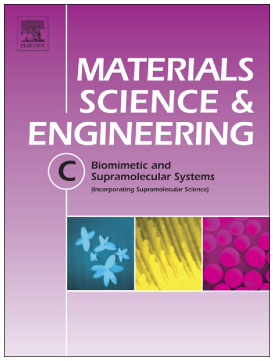

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Multilayer Porous UHMWPE Scaffolds for Bone Defects Replacement

Maksimkin A.V.[1], Senatov F.S.[1], Anisimova N.Yu.[1,2], Kiselevskiy M.V. [1,2], Zalepugin D.Yu.[3], Chernyshova I.V.[3], Tilkunova N.A.[3], Kaloshkin S.D.[1]

[1] National University of Science and Technology "MISIS", Moscow, Russian Federation
[2] N.N. Blokhin Russian Cancer Research Center, Moscow, Russian Federation
[3] State Plant of Medicinal Drugs, Moscow, Russian Federation

Abstract

Reconstruction of the structural integrity of the damaged bone tissue is an urgent problem. UHMWPE may be potentially used for the manufacture of porous implants simulating as closely as possible the porous cancellous bone tissue. But the extremely high molecular weight of the polymer does not allow using traditional methods of foaming. Porous and multilayer UHMWPE scaffolds with nonporous bulk layer and porous layer that mimics cancellous bone architecture were obtained by solid-state mixing, thermopressing and washing in subcritical water. Structural and mechanical properties of the samples were studied. Porous UHMWPE samples were also studied in vitro and in vivo. The pores of UHMWPE scaffold are open and interconnected. Volume porosity of the obtained samples was 79±2 %; the pore size range was 80-700 microns. Strong connection of the two layers in multilayer UHMWPE scaffolds was observed with decreased number of fusion defects. Functionality of implants based on multilayer UHMWPE scaffolds is provided by the fixation of scaffolds in the bone defect through ingrowths of the connective tissue into the pores, which ensures the maintenance of the animals' mobility

Keywords: UHMWPE; Scaffold; Multilayer; Bone defects; Biocompatibility

1. Introduction

People with traumas or bone diseases are in need for reconstruction of bone defects. Cancer patients need replacement of bone fragments as the result of the tissue destruction because of tumor or due to the extended surgical interventions involving resection of abnormal areas. Currently, reconstruction of the structural integrity of the damaged bone tissue is an urgent problem. Bone tissue has a natural ability to regenerate, but in case of large defects this ability is extremely limited. In these cases, additional exposure may require to restore bone tissues combining with the usual conservative methods to reduce local inflammatory processes. This is an actual problem: only in Russia more than 70 000 replacements are made per year [1].

Materials used for restoration of bone defects must have a whole set of specific properties: implants should be biologically compatible with organisms, have high mechanical properties, ensure the complete replacement of the bone defect and initiate repair processes. Therefore, there are many different materials which differ from each other according to the principle of bone defect substitution [2-3].

Ultra-high molecular weight polyethylene (UHMWPE) is a widely used material for implantology because of its bioinertness and high mechanical properties [4-6]. UHMWPE may be potentially used for the manufacture of porous implants simulating as closely as possible the porous cancellous bone tissue. But the extremely high molecular weight of the polymer does not allow to use traditional methods of foaming: chemical foaming with addition of foaming agents, such as azo compounds [7-8], peroxides [9-10], epoxides [11] salt [12-13], waxes, sugars [14-16], physical foaming at a high pressure by nitrogen [17-18], carbon dioxide [19-22], argon, [23], freon [24-26] or low-boiling hydrocarbons [27], method of phase separation [28] and sintering [29]. Application of porous UHMWPE as a material for implant imposes additional requirements on the purity and excludes the use of toxic pore-forming agents. A technique for obtaining a porous UHMWPE using a pore-forming filler (NaCl) was used in [38,41,53]. Such a method allows obtainung materials with a porosity of 60-70%. All the previously described porous UHMWPE materials [38,41,53] are used in orbital implants and for restoration of soft tissues and cartilage, which are not exposed to high mechanical stresses.

The bone can be considered as a composite material consisting of two structural layers formed of cortical and trabecular bone tissue. Cortical tissue provides the resistance to torsion, bending and compression. Trabecular tissue is highly porous and consists of the vascular system and interconnected network of trabeculae which are usually filled with bone marrow. The geometry of the formed porous structure will affect cell adhesion, proliferation, vascularization, nutrient delivery while restoring a damaged tissue. Pore size of 50 to 800 μm is required for unhindered penetration of tissue cells into implant [30-31]. High porosity of 60-80 % vol. and interconnection of pores are required for spreading of cells and nutrients throughout the structure [32-33]. Since the mechanical strength of the matrix decreases with increasing porosity, this structural parameter has to be balanced with the requirements of a particular tissue. Mechanical strength can be achieved due to the presence of a continuous reinforcing layer of UHMWPE.

Good biocompatibility, in particular hemocompatibility, is essential requirement for orthopedic implants. This property must be defined for each material or medical devices due to the potential blood contact. It's obvious that after implantation to recipient’s tissues, the scaffold will come into contact with blood and stromal cells and potentially trigger pathophysiologic processes. Hence, the blood compatibility of UHMWPE needs to be carefully investigated according to ISO 10993-4. In particular, in this work, the effects of UHMWPE on the hemolysis and cytotoxicity for lymphocyte *in vitro* had been measures for hemocompatibility evaluation. Biocompatibility of the tested scaffold was assessed after subcutaneous transplantation of the UHMWPE scaffolds in mice.

