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ADVANCED FUNCTIONALIZED POLYMERS AND COMPOSITES: FROM SYNTHESIS TO APPLICATION

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ADVANCED FUNCTIONALIZED POLYMERS AND COMPOSITES: FROM SYNTHESIS TO APPLICATION

POKROČILÉ FUNKCIONALIZOVANÉ POLYMERY A KOMPOZITY: OD SYNTÉZY K APLIKACI

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TABLE OF CONTENTS

1 INTRODUCTION 5

2 FUNCTIONALIZED SYNTHETIC POLYMERS 6

2.1 End-functionalized polymers for medical applications 6

2.1.1 Telechelic polyacrylate-based macromonomers 6

2.1.2 Telechelic polyester-based macromonomers 8

2.2 Degradable thermosets for industrial applications 11

2.2.1 Biodegradable polyurethanes 11

2.2.2 Thermodegradable polyurethane foams 15

3 MODIFIED NATURAL POLYMERS 17

3.1 Porous collagen scaffolds for tissue engineering 17

3.1.1 Hydroxyapatite modified collagen scaffolds for bone regeneration 18

3.1.2 Polycaprolactone nanofiber modified collagen scaffolds 22

3.1.3 Chitosan microfiber modified collagen scaffolds for cartilage regeneration 24

4 SUMMARY 25

5 REFERENCES 26

ABSTRACT 27

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Speciální syntézy a charakterizace nových polymerů, polymerních hydrogelů a kompozitů s řiditelnou dobou života, příprava nosičů buněk či léčiv z přírodních a syntetických polymerů pro regenerativní medicínu a tkáňové inženýrství

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1 INTRODUCTION

Nowadays, polymers dominate our rapidly developing daily needs and show enormous potential for the development of new technologies. It is therefore obvious that the future of polymer chemistry will be influenced by the elaboration of new functional polymers with unique properties or abilities. The development of various functional polymers is becoming increasingly important in specific areas of application in order to gain the desired physical, physicochemical, chemical and biological properties.

The submitted thesis presents the techniques for the generation functionalized or functional polymers by copolymerization, (end-)functionalization, particle modification and cross-linking methods to meet unique properties required either for industrial or biomedical applications.

In terms of industrial applications, thermosets that are very hard or impossible to recycle were functionalized in order to make them either biodegradable or thermodegradable to be easily discharged at the end of their life-time or to be used for recycling. Particularly, for the development of novel thermodegradable polyurethane foams we were recently granted by European and Czech patent.

Focusing on the constantly developing medical field, only specifically elaborated functional polymer materials can fulfill the specific challenges required of them for use as, for example bone adhesive. Typical examples of such materials include hydrogels and stimulus-responsive polymers which are also the basis of injectable drug delivery carriers. Recently, we have developed block copolymers from both hydrophilic and hydrophobic blocks resulting in amphiphilic hydrogels mimicking soft tissue. Moreover, prepared biodegradable hydrogels are thermo-responsive classified as "smart" polymers. They respond to the temperature by reversible changing the state from liquid to solid (gel) useful for injectable drug delivery carriers. However, their end-functionalization by both double bond and carboxylic groups can meet specific, novel properties like sensitivity to irradiation for enhancing mechanical properties and possibility to attach bioactive molecules promoted bioinduction useful in biomedical applications.

In 2006 we started collaboration with both University of Veterinary and Pharmaceutical Sciences Brno and Institute of Experimental Medicine in Prague on the development of novel bioconductive and bioinductive scaffolds useful in regenerative medicine of musculoskeletal system. Unique biodegradable scaffolds for tissue engineering of bones and cartilage based on collagen modified by either natural or synthetic polymers and fillers were prepared using both chemical and physical crosslinking meeting required hydrolytical stability *in vivo*. The Czech patent application was filled in 2013 for those successfully preclinically tested on animal models for accelerated bone regeneration and papers were written about surprising achievements in both hyaline and growth cartilages recovery.

