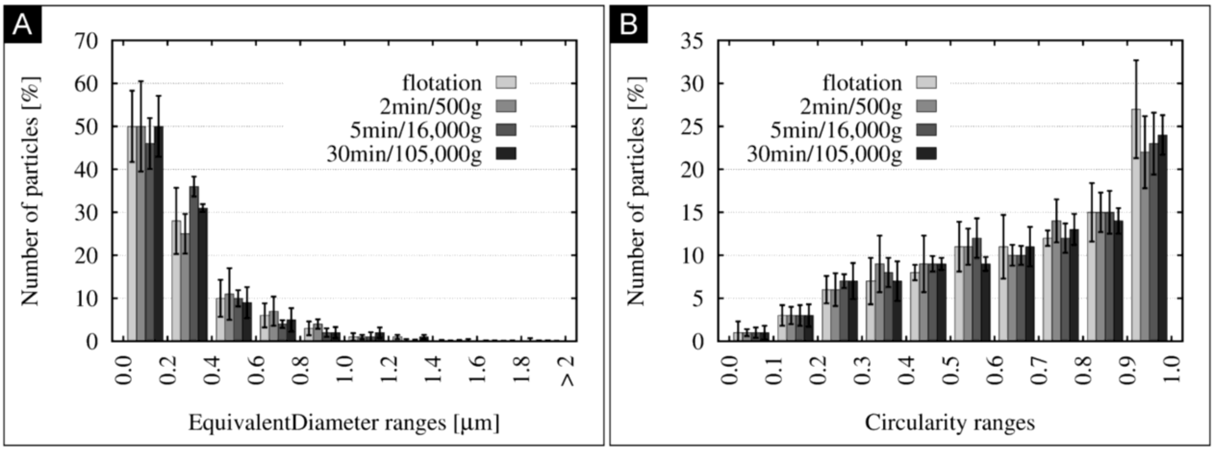


Figure 7. *Distributions of particle sizes (a) and shapes (b). Sizes are described by* EquivalentDiameter*, which gives average size of arbitrary particle; shapes are described by* Circularity*, which equals 1 for perfect spheres and decreases to 0 with increasing non-sphericity of the objects (for details see e.g. [C6]). Each column represents the average through all four patients. The columns are given in quadruplets: the columns in each quadruplet, from left to right, correspond to flotation, centrifugation at 500g, 16,000g, and 105,000g, respectively.*

There are two theoretical possibilities how centrifugation could have influenced wear particle morphology: (i) elongated particles might have been changed to spherical due to mutual collisions and collisions with vessel edges at very high centrifugation speeds and (ii) very small particles might have been isolated in higher amounts at higher centrifugation rates. Nevertheless, neither of the above mentioned effects was observed and even the ultracentrifugation at 105,000 g did not change the morphology of the UHMWPE wear debris, as evidenced in Fig. 7.

In [J6], we presented the first observation of nano-sized UHMWPE wear particles *in vivo* (Fig. 8). Due to continuous improvement of isolation methods and microscopic techniques worldwide, it has been possible to isolate and detect smaller and smaller wear particles. Scott et al. [34] proved that a significant fraction of in vitro UHMWPE wear particles can be smaller than 0.2 μm. Galvin et al. [45] found *in vitro* UHMWPE wear nanoparticles with sizes below 0.1 μm. In this study we found, for the first time, *in vivo* UHMWPE wear nanoparticles with average size below 0.05 μm (i.e. below the typical minimal size of pores in PC filters used for UHMWPE wear isolations). The nanoparticles were isolated from periprosthetic tissues of two different patients and had average particle size of 18.5 and 21.2 nm in the first and the second case, respectively. This was the first observation of *in vivo* nano-sized UHMWPE wear particles, which was confirmed almost at the same time by a group of British researchers [46].

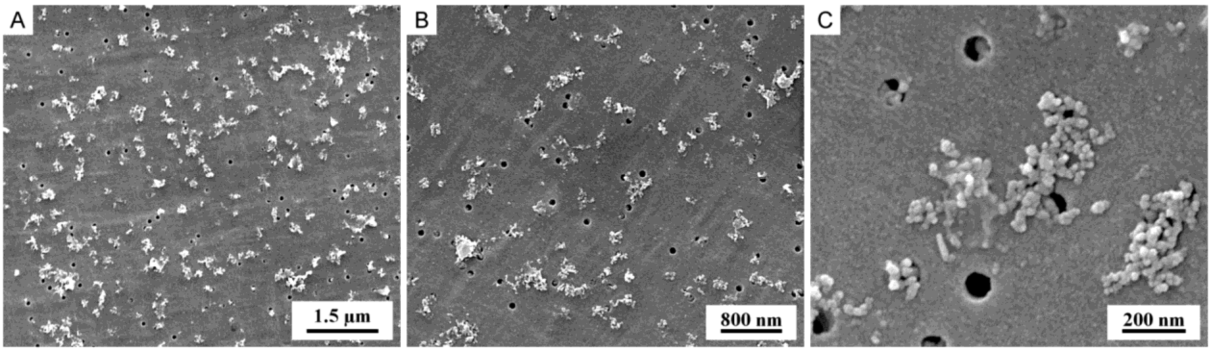


Figure 8. *FEGSEM micrographs showing polycarbonate membanes with isolated nano-sized UHMWPE wear particles at (a) medium, (b) high, and (c) very high magnification. These micrographs represent the first proof of in vivo UHMWPE wear particles with average size < 0.05 μm [J8].*

In [J7] we demonstrated that estimation of the total number of UHMWPE particles produced for a specific amount of volumetric wear is unreliable without knowledge of particle size distribution. We used software package named MDISTR, module VOL.PE to simulate various particle size distributions for given, fixed values of volumetric wear. The software package MDISTR was developed during the UHMWPE project by the author of this thesis. It was used above all for morphological analyses of UHMWPE wear debris with broad size distribution by means of "mathematical filtering" as mentioned above and published elsewhere [C6]. In this study [J7], we showed that total amount of PE wear particles decreases up to 4 orders of magnitude if the width of the distribution increases and total volumetric wear remains constant. This indicated that discrepancies among numerous orthopedic studies, taking into account only total volumetric wear and average particle size in order to determine "osteolytic threshold" [47, 48], might have been caused by different particle size distributions.

In [J8], we investigated the localization of the UHMWPE wear particles in periprothetic tissues from nine revisions of TJR in more detail. The tissues were joined and mechanically separated into granuloma tissue (containing hard granules visible to naked eye) and surrounding tissue (without visible granules). In the next step, the tissues were hydrolyzed by protease enzyme and granules were separated by filtration, which divided sample into four groups: (i) lysate and (ii) non-degraded large granules from granuloma tissue plus (iii) lysate and (iv) non-degraded small granules from the surrounding tissue. The UHMWPE particles were isolated using a combination of enzymatic hydrolysis (collagenase enzyme digesting granules) and acid hydrolysis (HNO_3 acid digesting everything). The complete isolation protocol description is to be found in the discussed paper [J8]. The purity of isolated particles was checked by SEM, EDX and IR as in the previous studies [J1–J6]. The results were surprising: (i) most of UHMWPE wear particles (69%) was found in granules, (ii) granules contained also bone fragments (isolated enzymatically, proved by EDX), (iii) certain differences were found also in the distributions of small (0.1–10 μm) and big (>10 μm) UHMWPE wear particles. We concluded that granuloma formation is not a dangerous process, but a natural defense mechanism for elimination of non-metabolizable particles (both UHMWPE and bone fragments). It is quite possible that individual differences among patients with similar total wear and different extent of damage of tissues around TJR might be caused, among other things, by different ability to encapsulate/eliminate the wear particles in granules.

At the very end of the UHMWPE project, we developed yet another method for quantification of in vivo UHMWPE wear particles [J10]. It is a "sister" method of IRc, but relies on UV/VIS spectroscopy, which more common technique in biochemical laboratories than IR. Development of colorimetric method was a logical continuation of our collaboration with the Department of Biochemistry, where Zolotarevova et al. [49] proved that hydrophobic human plasma proteins are bound to the surface of UHMWPE wear particles with strong hydrophobic interaction. Based on these results, we supposed that colored protein could bind to the wear particles in a similar way and be detected by UV/VIS spectroscopy. In colorimetric method, the UHMWPE particles are isolated as described in our previous studies [J3, J4, J9]. Then the suspension of isolated wear particles (50% iPrOH in water) is sonicated and centrifuged in special centrifugal filter devices (Ultrafree®-MC centrifugal filter units; Millipore). The isolated particles are caught on the filters during centrifugation, commercial dyes (FITC-BSA or ORO; Sigma Aldrich) are added, and the amount of colored particles is determined from the intensity of absorption at 488 nm and 492 nm for FTIC-BSA and ORO, respectively. Absolute mass of the particles can be determined from the previously measured calibration curves. The comparison of both colorimetric methods (1st with FITC-BSA, 2nd with ORO) with parallel IRc quantifications on the same samples (a suspension of isolated UHMWPE wear particles divided in three equal volumes) showed very good correlation between the three methods and suggested that the colorimetric methods might be even slightly more precise and accurate. Nevertheless, the colorimetric methods have not been used for a larger set of samples so far; as claimed above, the great majority of the results connected with UHMWPE wear particle monitoring was achieved with our well-established IRc method [J4].