In the present work the method for formation of highly porous multilayer scaffolds based on UHMWPE with required mechanical properties and tailored microstructure was proposed. The novelty of the suggested multilayer scaffold is determined by specific features of its

construction making it similar to tubular bones. There are two firmly adhered layers. The surface one is continuous and solid. This layer, just like a cortical layer of natural bones, makes the construction firm and provides support ability of extremity. The internal layer possesses pores of the determined size and may be colonized by recipient's cells thus accelerating intergrowth of his tissues into the construction and resulting fixing of the implant in the defect zone.

Therefore, the purpose of this study was to obtain a multilayer scaffolds with a nonporous bulk layer and a porous layer with three-dimensional structure of UHMWPE which can reconstruct the bone with defect and provides the functionality of the animal limb. Specifically, we asked (1) is it possible to form a UHMWPE porous structure that mimics cancellous bone architecture, maintaining its flexibility, (2) are the two-layer structures based on UHMWPE able to simulate different types of bone tissue by mechanical properties, (3) does the porous UHMWPE posses a toxic effect on animal cells after coincubation *in vitro*, (4) can the multilayer UHMWPE scaffold induce tissue reaction after implantation *in vivo* and reconstructed of the animal limb bone defect?

2. Materials and Methods

2.1 Preparation of test specimens

2.1.1 Porous UHMWPE

UHMWPE samples were obtained by solid-state mixing of UHMWPE powder (molecular weight of $5 \cdot 10^6$ g/mol, PJSC "Kazanorgsintez", Russia) and NaCl and subsequent thermopressing [34]. Mixing was performed in low energy conditions in a planetary ball mill Fritsch Pulverisette 5 (Fritsch GmbH, Germany), equipped with agate bowls (500 ml) and corundum grinding balls 10 mm in diameter to minimize penetration of impurities. NaCl crystalline powder with a particle size ranging from 80 to 700 μm was used as a soluble material. UHMWPE and NaCl powder was taken in the ratio 1:9 by weight.

Thermopressing was carried out under load of 70 MPa and temperature of 180 °C, as shown in Figure 1(a). UHMWPE/NaCl composites after thermopressing were washed with subcritical water (water flow rate of 3 ml / min) at a temperature of 120 °C and a pressure of 250 bar to remove salt [35]. The adsorbed water in the pores was removed by drying at 70 °C for 1 hour.

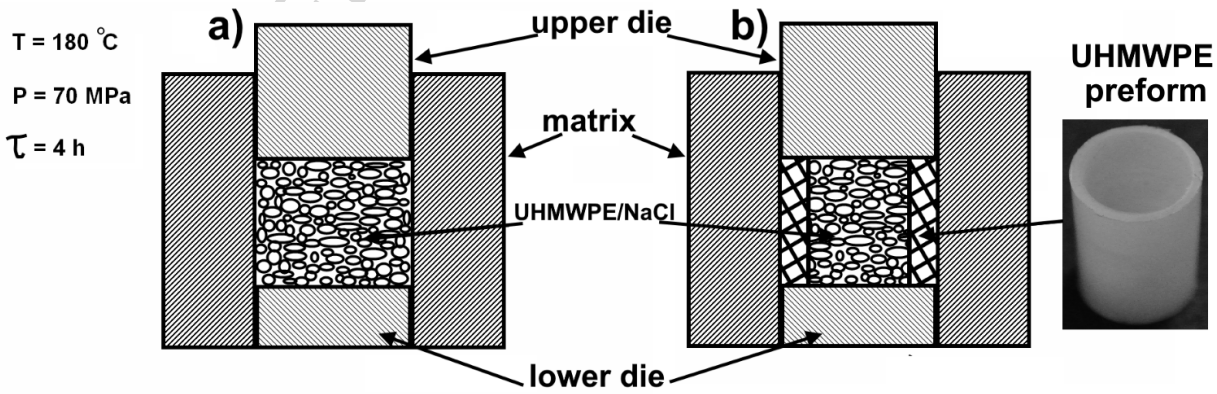

Figure 1 – Scheme of porous UHMWPE (a) and multilayer UHMWPE scaffold (b) preparation

2.1.2 Multilayer UHMWPE scaffold

Continuous UHMWPE layer of multilayer UHMWPE scaffolds was made of a preform in the form of a hollow cylinder, as shown in Figure 1 (b). UHMWPE preform was placed in a mold. Mixed UHMWPE / NaCl powder was loaded into the preform. Then it was compacted and

subjected to a thermopressing under conditions described in the previous section 2.1.1, as shown in Figure 1 (b). Removing of the salt was carried out by washing in subcritical water.

Prepared multilayer UHMWPE scaffolds were designed as a simulation of a tubular bone with an outer diameter of 10 mm and a height of 20 mm. The diameter of the UHMWPE porous layer was 6 mm.

The obtained porous and multilayer UHMWPE samples were studied with scanning electron microscopy (SEM) and tested for compressive strength. Volume porosity and pore size distribution were studied.

2.1.3. Sterilization

Porous UHMWPE and multilayer UHMWPE scaffolds samples were sterilized by steam in an autoclave MLS-2420U, Sanyo (Panasonic) at 120°C for 30 minutes. Then the scaffolds were washed three times with sterile medium RPMI-1640 (PanEco, Russia).

2.2. Structure characterization

Studies of porous UHMWPE and multilayer UHMWPE scaffolds were conducted by scanning electron microscopy (SEM). SEM was performed with VEGA 3 TESCAN microscope with an accelerating voltage of 5 and 20 kV using software VEGA TC. Pore size and size of interconnections was analyzed manually with statistical analysis performed using "ImageJ" software. To investigate non-conductive polymer samples, the surface of UHMWPE samples was covered with a layer of platinum (10–20 nm) by Auto Fine Coater JFC-1600 (Jeol, USA).

For determining the volume of the porous sample, V_{por}, five standard porous samples with dimensions of 10 × 10 × 1 mm were prepared. V_{por} was calculated by multiplying length, width and thickness of standard porous sample. From the data obtained the average V_{por} value was calculated.