This thesis summarized the main syntheses, modifications and characterizations of novel functionalized polymers and composites we have developed over the last 14 years which are presented in selected 16 scientific papers, 2 patents, 1 patent application and 1 utility. The chapters are divided into two main parts:

1) Functionalized **synthetic** polymers.
2) Modified **natural** polymers.

Both synthetic and natural polymers were modified by at least one of the followed approach:

- Coupling with another polymer to make amphiphilic copolymers
- Insertion of some functional groups into polymer chain
- Addition of inorganic micro- (nano)particles
- Addition of polymeric micro- (nano)fibres

All developed polymers and composites were finally chemically or physically crosslinked in order to increase mechanical properties and to adjust required stability.

2 FUNCTIONALIZED SYNTHETIC POLYMERS

Chapter 2.1 deals with selected newly developed end-functionalized block copolymers which can be end-linked resulting in amphiphilic hydrogels with specific function in biomedical engineering. Chapter 2.2 is directed to a novel bio- and thermo-degradable thermosets synthesized for their use as polyurethane foams or elastomers with controlled life-time in furniture or automotive industry.

2.1 END-FUNCTIONALIZED POLYMERS FOR MEDICAL APPLICATIONS

This chapter describes an investigation of synthesis of telechelic macromonomers (both ends double bond terminated low molecular weight polymers) via living Atom Transfer Radical Polymerization (ATRP)[LV-1,2] and Ring Opening Polymerization (ROP)[LV-2,3,4], which can be further photopolymerized (UV or VIS) without any cross-linker to form end-linked gels useful in biomedical/tissue engineering applications. In biomaterials and tissue engineering applications strict control of properties is critical since all functional units within the body are structurally and functionally very specific and dynamically interact with its environment in a synergistic manner.

2.1.1 Telechelic polyacrylate-based macromonomers

In 2003 in US patent application[LV-1] and later in paper[LV-2] we firstly reported about the synthesis of novel, end-linked amphiphilic temperature-sensitive polymers based on polyacrylate, which were synthesized via living Atom Transfer Radical Polymerization (ATRP).

Controlled "living" polymerizations offer the possibility of synthesizing polymers with precise control of the end groups, composition, functionality and architecture of polymer. However, living polymerizations based on anionic, cationic, or group transfer are very sensitive to moisture, oxygen and impurities and thus very difficult to operate. In 1995, the development of controlled "living" free radical polymerization technique known as Atom Transfer Radical Polymerization (ATRP)[1], one of the most successful and versatile system, has allowed for the synthesis of a variety of well-defined polymers with predetermined molecular weights (linear increasing with conversion) and low polydispersities ($M_w/M_n < 1.3$).

α,ω-allyl terminated macromonomers

As for macromonomers, we firstly described the synthesis of α,ω-allyl terminated (telechelic) poly(methacrylic acid) (PMAA) macromonomers (Scheme 1) that can be cured by an end-linking chemistry (e.g. by heating or radiation) to produce hydrogels of highly controlled molecular architecture[LV-1,2]. To avoid difficulties with acidic monomer (which can destroy the metal catalyst), we started with poly(tert-butyl acrylate) (PtBMA), where tert-butyl group acts as acid protecting group. Polymerization proceeded with Cu(I)Br/ hexamethyltriethyltetraamine (HMTETA) catalytic system in conjunction with an allyl-2-bromo-2-methylpropionate as the functional initiator. Then the active bromine at the end of the polymer chain was transformed to a second allyl group using by a free radical chain mechanism. This step was followed by successful deprotection of tert-butyl groups by trifluoroacetic acid (TFAA) affording α,ω-allyl terminated PMAA macromonomers, which we originally described in a paper[LV-2]. The polymerization of t-BMA was studied in different solvents and at different temperatures. Conversion increased with

[LV-1] VOJTOVÁ L.; KOBERSTEIN J.T.; TURRO N.T.: *Alpha, omega-allyl terminated linear poly(methacrylic acid) macromonomers for end-linked hydrogels and method for preparation*. Appl. Univ. Columbia USA, World Intellectual Property Organization, WO03101934 (A1), pp. 1 – 50. 2003-12-11. US patent appl.