3 MINIMIZATION OF UHMWPE WEAR

Since its introduction in 1960s, UHMWPE is regarded as the gold standard bearing surface for TJR. During 1980s, aseptic loosening and osteolysis in UHMWPE-based TJR emerged as major problems in arthroplasty (section 1). In 1990s it was accepted that UHMWPE wear particles play a role in initiating the osteolysis, although numerous problems connected with the wear particle quantification remained unresolved (section 2). In the first decade of the 21st century, some TJR manufacturers started to produce the 1st generation highly-crosslinked UHMWPEs with increased resistance to wear and oxidation. Recent intensive research resulted in the 2nd generation highly crosslinked UHMWPEs, which should have the same or higher wear and oxidation resistance as the 1st generation UHMWPE, without the small decrease in mechanical performance [24–27].

For the reasons given above, the **minimization of UHMWPE wear** is believed to prolong lifetime of total joint replacements. Other polymers, such as PTFE and polyethylene composites, did not prove successful. Chemical modifications of UHMWPE are impossible, as the implant manufacturers had to maintain the medical-grade purity of the material. The only exception is stabilization with a biocompatible stabilizer - vitamin E (α-tocopherol). Consequently, researchers in collaboration with implant manufacturers modify UHMWPE properties by a combination of irradiation, thermal treatment and modern sterilization techniques [4, 22–28].

This section deals with **material-scienece-related part** of the UHMWPE project (section 1.4). Firstly, partial results connected with the investigation of structure and properties of radiation modified UHMWPEs [J11–J14], are reported (subsection 3.1). Secondly, development our 1st generation UHMWPE is described [P1], its application in Czech TJRs is briefly mentioned [V1–V7, B1, 50, 51], and its comparison with recent 2nd generation polymer is given [J15, V6] (subsection 3.2) Finally, supplementary results [P2], alternatives to UHMWPE [C4, C5, B2] and possible developments in the field of total joint replacements are summarized (subsection 3.3).

3.1 MODIFICATIONS OF UHMWPE

There are two key steps in UHMWPE modification: irradiation and thermal treatment (section 1). Briefly, the irradiation is used for crosslinking (an increase in wear resistance) and the thermal treatment is used for removal of residual radicals (an increase in oxidation resistance). The impact of irradiation and thermal treatment on the UHMWPE structure and properties is strongly affected by conditions during the experiments, as demonstrated in the following two subsections.

Two kinds of irradiation are used for UHMWPE: accelerated electrons (section 3.1.1) and gamma radiation (section 3.1.2). Gamma radiation sources are commonly based on radioactive isotope ^{60}Co. Penetration of γ-photons into UHMWPE has almost no limitations, but the activity level of γ-sources limits the dose rate (usually < 10 kGy/h). Accelerated electrons (electron beam, e-beam) are produced in an electron gun (i.e. a cathode emitting electrons accelerated towards an anode). Penetration of e-beam into UHMWPE is limited by the energy of electrons (at 10MeV ≈ 4 cm), but the radiation dose rates are 2 orders of magnitude higher than in γ-sources [52].

Post-irradiation thermal treatment is either annealing or remelting. The *remelting* (RM; heating above UHMWPE melting point $T_m \approx 140$ °C) leads to additional decrease in crystallinity and mechanical performance, but removes all residual radicals. The *annealing* (AN; heating below T_m) maintains the crystallinity and results in smaller decrease in mechanical properties, but leaves certain amount of residual radicals, which can speed up the oxidative degradation [23, 23, 54].

3.1.1 Electron beam irradiation

Electron beam irradiation experiments were performed with an electron beam accelerator ELV-2 (Budker Institute of Nuclear Physics, Russia) installed at the Institute for Polymer Research (Dresden, Germany). We used high-energy electrons (1 MeV) and high dose rates (>2.5 kGy/min). The samples were irradiated in air, but high radiation dose rates minimized the oxidative degradation during the process. The irradiated material used in all experiments was UHMWPE Chirulen 1020 ($M_w = 3.5\times10^6$ g/mol; Ticona, Germany).

In our first set of experiments with e-beam irradiation [J11], we generated three series of UHMWPE samples in order to verify general trends and to test sensitivity of our structure characterization methods. Samples in the 1st series were irradiated only (conditions described in the previous paragraph), samples in the 2nd series were irradiated *and* remelted (hot press at 200 °C, 10 min, zero pressure), and the samples in the 3rd series were radiated at 50 kGy and remelted for 0, 2, 5, 10, 30, and 60 min at 150 °C. Immediately after the irradiation and between all experiments the samples were stored in the dark cold place with inert atmosphere (bags filled with N_2 in refrigerator at 5 °C) in order to minimize the oxidative degradation. Structural changes of the samples were characterized by a number of methods (light and electron microscopy: LM, SEM; small- and wide-angle X-ray scattering: SAXS, WAXS; infrared spectroscopy and electron spin resonance: IR, ESR; thermal techniques: DSC, TGA; solubility measurements); the tested methods were employed also in our later studies [J12–J15].

Fig. 9 shows changes of UHMWPE supermolecular structure as a function of the radiation dose and thermal treatment. Crystallinity (CR) and long period (LP) of non-modified polymer was within usual limits [7, 22, 53–55]. According to SWAXS (small- and wide-angle X-ray scattering), CR and LP of non-remelted samples monotonically increase with radiation dose, whereas CR and LP of remelted samples showed the opposite trend (Fig. 9a). The SWAXS results were confirmed also by DSC (not shown): shifts of the melting peak T_m corresponded to LP from SAXS and the areas under the melting peak corresponded to CR from WAXS. The observed shifts of LP were also confirmed qualitatively by the Fourier transform analysis of SEM micrographs showing UHMWPE etched surfaces (Fig. 9b,c; ref. [56]).

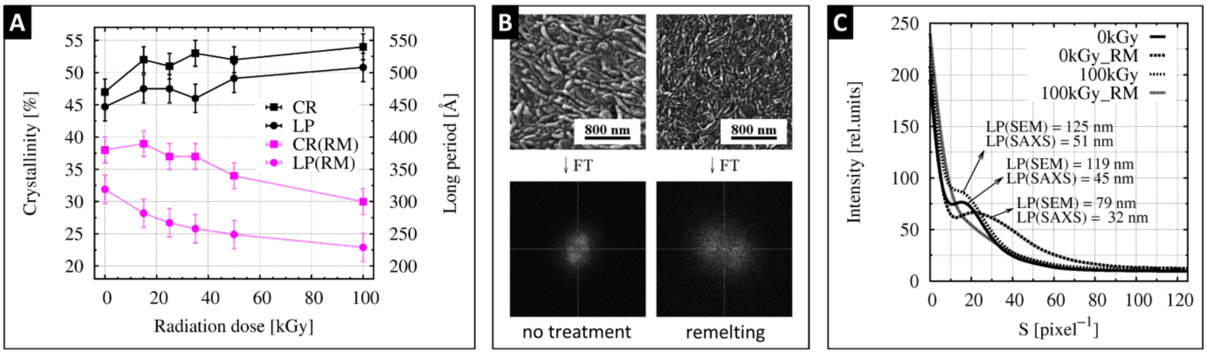


Figure 9. *Supermolecular structure of e-beam irradiated and remelted samples from ref. [J11]. (a) SWAXS results; CR = crystallinity, LP = long period, RM = remelting. (b) SEM micrographs (upper row) and their 2D-fourier transformations (lower row); left column = typical sample without thermal treatment, right column = typical remelted sample .(c)1D-Fourier transforms calculated from (b), using our own program MDFT [56].*

UHMWPE supermolecular structure (i.e. crystallinity and thickness of crystalline lamellae) were shown to be strongly influenced by irradiation and thermal treatment (Fig. 9). In addition, we managed to explain, summarize and generalize the observed changes of CR and LP by means of a model named MSSC (model of supermolecular structure changes; Fig. 10a, ref. [J11]). We also showed that the changes on supermolecular level are closely connected with those on molecular level that can be followed by IR spectroscopy (Fig. 10b).