Calculation of porosity was performed using the following equation:

$$P = (1 - V_{por}/V) \times 100\%, \quad (1)$$

where V_{por} – measured volume of a porous sample, V – calculated volume of a non-porous sample of the same size.

2.3. Study of mechanical properties

Mechanical testing was performed on a universal testing machine Zwick/Roell Z 020, (Zwick GmbH & Co. KG, Germany) using cylindrical samples 24 × 12.5 mm for compressive tests (ASTM D695). Bending tests were conducted for porous UHMWPE samples in accordance with ISO 178:210.

2.4. In vitro tests

For investigation *in vitro* were used the porous UHMWPE based pellets 10 × 10 × 2 mm.

2.4.1. Hemolysis assay

The hemolysis assay was done as described by *Henkelman S. et al* [36]. Pooled blood was collected from C57Bl/6 mice (n=3) in the tubes with heparin to prevent coagulation and centrifuged at 1000 rpm for 10 min at 40 °C. Plasma was removed carefully and the white buffy layer was completely removed by aspiration with a pipette with utmost care. The erythrocytes were then washed for additional three times with phosphate buffered saline (PBS), pH 7.4 for 5

min. Washed erythrocytes were mixed with PBS (1:9) and used within 1 h for the hemolysis assay. 500 uL of erythrocytes suspension were introduced into a well of a 24-well plate with the porous sample (n=6). 100 uL of PBS as negative control (0% hemolysis) or Triton-X-100 (Sigma-Aldrich, USA) as positive control (100% hemolysis) were added to 500 uL of erythrocytes suspension in different tubes. The tube with 1000 uL was used as intact control. The plate with samples was incubated at 37 °C. Probes for analysis were collected at 4h after start of the test. They were centrifuged at 300 rpm for 3 min and the resulting hemoglobin in supernatant was measured at 540 nm by plate reader Labsystems Multiscan MS to determine the concentration of hemoglobin. The percentage hemolysis induced by porous UHMWPE based pellets was calculated from the calibration curve.

2.4.2 Cytotoxicity Assessments

The mononuclear leukocytes (ML) were isolated from pooled blood of C57Bl/6 mice (n=3) with heparin according to *M'Bemba-Meka et al.* [37]. 1 ml of ML in RPMI-1640 medium supplemented with 10 % fetal bovine serum (Thermo Scientific, USA), 100 μg/ml penicillin, 100 μg/ml streptomycin, and 2 mM L-glutamine (all - Gibco, USA) at density of 5×10^5 cells/cm^2 were cultivated in a 24-well tissue culture plate (Nunc, USA) with porous UHMWPE based pellets (n=6) at 37 °C in a humidified atmosphere with 5% CO_2. Control cells were treated only with RPMI 1640. After incubation for 24h, 20 μL 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Sigma) with a concentration of 5 mg·mL^{-1}, was added into each well. The plates were incubated for 4 h under cell culture conditions. Subsequently, 500 μL dimethyl sulphoxide (PanEco, Russia) was added to the cells by replacement of the medium, to dissolve the formazan crystals. Optical density (OD) measurements were conducted at 540 nm using a plate reader Labsystems Multiscan MS in 96-well plates. The cytotoxicity of UHMWPE based pellets was calculated by applying the following formula:

$$\text{Cytotoxicity, \%} = 100 - \text{OD samples} / \text{OD control} \times 100. \quad (2)$$

2.4.3. Statistics

All experiments were performed with triplicate assays, i.e. at least three samples, and repeated three times using separate experiments in an attempt to achieve reliable and reproducible results. For statistical analysis dates were presented as mean ± standard deviation (SD). Comparisons between the two groups were made using Student's t-test. Significance was assigned at p-values 0.05.

2.5. In vivo test

2.5.1 Surgical procedure

Before implantation, the skin of the mouse was disinfected with 40% ethanol. The porous UHMWPE based pellets (n=5) with size of 5 × 5 mm by 2 mm in thickness were subcutaneously implanted into intracapsular region of the back for 30 days. One mouse underwent the implantation of only one sample, so 5 mice were used. Two months after implantation, the animals were sacrificed by cervical dislocation and after that the extraction of the implant with surrounding tissue was performed.

Study of reconstructive properties of porous UHMWPE were performed by implantation of cylindrical samples with average size 5 × 1.5-2 mm into formed defect of tibial bones of rats Wistar (n=5) for 30 days. Skin and muscles on rat's leg were dissected during the surgery in order to get access to the surface of tibial bone. After that a small defect (3 mm) was created

with a drill and sample was placed into the defect. Tibial bones with implants were taken out after implantation and then they were studied.

2.5.2. Histological analysis

The samples of implant with tissue were fixed in 10 % buffered formaline pH 7.4. Tibial bones with implants were decalcified in formic acid and sodium citrate. The tissue was then dehydrated in alcohol and embedded into paraffin. Sections of 3-6 μm were stained by haematoxillin and eosin (HE). Dehydrated portions were fixed on glass and then analyzed under light microscopy. In particular, the connection between implant and surrounding tissue was observed.

2.6. Ethics Statement

The use of animals, blood and procedures for this study was approved by the Ethics Committee of N.N. Blokhin Russian Cancer Research Center. All surgery was performed under ketamine and xylazine anesthesia, and all efforts were made to minimize suffering.

2.7 Animals

We used male C57Bl/6J laboratory mice - 3-4 months old, weight 22-24 g and male Wistar rats - 3 months old, weight 349-355 g. The animals were housed in polypropylene cages inside a well-ventilated room, provided with chow pellets and water ad libitum and maintained under a 12-hour light/dark cycle. All animal procedures were in accordance with the standards set forth in the guidelines for the care and use of experimental animals by our local Center Ethics Committee.