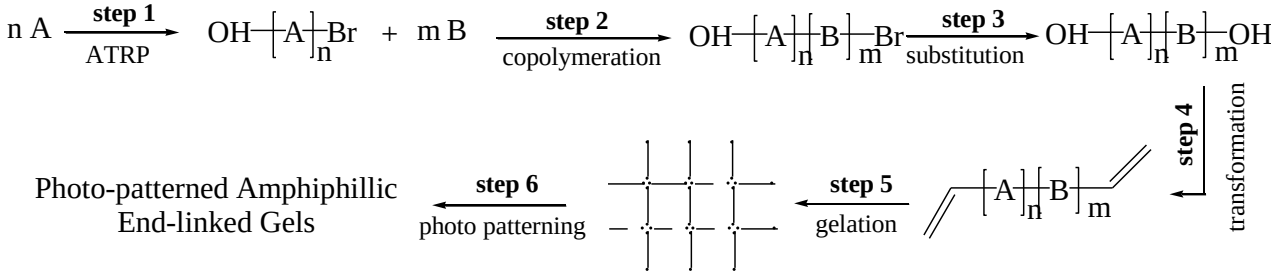
[LV-2] VOJTOVA, L.; TURRO, N.J.; KOBERSTEIN, J.T.: *ATRP Synthesis and Characterization of α,ω-allyl-terminated Macromonomers as Precursors for End-linked Gels and Hydrogels*. Mat Res Soc Proc (Materials Inspired by Biology), 2003, 774,59-73.

time linearly and, thus, a kinetic plot of ln ($[M]_0/[M]$) vs. time was first-order in all cases. The rates of reactions were very similar, even though reactions in THF and acetone were more homogeneous compared to the reaction in non-polar benzene. Conversions of monomer (calculated from a GC chromatogram using a GC standard) were approximately 88 % in 3 hrs. Prepared macromonomers were successfully end-linked (cross-linked without additional cross-linker) via thermoreaction using azobisisobutyronitrile (AIBN) initiator at 60 °C. However, irradiation by UV was not successful due to the high stability of allyl-end groups. Thus, a new macromonomers with α,ω-vinyl terminated groups were synthesized at that time.

α,ω-vinyl terminated macromonomers

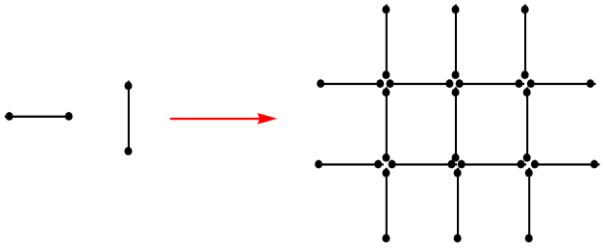
CH_2=CH–COO– (vinyl-) groups belong to acrylate end groups and are known to be very reactive in radical reactions. Thus, after curing by UV or visible light, functionalized end-linked amphiphilic gels with controlled chemical heterogeneity for biomedical/tissue engineering applications were prepared.

For synthesis of α,ω-vinyl terminated macromonomers we prepared new synthetic route (see Scheme 1).



Scheme 1 Procedure for preparation of photo-patterned amphiphillic end-linked gels where A stands for tert-butyl methacrylate and B for styrene monomers.

In this case, vinyl group cannot be a part of initiator because of their high reactivity with radicals. Therefore, hydroxy-functional initiator was used for the ATRP of t-BMA (step 1) where the OH group can be easily replaced later by acryloyl chloride to incorporate CH_2=CH–COO– to the end of polymer (step 4). In the step 2 the t-BMA was copolymerized with styrene (St) as well via ATRP. The second vinyl group was added through OH group that previously replaced terminal bromine by nucleophilic substitution (step 3). The successful reaction was confirmed by ^{1}H NMR spectroscopy. Thus, new α,ω-vinyl terminated poly(t-BMA)-*b*-poly(St) (PtBMA-PS) macromonomers with "hot" double bonds for photopolymerization were synthesized and were finally used for end-linked gels (Scheme 2). These reactions carried out in a mixture of the α,ω-functional macromonomer, suitable UV-initiator and solvent by UV irradiation at 355 nm. There is no need for cross-linker, and thus the molecular structure is well-determined by the macromonomer molecular weight and composition.