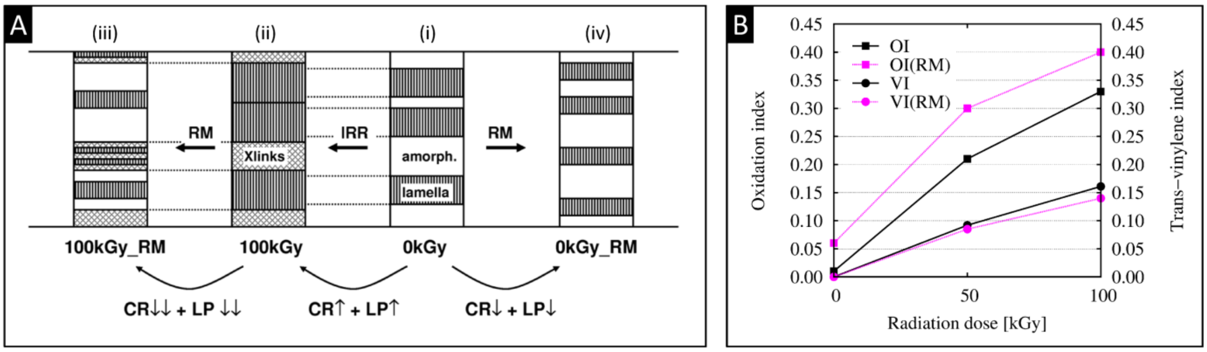


Figure 10. *Changes of UHMWPE structure after irradiation and thermal treatment. (A) MSSC = model of supermolecular structure changes: (i) virgin PE, (ii) irradiated PE, (iii) irradiated and remelted PE, and (iv) only remelted PE. (B) Infrared spectroscopy, which shows changes at molecular level: oxidation index (OI) and trans-vinylene index (VI) characterize level of oxidation and crosslinking, respectively.*

The MSSC model in Fig. 10a is based on the assumption that three different phases – amorphous, crystalline, and crosslinked – exist in UHMWPE. In our study [J11], crosslinking predominated over chain scissions as confirmed by swelling experiments. The small increase in CR after irradiation (Fig. 10a, transformation i→ii) is usually attributed to preferential chain scissions of highly constrained entanglements [57], tie molecules [58, 59], and the loops on the surface of crystallites [59, 60]. These chain scissions are followed by additional crystallization as discussed elsewhere [58–60, J11] and confirmed experimentally in Fig.9. The small increase in LP after irradiation (Fig. 10a, transformation i→ii) probably results from the combined effect of lamellar melting (due to elevated temperature induced by radiation [52]) and lamellar merging (scissions of loops and tie molecules [58–60]). The drop of both CR and LP after remelting (Fig. 10a, transformation i→iv) is caused by the change of the thermal history of the sample. Virgin bulk UHMWPE is consolidated from resin at elevated temperatures and pressures followed by annealing to remove residual stresses [61]. Consequently, the virgin annealed polymer has high CR and LP, which both decrease after our RM procedure (Fig. 9) that leads to higher amount of thinner lamellae. Further drop of CR and LP after irradiation followed by remelting (Fig. 10a, transformation i→iii) results from two facts: Firstly, our RM procedure leads to lower CR and LP and secondly, the crystallization in the irradiated samples is further constrained due to crosslinks in the polymer. As the crosslinks are formed preferentially in the amorphous region [57–60], the lamellae formed in the crosslinked phase are especially thin, while the lamellae formed in the remelted crystalline phase are thinner due to different thermal history as described above.

Figure 10b illustrates changes of UHMWPE structure at molecular level. Primary product of an irradiation are alkyl radicals ($-CH_2-CH^*-CH_2-$), which enter a complex system of reactions such as: (i) the reaction with oxygen followed by chain scissions (which results in deterioration of mechanical properties; section 1.2), (ii) transformation to more stable allyl radicals ($-CH_2-CH^*-CH=CH-$), (iii) double bond formation, and (iv) crosslinking [62–66]. Some more stable radicals, such as allyl, polyenyl and peroxy radicals (so called *residual radicals*), can survive in the polymer even for several years and their mixture is detectable by ESR in the form of residual/free radicals concentration (FRC; refs. [14, 65, J11]; Fig. 11). Final oxidation damage and absorbed radiation dose can be estimated from IR spectra in the form of oxidation index (OI, concentration of C=O groups, ref. [9, 13, 67, 68]) and trans-vinylene index (VI; concentration of C=C groups; ref. [13, 68]), respectively. Oxidation (estimated in the form of OI) is connected with chain scission and, subsequently, with the *increase* in CR and LP, while total dose/crosslinking (estimated in the form of VI, [J11]) is associated with formation steric constraints (crosslinks), which hinder crystallization and lead to *decrease* in CR and LP (compare Figs. 9 and 10).

The increase in OI was observed after remelting of both non-irradiated and irradiated samples (Fig. 10b, OI), which indicated that the selected thermal treatment (200 °C for 10 min) was too strong. This was confirmed by further TGA and ESR experiments: TGA analysis of virgin polymer documented that oxidation damage depended more on temperature than on time (Fig. 11a). ESR experiments showed that much lower temperature (150 °C) was quite sufficient to eliminate all residual radicals (Fig. 11b). These two results were of particular importance for our patented UHMWPE modification procedure [P1].

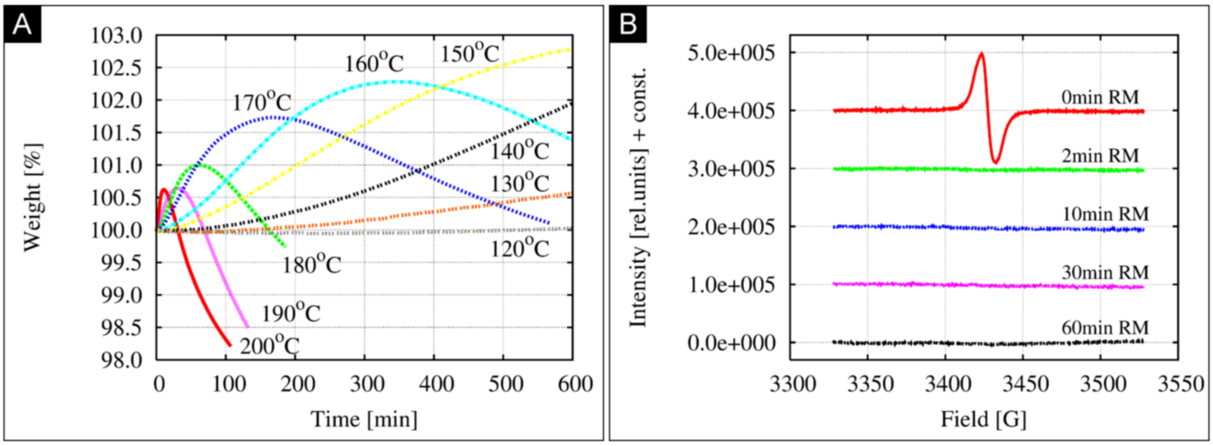


Figure 11. *Thermal treatment of UHMWPE: (a) Thermogravimetric analysis of virgin UHMWPE at various temperatures. (b) Electron spin resonance spectra of 50kGy-irradiated samples with various remelting times.*

In our second study dealing with e-beam irradiated UHMWPE [J13], we developed a new microscopic staining technique for visualization of UHMWPE crystalline lamellae in TEM. The technique consisted in one-step staining with oleum (H_2SO_4 solution of SO_3) for four days, followed by cryo-ultramicrotomy (sample temperature -140 °C, knife temperature -60 °C) and TEM (bright field imaging at 100 kV). This yielded high-contrast micrographs (Fig. 12, left) and was significantly simpler than previously described techniques (mostly two-step procedures including staining with chlorosulfonic acid and post-staining with uranyl acetate or osmium tetroxide [69–74]). The lamellar thicknesses measured from the final TEM micrographs correlated with the lamellar thicknesses determined from a combination of small- and wide-angle X-ray scattering (SWAXS). The correlation was linear within the estimated standard deviations (Fig. 12, right). The higher estimated standard deviations of lamellar thickness from TEM (Fig. 12, d(TEM)) resulted from variations of lamellar thicknesses in different locations within each sample. The intercept of the linear regression curve was >0, which was attributed to the fact that the thinnest lamellae were invisible in the ultrathin sections in TEM (the thickness of ultrathin sections ~50 nm; resolution in TEM is approx. 1/10 of the sample thickness).