3. Results

Figure 2 (a) shows micrographs of porous UHMWPE and multilayer UHMWPE scaffold. SEM micrograph of porous UHMWPE shows no visible boundaries between the sintered polymer particles, Figure 2 (b). All the UHMWPE particles were sintered. Possibility of producing multilayer materials combining non-porous reinforcing UHMWPE layer and porous layer was also demonstrated in Figure 2 (a). Multilayer UHMWPE scaffold consists of an outer continuous layer and porous layer. Figure 2 (c) shows SEM micrograph of cut in half multilayer UHMWPE scaffold. SEM shows the formation of a transition layer between the continuous and porous structures of UHMWPE, whereby there was a strong connection of the two layers.
The pores of UHMWPE are open and interconnected. Volume porosity of the obtained samples was 79±2 %. The pore size range was from 80 to 700 μm. The average pore size was 350 μm; size of interconnections was 8±2 μm. Figure 3 demonstrates the distribution of pore sizes in porous UHMWPE estimated from image analysis of the SEM micrographs. The distribution can easily be varied by introducing the desired size of salt particles which determines the pore size.

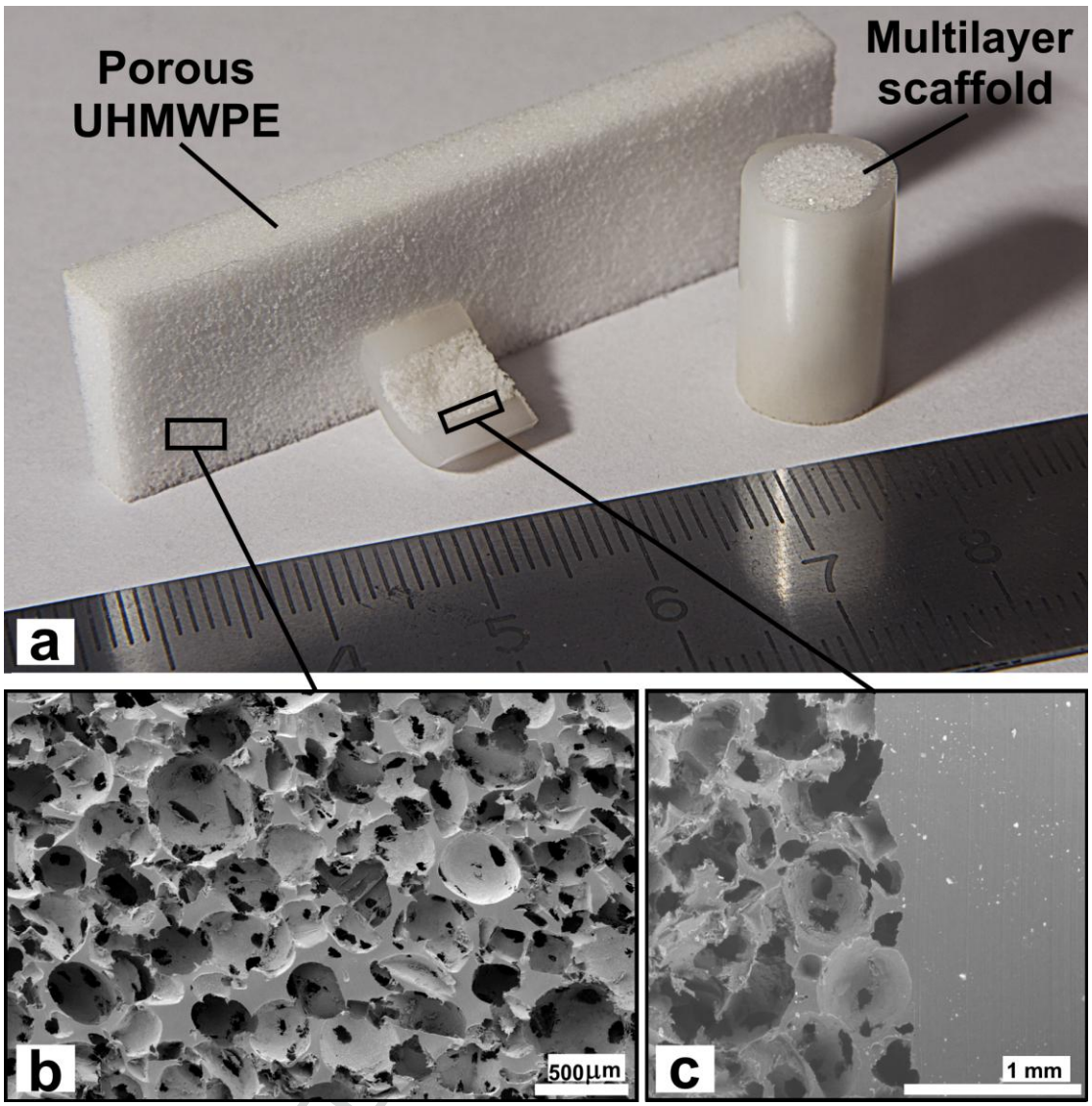

Figure 2 – (a) Porous UHMWPE and multilayer UHMWPE scaffold, (b) SEM of porous UHMWPE and (c) SEM of multilayer UHMWPE scaffold