Scheme 2 End-linked gel formed from telechelic macromonomers without the use of cross-linker.

To prepare **amphiphilic hydrogels**, the hydrophobic end-linked gel was treated with photo acid generator (PAG - triarylsulfonium triflate). The PAG acetone solution (0.2% w/v) was dropped on the top of cross-linked polymer through the copper grid 1GC100 and the excess of acetone was evaporated. The sample was exposed to 254 nm UV light for 20 minutes. After this deprotection step the grid was gently removed from the cross-linked sample (Figure 1).

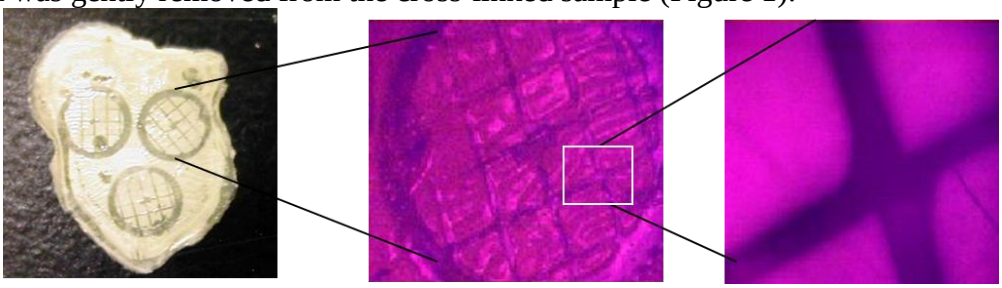


Figure 1 Photo patterning of PtBMA-PS by PAG to obtain PMAA-PS. A: Gel after VIS-irradiation and grid removing, B: staining hydrophobic PtBMA-PS dark violet and hydrophilic PMAA-PS pink, C: magnification of picture B.

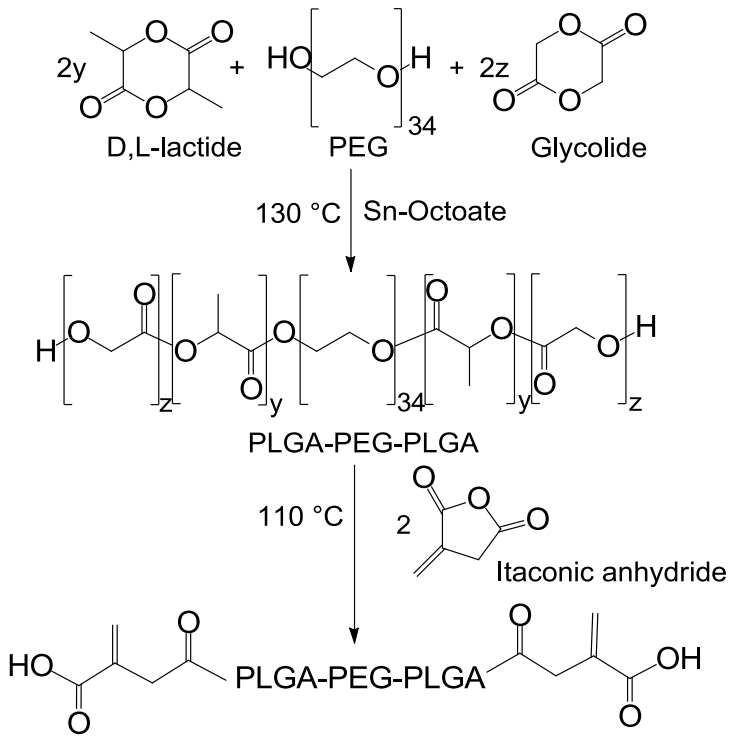
New hydrogels with controlled mechanical properties were prepared by end-linking hydrophobic PtBMA-PS copolymer, where PtBMA was transformed to hydrophilic poly(methacrylic acid) PMAA by PAG resulting in amphiphilic PMAA-PS. In addition, the carboxyl functional groups of PMAA hydrogels might serve as reactive sites for attaching peptides and bio-active compounds required for biomedical and tissue engineering applications.