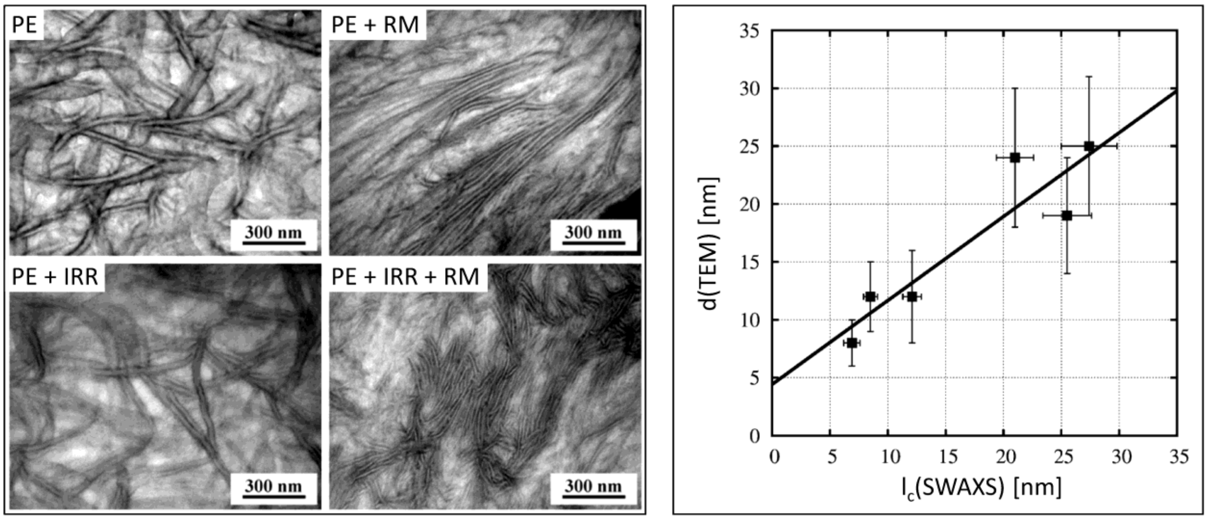


Figure 12. *New method for staining and visualizing UHMWPE crystalline lamellae in TEM. Left: TEM micrographs of virgin polymer (PE), remelted polymer (PE+RM), irradiated polymer (PE + IRR), and irradiated* plus *remelted polymer (PE + IRR + RM). Right: correlation between the lamellar thickness from X-ray diffraction, l_c(SWAXS) and the lamellar thickness determined from image analysis of TEM micrographs, d(TEM).*

In conclusion, the studies of UHMWPE irradiated with accelerated electrons [J11, J13] brought the following results: (i) If the *radiation dose* is high (> 25 kGy/min), crosslinking predominates over chain scission even in oxygen atmosphere, as evidenced directly by solubility measurements and indirectly by changes of molecular and supermolecular structure. (ii) The importance of inert *atmosphere* and suitable *temperature* was confirmed. Temperatures higher than 160 °C resulted in severe oxidation, whereas longer remelting times were acceptable if the remelting temperature was just slightly above the UHMWPE melting point. (iii) Moreover, we introduced a universal model of supermolecular structure changes [J11], developed a program for automated, semi-quantitative evaluation of lamellar thickness from SEM micrographs [J11], and introduced a novel and efficient method of UHMWPE morphology visualization using TEM [J13].

3.1.2 Gamma irradiation

Gamma irradiation experiments were carried out in a container with ^{60}Co γ-emitter in Nuclear Research Institute (Řež, Czech Republic). All samples were irradiated at room temperature, either in air or in the nitrogen atmosphere. Two different dose rates were tested: 2.5 kGy/h and 0.25 kGy/h, the first of which was the highest dose possible with given device. The same UHMWPE polymer (Chirulen 1020) was used for all experiments.

In [J12] we investigated supermolecular structure and microhardness of UHMWPE with various modifications. The polymer was gamma-irradiated in nitrogen (0, 25, 50 and 100 kGy) with two different dose rates (0.25 kGy and 2.5 kGy). The thermal treatment included no modification (NN-samples, i.e. samples denoted as NN), the annealing at low-oxygen atmosphere

(AN-samples; 120 °C for 10 min), and the remelting at low-oxygen atmosphere (RM-samples; 150 °C for 10 min). The samples were characterized by WAXS, SAXS, DSC, and Vickers microhardness (MH) testing.

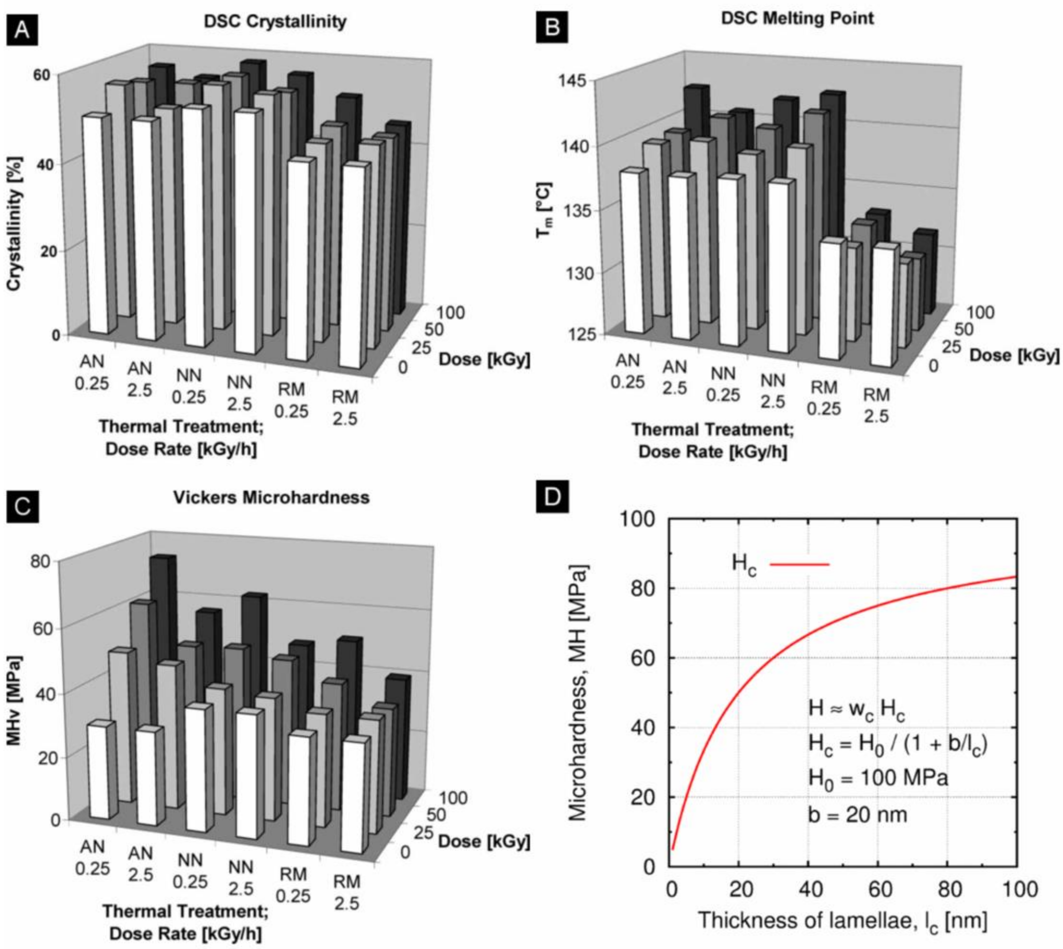


Figure 13. *Characterization of the UHMWPE samples, which were irradiated (three doses: 25, 50, 100 kGy; two dose rates: 0.25, 2.5 kGy) and then left without thermal treatment (NN), annealed (AN) or remelted (RM): (a) crystallinity from DSC, (b) melting point from DSC, (c) Vickers microhardness and (d) theoretical prediction of microhardness of a semicrystalline polymer above* T_g; *H = total microhardness,* H_c *= microhardness of crystalline phase,* w_c *= weight fraction of crystalline phase ≈ crystallinity,* l_c *= thickness of lamellae,* H_0 *= microhardness of infinitely thick crystal, and b = constant related to surface energy; the constants* H_0,*b were adjusted to UHMWPE according to ref. [75]).*