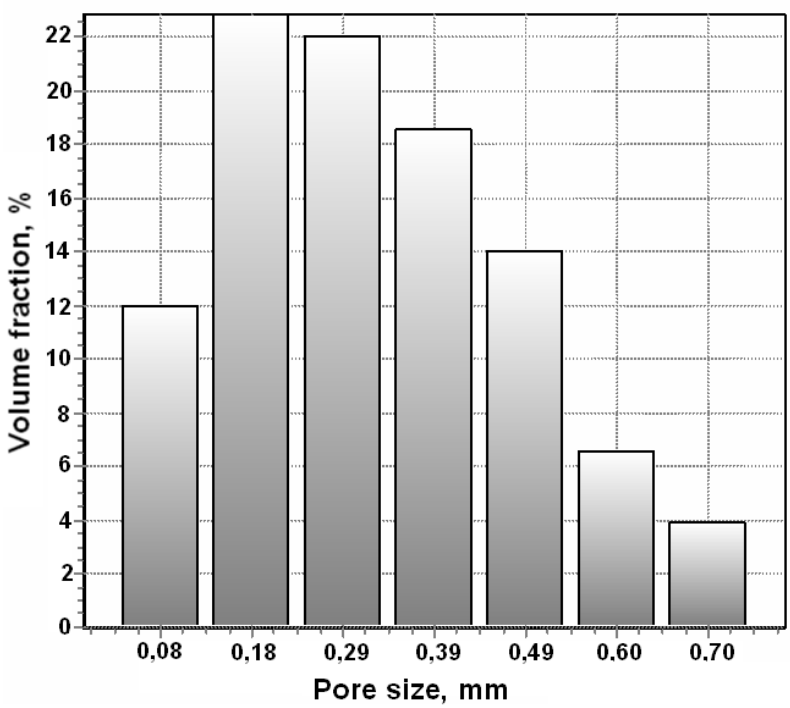

Figure 3 – Distribution (%) of pore sizes in porous UHMWPE

These porous samples had high plasticity with increased fracture-resistance and could be bent several times, as shown in Figure 4. The results of bending tests of porous UHMWPE are shown in Table 1. Porous UHMWPE samples did not destroy under bending and there was no loss of individual particles of UHMWPE because of complete sintering between individual polymer particles in point contact and with decreased number of fusion effects. The samples recovered the dimensions and shape after unloading.

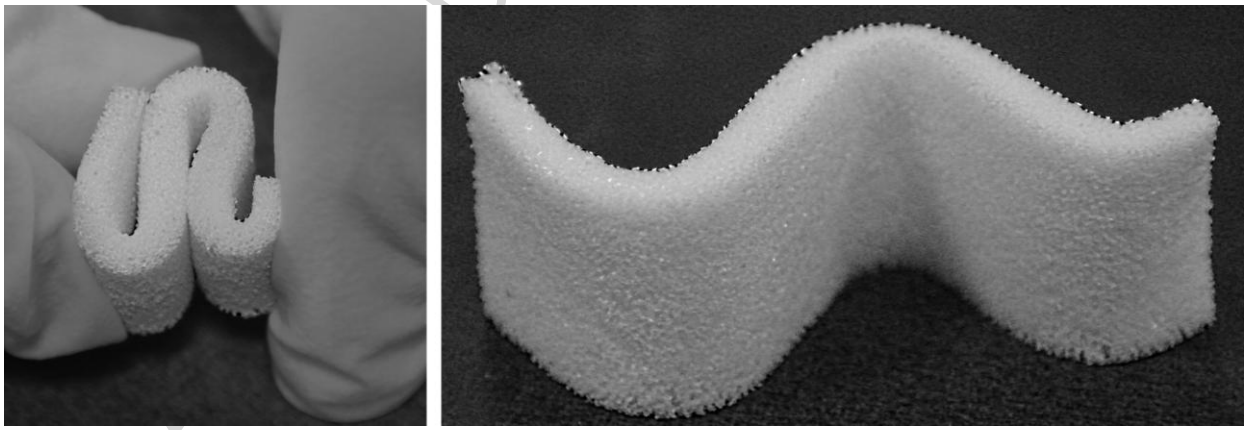

Figure 4 – Photographs demonstrating high plasticity of the porous UHMWPE samples

Table 1. Flexural properties of porous UHMWPE

Material	Flexural Modulus, MPa	Flexural stress, MPa	Flexural strain, %
Porous UHMWPE	14.7±2	0.61±0.03	10

The porous UHMWPE due to the high content of pores exhibits a low ability to resist compression. The compressive strength is 1.2 MPa upon reaching deformation of 40 %. Multilayer UHMWPE scaffold showed compression strength of 70 MPa due to the presence of a continuous UHMWPE layer. Figure 5 shows a photograph of multilayer UHMWPE scaffold at the end of the test, which demonstrates that there was no delamination of porous layer of

continuous layer even at 40 % of strain and loss of sample stability. Multilayer UHMWPE scaffold showed compression strength of 70 MPa due to the presence of a continuous UHMWPE layer. The strength of solid bulk UHMWPE is 150 MPa. As multilayer scaffolds are suggested to be used as implants for reconstruction of tubular and plane bones in medicine and veterinary, their strength was compared to the strength of native tubular bone of a dog, which was measured in our previous paper [43]. The strength of tubular bone is lying in the range of 80-115 MPa. The strength of multilayer scaffold is on the border with the strength of native tubular bone [43], and can be increased due to the regulating the thickness of a continuous layer of UHMWPE.