2.1.2 Telechelic polyester-based macromonomers

In order to satisfy all the conditions, further chapters deal with the study of the synthesis and characterization of well-defined multifunctional biocompatible and biodegradable polymer materials with controlled life time additionally modified by renewable raw materials.

In 2010[LV-3] we utilized the novel approach for "one pot" synthesis of biodegradable thermosensitive poly(D,L-lactic acid-*co*-glycolic acid)-*b*-poly(ethylene glycol)-*b*-poly(D,L-lactic acid-*co*-glycolic acid) (PLGA–PEG–PLGA) copolymers via living Ring Opening Polymerization (ROP) both-end terminated with itaconic acid bringing both carboxyl groups (–COOH) and double bonds (–CH=CH_2) (Scheme 3). Itaconic anhydride (ITA), an unsaturated cyclic anhydride, can be obtained from renewable resources both by distillation of citric acid and pyrolysis of itaconic acid. It is generally known that itaconic anhydride passes to nontoxic degradation products under physiological conditions, when it initially hydrolyzes to itaconic acid followed by oxidation to acetate, lactate and carbon dioxide[2]. After optimizing the reaction conditions, obtained well-defined ITA/PLGA–PEG–PLGA/ITA macromonomers with molecular weight of 7.2 $kg.mol^{-1}$, narrow polydispersity (M_w/M_n = 1.20), weight ratio of PLGA/PEG = 2.5 and molar ratio of LA/GA = 3.0 were characterized using ^1H NMR, FTIR, TGA and DSC analysis. The highest amount of ITA (76.6 mol%) bonded to PLGA–PEG–PLGA copolymer was obtained with ITA purified by sublimation in a bulk at 110 °C after 1.5 hours.

[LV-3] MICHLOVSKÁ, L.; VOJTOVÁ, L.; MRAVCOVÁ, L.; HERMANOVÁ, S.; KUČERÍK, J.; JANČÁŘ, J.: *Functionalization conditions of PLGA-PEG-PLGA copolymer with itaconic anhydrite*. Macrom Symp. 2010, 295 (1), 119-124.



Scheme 3 ‘‘One-pot’’ functionalization resulting in ITA/PLGA–PEG–PLGA/ITA macromonomer.

A series of both PLGA–PEG–PLGA and ITA/PLGA–PEG–PLGA/ITA copolymers with variable LA/GA and PLGA/PEG ratios were consequently evaluated in terms of sol-gel transition measurement to find whether and how the ITA modification affected the gelation temperature. Phase diagrams of these polymers in water were recorded in detail[LV-4]. Results indicated that the critical gel concentration (CGC) of PLGA–PEG–PLGA triblock copolymer-water systems is mainly determined by the LA/GA ratio while the critical gel temperature (CGT) is more affected by changing the PLGA/PEG ratio. All itaconic acid functionalized copolymers displayed better sol-gel characteristics then original unmodified copolymers by approximating CGT to body temperature. The best gelation behaviors exhibited ITA/PLGA–PEG–PLGA/ITA copolymer with PLGA/PEG weight ratio of 2 and LA/GA molar ratio equal to 3 having CGC = 6 wt % and CGT = 36 °C.

In order to study the gel stiffness (as a storage modulus G’) the visco-elastic properties of both copolymers were evaluated by rheology (Figure 2). The gel stiffness (G’max) increased with the polymer concentration moving the gel point of copolymer to the lower temperature. ITA functionalization improved both the gel stiffness and sol-gel characteristics of original PLGA–PEG–PLGA copolymer by approaching gel phase to 37 °C. Water solutions of this functionalized copolymer at concentrations higher than 6 wt% might be a suitable material for biomedical applications as injectable temporary implants.