The results of DSC and MH measurements are summarized in Fig. 13. Crystallinity (CR) from WAXS and long period (LP) from SAXS are not shown; both CR and LP just decreased after RM and otherwise did not show any specific trend. Also the CR from DSC (Fig. 13a) showed just decrease after RM; the reasons were discussed in the previous section and in refs. [J11, J13]. Melting points from DSC (T_m; Fig. 13b) decreased after RM (like long periods), but grew with radiation dose in case of NN and AN samples. This could be attributed to preferential scissions of loops at lamellar surface followed by additional crystallization (refs. [57–66, 76]; Figs. 9,10), because neither NN nor AN melt the thickest lamellae. Microhardness (Fig. 13c) was quite sensitive to all parameters (dose, dose rate, thermal treatment). This can be explained by three facts: (i) The microhardness of semicrystalline polymers above glass transition (T(measurement) > T_g) is proportional to crystallinity (MH ≈ $w_c H_c$ ≈ CR; w_c and H_c are the weight fraction and the microhardness of crystalline phase, respectively); this results from the microharness additivity law [75]. (ii) H_c grows with lamellar thickness l_c, as shown in Fig. 13d (justification in [75]). (iii) Crosslinking toughens the amorphous phase, which seems to increase the MH. Therefore: (a) MH of NN-samples and AN-samples is higher than MH of RM-samples due to higher crystallinity, (b)

MH of all samples increases with radiation dose due to crosslinks in amorphous phase, (c) MH of NN/AN-samples increases with dose even more due to additional crystallization and thicker lamellae, which are in agreement with theoretical prediction illustrated in Fig. 13d, and (d) lower radiation dose rates mean longer exposure to traces of oxygen, higher oxidation, more chain scissions, more intensive additional crystallization and, finally, higher MH. Microhardness proved to be a very sensitive tool for studying of irradiation-induced changes of UHMWPE.

In [J14], we studied the impact of dose rate on the structure and properties of modified UHMWPE in more detail. UHMWPE samples were gamma-irradiated with high radiation dose rate (2.5 kGy/h) in nitrogen (Series I), low radiation dose rate (0.25 kGy/h) in nitrogen (Series II), and low radiation dose rate in air (Series III). Each sample was then cut into two halves, the first was left as it was (NN-samples) and the second was remelted (RM-samples). The changes of UHMWPE after modification were checked by SAXS, WAXS, IR, ESR, swelling measurements, rheological experiments and wear testing.

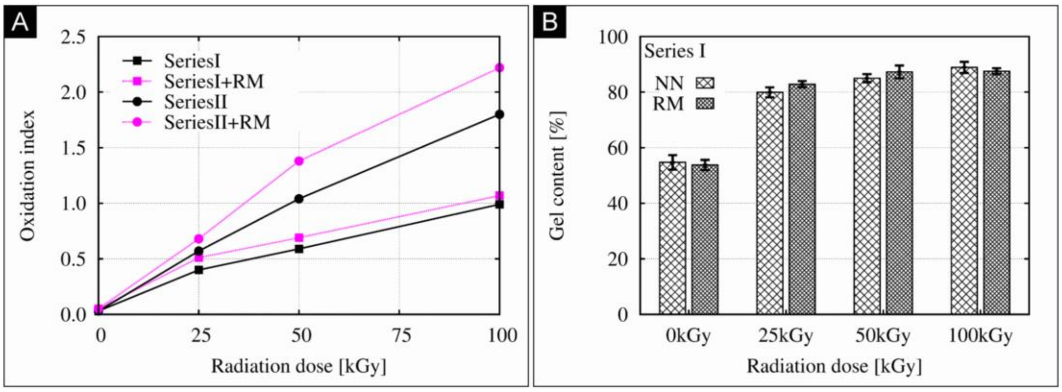


Figure 14. *Impact of modification conditions on oxidative degradation and crosslinking of UHMWPE: (a) IR spectroscopy – oxidation index as a function of radiation dose and dose rate; samples from Series I and II, (b) swelling experiments – gel content as a function of radiation dose and thermal treatment; samples from Series I.*

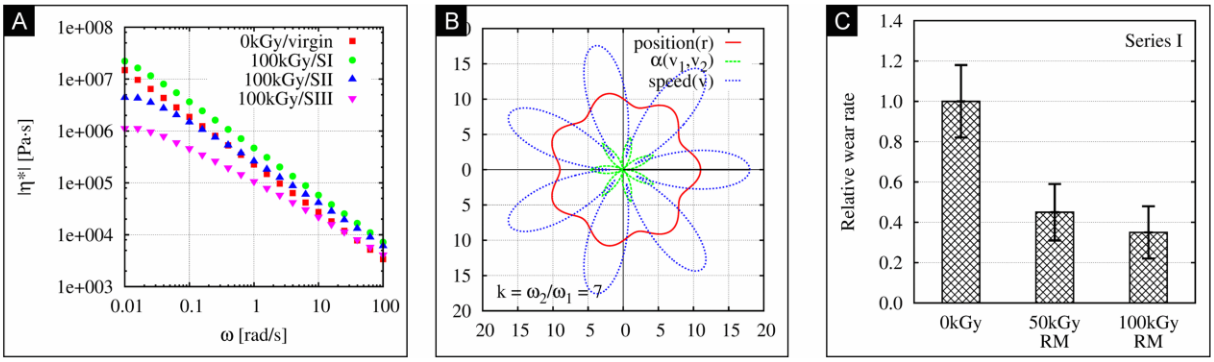


Figure 15. *Impact of modification conditions on crosslinking density and wear resistance. (a) Rheological measurements - complex viscosities |η*| from oscillatory shear measurements; the values of |η*| evidence that the most efficient crosslinking was achieved in Series I. (b) Multidirectional pin-on-disk wear testing simulation: a calculation describing final movement of a small UHMWPE pin on a big metal disk. This illustrative calculation documents that the pin position (red), relative direction (green) and velocity (blue) change during its movement (more details in [77]). (c) Multidirectional pin-on-disk wear testing experiment results of UHMWPE samples from Series I: relative wear rates (WR); WR is the weight loss of measured sample (Δm_M) divided by the weight loss of standard (non-modified UHMWPE; Δm_0); error bars give 95% confidence intervals (more details in [J14]).*

The changes of supermolecular structure will not be re-discussed here, because they followed similar trends as described in our previous studies [J11–J13]. Instead, we focus our attention on IR, swelling, wear testing and rheology experiments. Infrared spectroscopy confirmed that higher dose rates result in lower oxidation damage and that remelting should be carried out in inert atmosphere (Fig. 14a). Swelling experiments in hot xylene (138 °C for 8 h) proved that for high dose rates (Series I) the crosslinking predominates over chain scissions (Fig. 14b). Rheological experiments evidenced that the ratio of crosslinking to chain scissions decreased in the row: Series I > Series II > Series III (Fig. 15a; increase and decrease in complex viscosity indicated crosslinking and chain scissions, respectively). In series III (irradiation with low dose rate in air) the chain scissions even started to predominate at dose rates >25 kGy [J14]. In this study we also reported the first successful measurements of the UHMWPE wear resistance with a multidirectional pin-on-disk tester, which was built in collaboration with Beznoska company within the UHMWPE project (Figs. 15b,c). The wear tests confirmed that the crosslinking lead to increase of wear resistance of radiation-modified polymers of Series I (Fig. 15c).

We conclude that studies of gamma-irradiated UHMWPE [J12, J14], pointed out the following aspects of the polymer modification: (i) The level of crosslinking is determined not only by total radiation dose, but also by the dose rate in combination with ambient conditions. Low dose rates result in oxidative degradation, chain scission and weak crosslinking. (ii) The importance of inert atmosphere during gamma irradiation was verified. Lower dose rates of gamma radiation in comparison with accelerated electrons makes the irradiation process more sensitive to oxidation, chain scissions and deterioration of mechanical properties. (iii) In addition, we verified that microhardness [J12] and rheology [J14] are sensitive methods of UHMWPE characterization; both methods detect subtle differences in radiation dose, dose rate and thermal treatment. Last but not the least, during this stage we proposed, build and tested the first multidirectional pin-on-disk wear testing device optimized for UHMWPE in the Czech Republic.