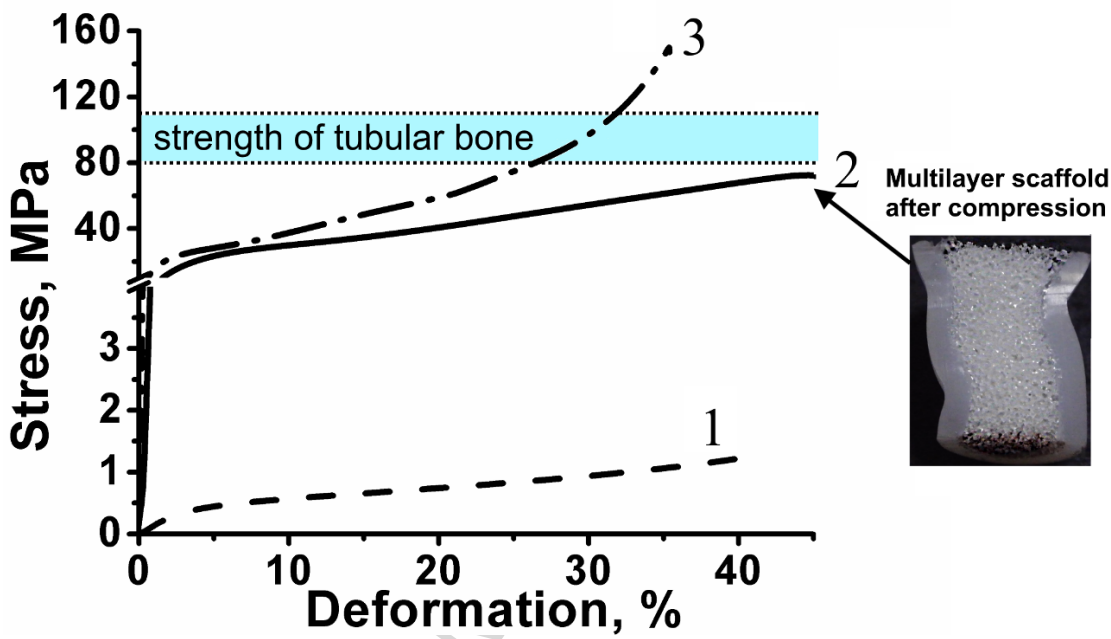

Figure 5 – Stress-deformation diagrams: 1 - porous UHMWPE, 2 - multilayer UHMWPE scaffold, 3 – bulk UHMWPE. Data for tubular bone strength was obtained in [43] for the radius and humerus bones

Hemolysis refers to the damage of red blood cells (RBC) result in the release of intracellular erythrocyte content into blood plasma. A dominant intracellular protein of RBC is hemoglobin. It plays a key role in carrying oxygen to other cells and tissues. However, extracellular hemoglobin released upon hemolysis may be toxic for vascular, renal, myocardial and central nervous system tissues. Therefore, all medical materials which come into contact with blood are required to be tested for potential hemolytic properties.

To assess the impact of processed UHMWPE based pellets on RBS, hemolysis test was performed by spectrophotometric measurement of hemoglobin release. The performance of hemolysis assay was tested on 4 h after start the incubation tested samples with RBC. It was shown that no significant differences were observed for both UHMWPE based pellets and control with intact cells: 5.5 ± 3,2 % versus 1.4 ± 2.4 %, respectively, p=0,062. Thus, the tested multilayer scaffold didn`t stimulate the significant hemolysis and was non-toxic for animal erythrocytes.

To further explore the safety of processed porous UHMWPE in the bloodstream, we investigated to assess the effects of tested samples on the basic immunological cells - ML. Peripheral blood ML were incubated with tested samples for 24h and measured by MTT assay comparing with control with intact cells. It was demonstrated that UHMWPE pellets for 24 hours did not significantly affect on the cell viability: their cytotoxicity was 2 ± 2,8%. Therefore, porous UHMWPE was shown to be non-toxic for animal blood cells *in vitro*. It allows recognizing this material as hemocompatible.

Histological study of the implants demonstrated that the synthetic samples induced the formation of the thin layer of a fibrovascular tissue after subcutaneous implantation to mice. The

granulation tissue that filled the subcutaneous matrix with surrounding tissue was composed of spindle-shaped fibroblasts, microvessels, and polynuclear giant cells embedded in an extracellular matrix (Fig. 6). At the same time there was no evidence of the local edema or abscess formation in surrounding tissue. As a result, the samples didn't induce negative tissue reaction after implantation *in vivo*.

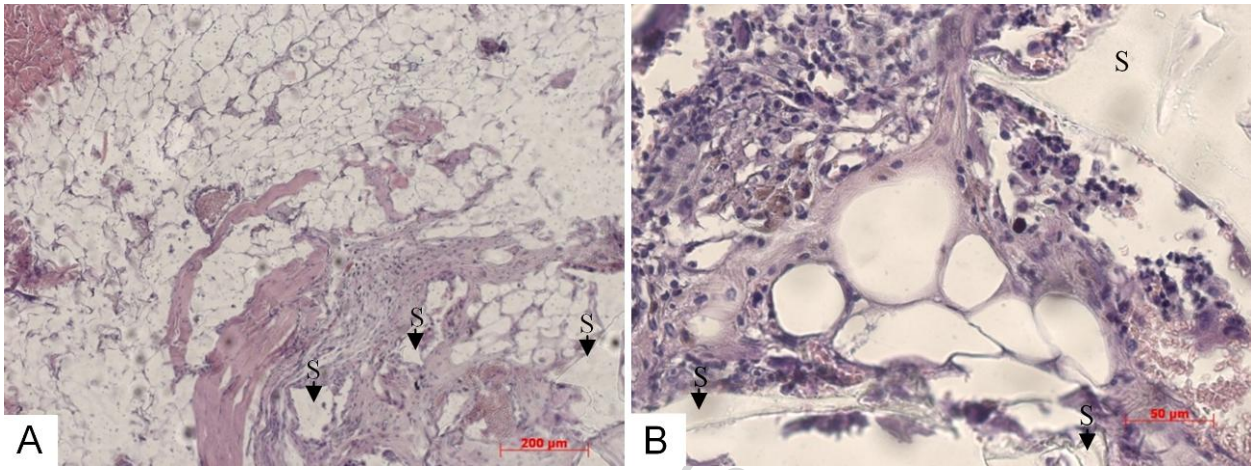

Figure 6 - Histological analysis of porous UHMWPE scaffolds (S) with surrounding tissue after 60-day of the subcutaneous implantation in mice (stained with HE). A- magnification ×200; B- magnification ×400

Generally, the collected data allow characterizing the porous UHMWPE as a biocompatible material, stimulating the adhesion and proliferation of recipient mesenchymal stromal cells followed by colonization of scaffold after implantation into tissue of recipient.