3.2 UHMWPE WITH OPTIMIZED PROPERTIES

The UHMWPE structure and properties can be modified using laboratory-scale or industrial-scale procedures. The laboratory-scale modifications of UHMWPE were described in the previous section (section 3.1, refs. [J11–J14]). The industrial-scale modifications of UHMWPE were carried by standard, certified, commercial procedures. The results dealing with commercially modified samples were summarized in (mostly confidential) research reports for Beznoska company [V1–V9], which are not part of this habilitation thesis. Nevertheless, combination of all results [J11–J14; V1–V9] allowed us to propose an industrial-scale modification procedure, which yielded UHMWPE with increased wear resistance and oxidation stability. The procedure was patented [P1] and employed for the production of TJR [50, 51]. In this section, we briefly describe the principle of our patent (section 3.2.1; ref. [P1]) and demonstrate that our 1st generation UHMWPE (PE-IMC) is comparable with other commercial polymers (section 3.2.2.; refs. [J15, V4, V7]).

3.2.1 PE-IMC: 1st generation highly-crosslinked UHMWPE

The UHMWPE handbook [2] reports >10 types of highly crosslinked UHMWPEs. Most of the polymers belongs to 1st generation highly-crosslinked UHMWPEs; three of them are claimed to be the 2nd generation highly-crosslinked UHMWPEs with further improved oxidation resistance and mechanical performance. Each of the polymers is produced by a combination of irradiation, thermal treatment and sterilization under specific conditions (dose, ambient atmosphere, temperature etc.). In one case the modification includes stabilization with biocompatible vitamin E. The exact procedures are usually proprietary and protected by patents.

In fact, almost every manufacturer of total joint replacements uses its own, proprietary highly-crosslinked UHMWPE. In our UHMWPE project (section 1.4) we developed yet another modification procedure, which yields 1st generation highly-crosslinked UHMWPE [P1]. The characteristic feature, which distinguished our procedure from the previous patents, was the *radiation dose rate*. The patent [P1] protects the general idea of optimal radiation dose rate, while the associated research reports [V2–V6] deal with optimization of modification conditions so that the best combination of wear resistance, oxidation stability, and mechanical properties was achieved. This is briefly illustrated in Fig. 16, which shows a part of the results of series M1 (irradiated and remelted UHMWPEs, prepared by industrial modifications; M11 = virgin polymer; M12 = remelted polymer; M13–M17 = irradiated and remelted polymers). The irradiation followed by thermal treatment resulted in improved wear resistance (Fig. 16a; in most samples improvement >50%), but it also decreased mechanical performance (Fig. 16b; yield stress σ_Y, stress at break σ_B, and elongation at break ϵ_B are lower by approx. 10, 30, and 50 %, respectively). Briefly, the wear resistance was optimized by suitable conditions during irradiation (including radiation dose rate according to [P1]), the oxidation stability was achieved by suitable thermal treatment in combination with ethylenoxide sterilization (which resulted in zero concentration of residual radicals; [V3]), and the mechanical properties were optimized by combination of all parameters so that their unavoidable radiation-induced decrease was minimized [V2, V7].

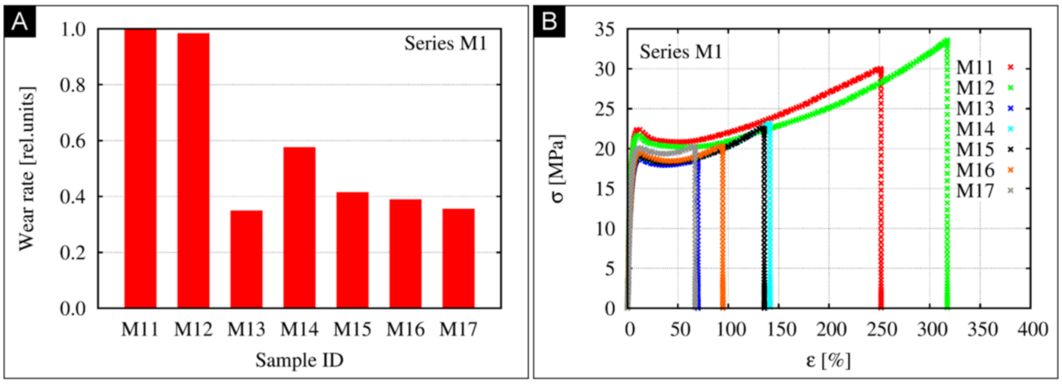


Figure 16. *Characterization of UHMWPE, series M1 (industrial-scale modification); sample M11 = virgin polymer, sample M12= remelted polymer, samples M13-M17 = polymers that were irradiated and remelted at various conditions. (a) Wear testing carried out as illustrated in Fig. 15, (b) Tensile testing according to ISO 5834-2.*

We concluded that our patented procedure [P1, V2] lead to UHMWPE with an increased wear resistance, improved oxidation stability, and acceptable mechanical properties. The modification addressed two main reasons of total *hip* replacement failures. As a result, our highly-crosslinked UHMWPE should contribute to longer lifetime of THRs [J9, B1, B2]. As for the total *knee* replacements, in which the wear resistance does not seem to be a major reason of failures (section 2.2, [41, J9, B2]), we decided not to use crosslinked polymer [V4–V6]. Nonetheless, UHMWPE for TKR was also improved because we exchanged old gamma sterilization for newer ethylenoxide sterilization, in order to produce TKRs with higher oxidation stability [V3, V6, V7].

3.2.2 Comparison of PE-IMC with other highly-crossliked UHMWPEs

In [J15], we compared our 1st generation highly-crosslinked ultrahigh molecular weight polyethylene (PE-IMC; produced by Beznoska, Czech Republic) with another commercial 2nd generation highly-crosslinked polymer (PE-X3; produced by Stryker, USA). PE-IMC was briefly described in section 3.2.1; essentially it is a standard highly-crosslinked and remelted UHMWPE, sterilized by ethylenoxide (EtO). PE-X3 is sequentially-crosslinked and annealed UHMWPE, sterilized by EtO [24, 27]. Sequential crosslinking (SXL; also called sequential irradiation) consists of three cycles; within each cycle the material is irradiated to 1/3 of the desired total dose and then annealed [27, 78]. According to the authors of SXL process [27, 78–80] the polymer exhibits improved wear resistance, oxidation stability and mechanical properties and belongs among 2nd generation highly-crosslinked UHMWPEs.

In the first set of experiments within [J15], we prepared **model samples**: virgin UHMWPE (sample M0); irradiated and *remelted* UHMWPE (M1); irradiated and *annealed* UHMWPEs, with irradiation in one (M2), two (M3), and three steps (M4); and finally the sample like M4, in which the last thermal treatment step was exchanged for *remelting* (M5). Hence, sample M1 was analogous to PE-IMC (single-step irradiation + remelting) and sample M4 was analogous to PE-

X3 (three-step irradiation + annealing). Total radiation dose in all samples was the same (75 kGy). The samples were characterized by a number of techniques (SEM, TEM, IR, ESR, SAXS, WAXS, DSC, small-punch test (SPT) and microhardness), which were tested previously [J11–J14]; selected results are shown in Fig 17.

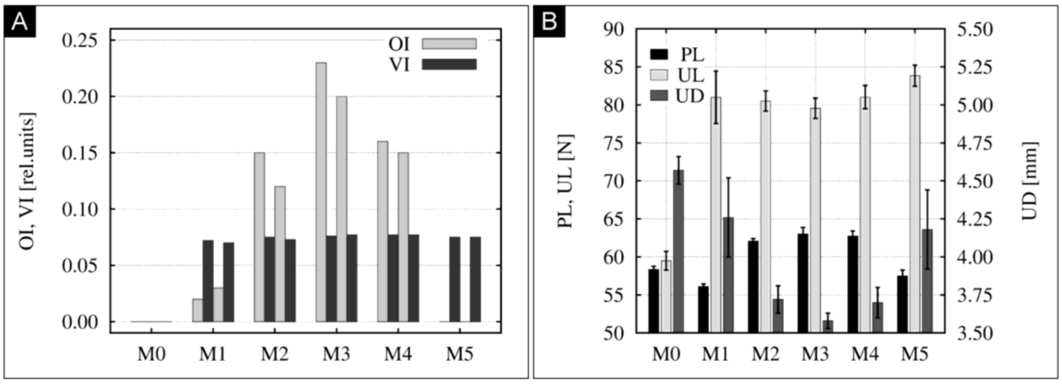
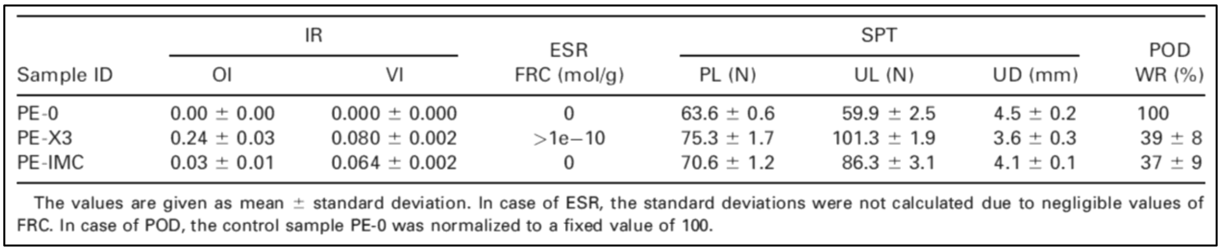