Study of reconstructive properties of multilayer UHMWPE scaffolds was performed by implantation into formed defect of tibia bones of rats for 30 days. Starting from day 5 after operation no signs of inflammation or rejection (hyperthermia, edema, pain reflex, and abscess) were detected in the legs with reconstructed bones. All animals moved with no any lameness. No symptoms of defected bone fractures were registered. Histological study of HE stained sections indicated good integration of the implants with the surrounding bone with the formation a limited amount of new bone sites mainly at the periphery of the scaffolds. Blood vessels were detected within porous part of the implanted multilayer scaffolds with complete filling of all pores by connective tissue, but with no signs of cellular infiltration by neutrophils (Fig.7A). There was documented a formation of the tissue layer on the scaffold's external surface (Fig.7B).

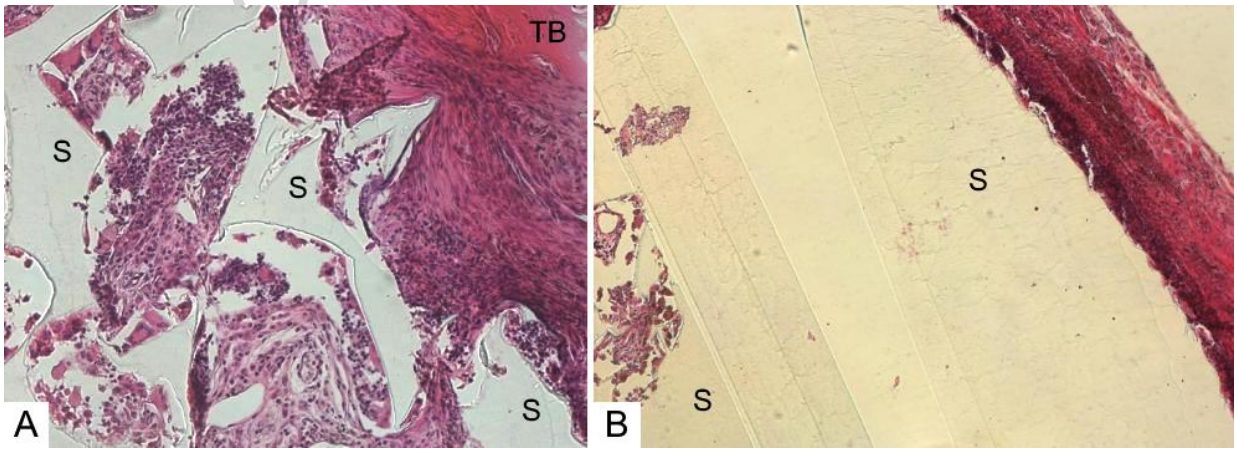

Figure 7 – Histological analysis of multilayer UHMWPE scaffolds (S) with surrounding tissue after 30-day of the implantation into rat tibia bone (TB). A – porous part of scaffold; B – tissue converted external surface layer of the nonporous bulk part of scaffold. Stained with HE, magnification ×100

4. Discussion

Development of scaffolds with architecture of bone tissue and capability to function under the mechanical load is a promising direction for solving bone reconstruction problem, but a complex engineering task. The effectiveness of porous scaffolds is largely determined by their architecture, which includes the type of porous structure, three-dimensional pore content and size distribution. Architecture of porous scaffold creates favorable conditions for the formation of new tissue. The technology of producing porous UHMWPE scaffolds with wide range of quantity and size of pores was developed. The uniqueness of the proposed approach, providing the combination of mechanical strength of synthetic scaffold and high porosity is designed to provide acceleration of the repair of damaged tissue with preserving its functionality during integration process.

There were several limitations to this study. The developed technology allows forming porous scaffolds with porosity more than 30 % vol. only, since salt washing process is significantly limited at a higher content of soluble filler when salt particles are surrounded by polymer matrix without connection to each other [38]. Therefore, only UHMWPE scaffolds with high porosity may be produced by such technology. But this is not a problem in the case of reproducing the tissue architecture of trabecular bone, which is highly porous.

The results of our study support that the size of the formed pores and their distribution in the porous UHMWPE depends on the size of the soluble filler particles. The use of low energy modes of ball milling of polymer / soluble filler composite powders allows to save the distribution of particles size, which greatly simplifies the programming of required pore size. The method allows obtaining porous bulk samples with high porosity (up to 80 % vol.), a wide distribution of pore size from 80 to 700 μm. In this case, the pores are open and interconnected with each other with network of channels and cavities. This allows cells to penetrate into the implant and remain on the surface.

There are several studies [38,41,53], which describe similar approaches to the formation of the porous structure of UHMWPE using a pore-forming filler. However, besides the formation of the porous structure, it is necessary to achieve complete sintering of the polymer particles. Otherwise loss of individual polymer particles during loading may occur in the porous UHMWPE. In the study of *Plumlee et al* [38] NaCl was also used as a pore-forming filler for obtaining UHMWPE porous structure. However, the boundary between sintering polymer particles becomes visible at high SEM magnification of porous scaffold [38] that may eventually lead to the destruction of the implant (partial or total). A possible reason for this may be the use of excessive pressure of 100 MPa, which is close to the locking pressure for UHMWPE, when the diffusion of macromolecules is extremely limited. The visible boundaries between the particles and fusion defects in a porous UHMWPE have also been found in other studies of *King et al* [39] and *Wellisz et al* [40]. The main advantage of the developed porous scaffold compared to its analogues based on UHMWPE is the lack of visible boundaries of the sintering polymer particles, which guarantees the preservation of the structural integrity of the material for a long period, and also provides the ability to withstand the cycle compressive loading without failure. Another important advantage of the developed porous scaffold is the achieved porosity up to 80 %, whereas it does not exceed 60-70 % in some works about obtaining porous UHMWPE [38-41].

An important characteristic of the material when it is functioning as a part of an implant in contact with bone is the ratio of elastic modulus in compression of the material to the modulus of bone tissue. If the difference in elastic modulus is large, it leads to microstrain formation in the border bone/material and stress shielding that may cause destruction of bone and loosening of the implant [42]. Elastic modulus of trabecular bone tissue is lying in the range from 1.1 MPa [43-44] to 5 GPa [45-47]. Elastic modulus of cortical bone tissue is lying in the range 11 ÷ 21 GPa (longitudinal) and 14 ÷ 25 GPa (transverse) [48-52]. Creating of a multilayer UHMWPE

scaffold demonstrates broad prospects for the design of implants based on porous UHMWPE to replace the different bone defects. Young's modulus of multilayer porous UHMWPE scaffold was 0.7 ± 0.05 GPa. Therefore, mechanical properties, particularly a Young's modulus of UHMWPE, are close to the trabecular bone, but not to the cortical. A continuous layer of multilayer UHMWPE scaffold may be made reinforced, for example, by titanium, depending on the desired mechanical properties of the construction to imitate properties of cortical bone tissue.

Biological study of the porous UHMWPE samples showed that the scaffold did not induce cytotoxicity or hemolysis, i.e. did not posses of damaging effect on isolated blood cells (erythrocytes and lymphocytes). Therefore, the porous UHMWPE could be considered as hemocompatible scaffold. Subcutaneous implantation of the porous scaffold in mouse triggered a series of host reactions at the injury site that include interactions material/tissue, provisional matrix formation, granulation tissue development, fibrosis and fibrous capsule development. The results showed that the porous UHMWPE actively attracted the cells to implantation area stimulated the granulation and vascularization of the synthetic scaffold porous part. There were no signs of escalation of negative tissue reaction after implantation of the sample *in vivo*. The implantation of multilayer UHMWPE scaffolds into tibia of rats demonstrated their functionality for the reconstruction of small bone defects. Strong fixation of the implant in the bone led to the possibility of good mobility of limbs without lameness and bone fractures of all experimental animals.

The main idea of the suggested multilayer UHMWPE scaffold is determined by specific features of its design making it similar to tubular bones. There are two firmly adhered layers. The surface one is continuous and solid. This layer, just like a cortical layer of natural bones, makes the construction firm and provides support ability of extremity. The internal layer possesses pores of the determined size and may be colonized by recipient's cells thus accelerating intergrowth of his tissues into the construction and resulting fixing of the implant in the defect zone. Namely these specific features of the construction besides described earlier polymer characteristics determine the novelty of the suggested scaffold. The data obtained prove that the suggested multilayer scaffold is promising for creation of medical products: inner prostheses and bioimplants.

5. Conclusions

Highly porous UHMWPE scaffold, formed by thermopressing with NaCl and washing with subcritical water, mimics cancellous bone architecture, maintaining its flexibility, was obtained. Porous UHMWPE may be characterized as a biocompatible material. Multilayer UHMWPE scaffolds with nonporous bulk layer and porous layer with three-dimensional structure of UHMWPE with the ability to control the pore size were developed for the first time. Multilayer UHMWPE scaffold based on porous UHMWPE is able to simulate at macro scale different types of bone tissue. Functionality of implants based on multilayer UHMWPE scaffolds is provided by the fixation of scaffolds in the bone defect through ingrowths of the connective tissue into the pores, which ensures the maintenance of the animals' mobility.

Therefore, multilayer UHMWPE scaffolds can be used for the formation of medical devices (implants, bioimplants) for reconstructive medicine. But, it can be recommended to reinforce a continuous UHMWPE layer to achieve the mechanical properties as close as possible to the cortical bone. This is an important and challenging problem for future studies.

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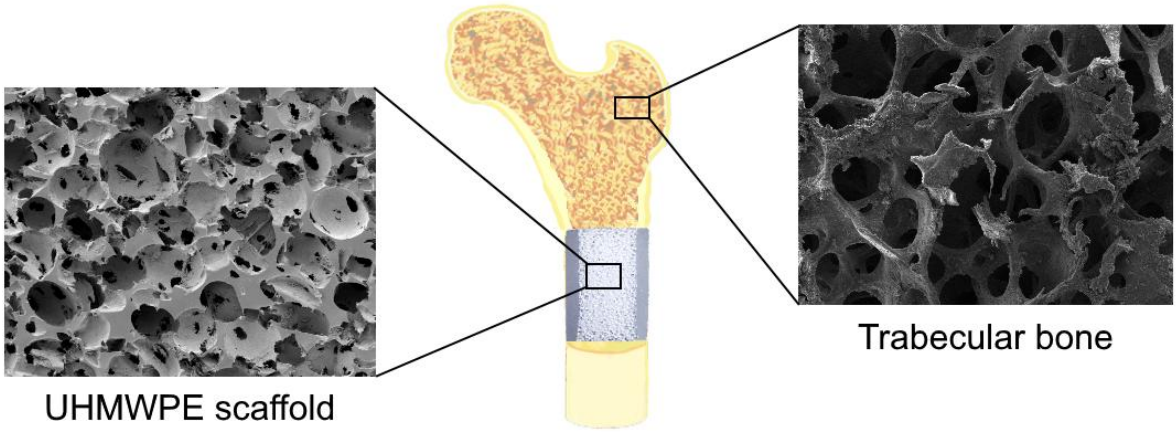

Graphical abstract

Highlights

Porous UHMWPE scaffold mimics cancellous bone architecture, maintaining its flexibility

Multilayer UHMWPE scaffold is able to simulate different types of bone tissue

Fixation of scaffolds in the bone provides through ingrowths of the connective tissue into pores

Multilayer UHMWPE scaffolds can be used for the formation of bone implants