Figure 17. *Comparison of standard and sequential irradiation: M0 = virgin polymer, M1 = irradiated and remelted polymer, M2-M4 = sequentially irradiated polymer, M5 = sequentially irradiated polymer, which was remelted in the last step. (a) IR results (OI = oxidation index, VI = trans-vinylene index). (b) SPT results (PL = peak load, UL = ultimate load, UD = ultimate displacement; SPT measurement according to ASTM F2183-02).*

The complete discussion of the results is quite complex [J15], but the basic conclusions from Fig. 17 are evident: according to both structure and properties, the samples split into three groups, (i) the first group is formed by non-modified polymer (M0), (ii) the second group is formed by AN-samples, i.e. by the samples, whose last thermal treatment step is annealing (M2, M3, M4), (iii) the last group is formed by RM-samples, i.e. by the samples, whose last thermal treatment step is remelting (M1, M5), and (iv) *the final structure and properties is determined by the last thermal treatment step and not by the number of irradiation steps*.



Sample ID	IR		ESR	SPT			POD
	OI	VI	FRC (mol/g)	PL (N)	UL (N)	UD (mm)	WR (%)
PE-0	0.00 ± 0.00	0.000 ± 0.000	0	63.6 ± 0.6	59.9 ± 2.5	4.5 ± 0.2	100
PE-X3	0.24 ± 0.03	0.080 ± 0.002	>1e−10	75.3 ± 1.7	101.3 ± 1.9	3.6 ± 0.3	39 ± 8
PE-IMC	0.03 ± 0.01	0.064 ± 0.002	0	70.6 ± 1.2	86.3 ± 3.1	4.1 ± 0.1	37 ± 9

The values are given as mean ± standard deviation. In case of ESR, the standard deviations were not calculated due to negligible values of FRC. In case of POD, the control sample PE-0 was normalized to a fixed value of 100.

Figure 18. *Copy of the final table from [J15], which compares properties of commercial samples: PE-0 = virgin medical-grade UHMWPE, PE-X3 = sequentially irradiated UHMWPE (Stryker, USA) and PE-IMC = irradiated and remelted UHMWPE (Beznoska, Czech Republic).*

In the second set of experiments within [J15], we compared **commercial samples**: non-modified UHMWPE (PE-0), the sequentially irradiated UHMWPE (PE-X3) and our 1st generation UHMWPE (PE-IMC). The most important results are summarized in Fig.18. Neat polymer (PE-0) showed no oxidation damage (OI), no indication of irradiation (VI) and no residual radicals. PE-X3 sample exhibited certain oxidation damage (OI), clear indication of irradiation (VI) and detectable amount of residual radicals. PE-IMC sample had very low oxidation damage (OI), clear indication of irradiation (VI), and no detectable amount of residual radicals. Mechanical properties investigated by SPT corresponded to those observed in model samples (Fig. 17): peak load (PL) of all materials was similar, ultimate load (UL) of the neat polymer (PE-0) was lower in comparison with both irradiated samples (PE-X3, PE-IMC) due to strain hardening, and ultimate displacement (UD) of the neat polymer was the highest due to the highest drawability and ductility of non-crosslinked material. Multidirectional pin-on-disk experiments (POD) suggested that the wear rate

(WR) of both highly-crosslinked samples (PE-X3, PE-IMC) decreased to approximately same level in comparison with neat polymer (PE-0). These results were in agreement with the recent papers [28, 81], claiming that thermal treatment temperature affects mechanical properties but not wear resistance. Moreover, comparison of our 1st generation UHMWPE (PE-IMC) with other commercially available highly-crosslinked UHMWPEs confirmed that the properties of our material are quite comparable, if not better, than those of the competing products [V7].

We concluded that the sequential irradiation did not bring any apparent benefit in comparison with the single dose irradiation on a series of the model samples irradiated with the same total dose at the same conditions (Figs. 17,18). In the commercially available samples, the sequentially crosslinked polymer showed properties of a standard, single-dose irradiated and annealed polymer, which means improved mechanical properties, but higher oxidative degradation. Therefore, our 1st generation highly-crosslinked polymer (PE-IMC) is fully comparable to the tested 2nd generation highly-crosslinked polymer produced in USA [J15] and also with other commercial products [V7].

4 SUMMARY

The habilitation thesis deals with ultrahigh molecular weight polyethylene (UHMWPE) for total joint replacements (TJR). UHMWPE is the most popular bearing component of the contemporary TJRs. However, the release of microscopic particles from the UHMWPE surface during mutual motion of the TJR components (wear) limits the lifetime. The particles are released to the surroundings of TJR, where they cause a tissue damage, osteolysis, and loosening of the implant. The orthopedics part of the research was focused on the *monitoring of UHMWPE wear*, while the materials science part concentrated on the *minimization of UHMWPE wear*.

The main objective in the field of *UHMWPE wear monitoring* was to find the correlation between the concentration of UHMWPE wear particles in specific zones around TJR and the extent of tissue damage in these zones. In order to achieve this goal, we had to develop new, more efficient techniques of wear particle sampling, isolation, and quantification [J1, J3, J4, J10]. During the development of the methods, we found that metal nanoparticles with tunable size [J2] can be employed in quite different field of multiple immunolabeling in biology [P3, 36, 37]. We also proved that centrifugation and ultracentrifugation techniques used during the wear particle isolation do not influence particle morphology [J5]. Our laboratory was the first (together with an independent group of British investigators), in which the *in vivo* nanometer-sized UHMWPE wear particles were observed [J6]. We demonstrated that not only average particle size, but also particle size distribution width is necessary to precisely evaluate concentration of UHMWPE wear particles causing osteolysis [J7]. Moreover, we suggested that encapsulation of polyethylene wear particles inside collagen grana is not a dangerous process, but a natural defense mechanism of the human body [J8]. Finally, we proved that the distribution of wear particles in tissues around TJR is very non-homogeneous [J1, J3, J4] and that the correlation between concentration of particles and tissue damage around THR not only exists [J3, J4] but is statistically significant [J9].

The main objective in the field of *UHMWPE wear minimization* was to develop an original modification procedure, which would yield a highly-crosslinked UHMWPE. The highly-crosslinked polymer should have had the following features: (i) increased wear and oxidation resistance, (ii) all other properties compatible with analogous foreign highly-crosslinked polymers, and (iii) should have been usable for production of total joint replacements in the Czech Republic. In the first step, we investigated impact of irradiation and thermal treatment under a broad range of conditions on the UHMWPE structure and properties [J11–J14]. As a by-product of our research, we prepared and investigated biocompatible filler for polymers – titanate nanotubes [82], which are suitable for another types of implant materials [P2]. As for UHMWPE modifications, we used both e-beam [J11, J13] and gamma irradiation [J12, J14] and found that radiation dose rate strongly influences the final properties of the irradiated polymer. Consequently, we protected the general idea of the optimal radiation dose rate during UHMWPE modifications [P1], while further research in collaboration with Beznoska company (Kladno, Czech Republic) resulted in so-called 1st generation highly-crosslinked UHMWPE (denoted as PE-IMC) with the increased wear resistance and oxidation stability. The PE-IMC polymer has been introduced in the production of the Czech total joint replacements since the end of the year 2007 [50, 51]. In our recent work [J15] we investigated a sequentially-crosslinked 2nd generation UHMWPE and found that the sequential irradiation brought no apparent benefits in comparison with single-dose irradiation used in our material [P1]. Also another comparison of PE-IMC with highly-crosslinked polymers from USA confirmed that our material exhibited similar or even better properties [V7]. We concluded that the wear resistance, the oxidation stability and the mechanical performance of our polymer were fully compatible with the foreign products and that the TJRs with the polymer modified according to our procedure [P1] should exhibit longer lifetime.

5 PAPERS INCLUDED IN THE HABILITATION THESIS

5.1 IMPACTED JOURNALS

[J1] **Slouf M**, Sloufova I, Entlicher G, Horak Z, Krejcik M, Stepanek P, Radonsky T, Pokorny D, Sosna A: New fast method for determination of numbers of UHMWPE wear particles. *J. Mater. Sci.-Mater. Med.* 15 (2004) 1267-1278. [IF = 2.0]

[J2] **Slouf M**, Kuzel R, Matej Z: Preparation and characterization of isometric gold nanoparticles with precalculated size. *Z. Kristallogr. Suppl.* 23 (2006) 319-324. [IF = 1.5]

[J3] **Slouf M**, Eklova S, Kumstatova J, Berger S, Synkova H, Sosna A, Pokorny D, Spundova M, Entlicher G: Isolation, Characterization and Quantification of Polyethylene Wear Debris from Periprosthetic Tissues around Total Joint Replacements. *Wear* 262 (2007) 1171-1181. [IF = 1.9]

[J4] **Slouf M**, Pokorny D, Entlicher G, Dybal J, Synkova H, Lapcikova M, Fejfarkova Z, Spundova M, Vesely F and 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* (2008) 265, 674-684. [IF = 1.9]

[J5] Zolotarevova E, Fejfarkova Z, Entlicher G, Lapcikova M, **Slouf M**, Pokorny D, Sosna A: Can centrifugation affect the morphology of polyethylene wear debris? *Wear* 265 (2008), 1914-1917. [IF = 1.9]

[J6] Lapcikova M, **Slouf M**, Dybal J, Zolotarevova E, Entlicher G, Pokorny D, Gallo J, Sosna A: Nanometer size wear debris generated from ultrahigh molecular weight polyethylene in vivo. *Wear* 266 (2009) 349-355. [IF = 1.9]

[J7] Gallo J, **Slouf M**, Goodman SB: The Relationship of Polyethylene Wear to Particle Size, Distribution, and Number: A Possible Factor Explaining the Risk of Osteolysis after Hip Arthroplasty. *J. Biomed. Mater. Res. Part B* 94B (2010) 171–177. [IF = 2.5]

[J8] 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 replacements. *Acta Biomater.* 6 (2010) 3595–3600. [IF = 4.2]

[J9] Pokorny D, **Slouf M**, Vesely F, Fulin P, Jahoda D, Sosna A, Belacek J, Landor I, Zolotarevova E, Popelka S: Distribuce otěrových částic UHMWPE v periprotetických tkáních u TEP kyčelního kloubu. *Acta Chir. Orthop. Traumatol. Cechoslov.* 77 (2010) 87–92. [IF = 1.6]

[J10] Vesely F, Zolotarevova E, Spundova M, Kaftan F, **Slouf M**, Entlicher G: Simple colorimetric methods for determination of sub-milligram amounts of ultrahigh molecular weight polyethylene wear particles. *Acta Biomater.* 8 (2012) 1935–1938. [IF = 4.9]

[J11] **Slouf M**, Synkova H, Baldrian J, Marek A, Kovarova J, Schmidt P, Dorschner H, Stephan M, Gohs U: Structural Changes of UHMWPE after e-Beam Irradiation and Thermal Treatment *J. Biomed. Mater. Res. Part B*, 2008, 85B, 240-251. [IF = 2.0]

[J12] Lednicky F, **Slouf M**, Kratochvil J, Baldrian J, Novotna D: Crystalline character and microhardness of gamma-irradiated and thermally treated UHMWPE. *J. Macromol. Sci. Part B-Phys.*, 2007, 46, 521-531. [IF = 0.8]

[J13] Stara H, **Slouf M**, Lednicky F, Pavlova E, Baldrian J, Stary Z: New and Simple Staining Method for Visualizing UHMWPE Lamellar Structure in TEM. *J. Macromol. Sci. Part B-Phys.*, 47 (2008) 1148–1160. [IF = 0.8]

[J14] **Slouf M**, Mikesova J, Fencl J, Synkova H, Baldrian J, Horak Z (2009). Impact of dose-rate on rheology, structure and wear of irradiated UHMWPE. *J. Macromol. Sci. Part B-Phys.* 48 (2009) 587–603. [IF = 0.8]

[J15] **Slouf M**, Kotek J, Baldrian J, Kovarova J, Fencl J, Bouda T, Janigova I: Comparison of one-step and sequentially irradiated ultra-high molecular weight polyethylene for total joint replacements. *J. Biomed. Mater. Res. Part B*, (2012) in press. [IF = 2.1]

5.2 PATENTS

[P1] Horak Z, **Slouf M**, Krulis Z & Fencl J (2005): Způsob modifikace ultravysokomolekulárního polyethylenu pro výrobu kloubních náhrad se zvýšenou životností. Czech patent CZ 297700 (from 2007).

5.3 OTHER PUBLICATION RELATED TO HABILITATION THESIS

List of other author's patents and publications, which are related to this habilitation thesis, but are not included in it (long book chapters; confidential research reports; patents, which were by-products of our research on UHMWPE; non-impacted publications). More detailed explanation concerning these publications is in the full version of the habilitation thesis.

5.3.1 Book chapters

[B1] **Slouf M**: Náhradní díly pro člověka: umělé klouby. In: *Makromolekuly pro bioaplikace a lékařství. Soubor deseti publikací vědců ÚMCH.* Vydal Ústav makromolekulární chemie Akademie věd ČR, v.v.i., Praha 2011, ISBN: 978-80-85009-65-1.

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5.3.2 Publications in local orthopedic journal

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7 ABSTRACT

This habilitation thesis summarizes major results of the applicant, which are associated with ultrahigh molecular weight polyethylene (UHMWPE) for total joint replacements (TJR), and which were published during 2004–2012 (on the whole 15 impacted publications and 1 Czech patent). The first chapter explains why the UHMWPE is regarded as *gold standard* in the field of joint replacements. It is also shown why the UHMWPE remains a subject of intensive research and how important is to monitor and minimize its wear.

The second chapter deals with *monitoring of in vivo UHMWPE wear particles*. It describes the novel methods, which had to be developed in order to quantify wear particles efficiently. The new methods were employed in the analysis of failed TJRs and confirmed that there is a correlation between amount of wear particles in specific zones around TJR and extent of tissue damage in these zones. This result confirmed that the UHMWPE wear particles are the major material-related cause of TJR failures.

The third chapter focuses on *minimization of UHMWPE wear*. It shows how the combination of irradiation and thermal treatment under precisely defined conditions influences molecular structure, supermolecular structure and properties of the polymer in such a way that it exhibits increased wear and oxidative resistance. It also describes the development of an original modification procedure, which results in *highly-crosslinked* UHMWPE for total joint replacements with increased lifetime. The modified polymer was introduced in the production of TJRs in the Czech Republic. It has been demonstrated our material is fully comparable with foreign competing types of UHMWPE.

Abstrakt

Tato práce shrnuje podstatné výsledky vědecké činnosti předkladatele, které se týkají ultravysokomolekulárního polyetylenu (UHMWPE) pro kloubní náhrady (TJR) a byly publikovány v letech 2004–2012 (celkem 15 impaktovaných publikací a 1 udělený český patent). Úvodní kapitola vysvětluje, proč je UHMWPE na poli kloubních náhrad ve světě považován za tzv. zlatý standard. Současně ukazuje, proč je UHMWPE pro TJR stále předmětem intenzivního výzkumu a jak je důležité sledovat a minimalizovat jeho otěr.

Druhá kapitola se zabývá *monitorováním in vivo otěrových částic UHMWPE*. Popisuje nové metody, které bylo nutno vyvinout pro efektivní kvantifikaci otěrových částic. Pomocí zmíněných metod bylo prokázáno, že existuje statisticky významná korelace mezi množstvím otěrových částic v jednotlivých zónách v okolí TJR a stupněm poškození tkání v těchto zónách. Dosažený výsledek potvrdil, že otěrové částice UHMWPE jsou z materiálového hlediska hlavní příčinou selhání kloubních náhrad.

Třetí kapitola se věnuje *minimalizaci otěru UHMWPE*. Ukazuje, jak je možno pomocí ozařování a tepelných úprav za přesně definovaných podmínek modifikovat molekulární strukturu, nadmolekulární strukturu a vlastnosti polymeru tak, že vykazuje zvýšenou odolnost vůči otěru a oxidativní degradaci. Popisuje, jak byl vyvinut originální modifikační postup, který vede tzv. vysoce síťovanému UHMWPE pro kloubní náhrady s vyšší životností. Modifikovaný polymer byl zaveden do výroby kloubních náhrad v ČR a tato práce demonstruje, že je plně srovnatelný s konkurenčními materiály ze zahraničí.