[LV-4] MICHLOVSKÁ, L.; VOJTOVÁ, L.; MRAVCOVÁ, L.; JANČÁŘ, J.: *Thermogelling water solutions of multifunctional macromonomers based on PLGA-PEG-PLGA triblock copolymers*. Challenges of modern technology, 2011, 2(1), 12-15.

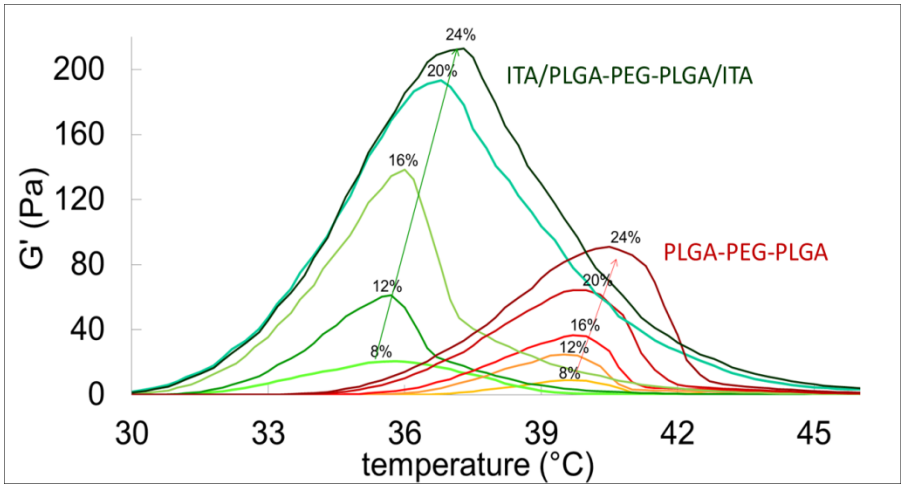


Figure 2 Dependence of storage modulus G' on the temperature measured at different PLGA–PEG–PLGA and ITA/PLGA–PEG–PLGA/ITA concentration in water.

End-linked biodegradable hydrogels

Followed Czech utility[LV-5] granted in 2014 relates to the preparation of chemically crosslinked hydrogels from ITA/PLGA–PEG–PLGA/ITA without cross-linker by blue light. The effect of amount of bonded itaconic acid on the cross-linking efficiency and of the blue light exposition time on the swelling and hydrolytic stability of resulted end-linked hydrogels was investigated. It was found that hydrogels were able to increase water absorption from 717 to 3581 % by increasing the amount of bonded ITA from 37 to 63 mol% and by prolonging the crosslinking time from 5 to 40 minutes (see photos in Figure 3). Chemically crosslinked samples were stable in water at room temperature up to 32 days (63 mol% ITA, 40 min blue light exposition).

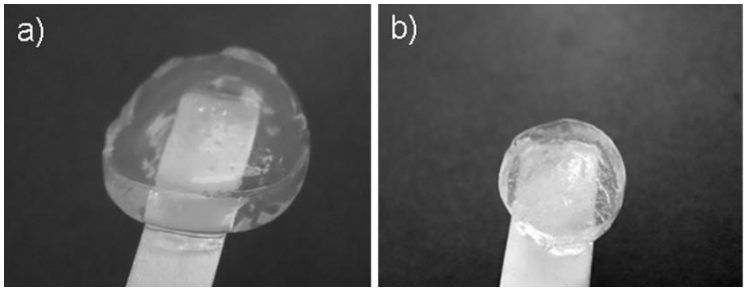


Figure 3 Crosslinked samples a) hydrogels with 37 mol% of ITA and b) hydrogels with 63 mol% of ITA in swelled state at 11th day.

Structural changes, loss of double bonds and formation of new RRC–CHR bonds were determined by attenuated total reflection infrared spectroscopy (ATR-FTIR). Transformation of double bonds to the single bonds was most evident at samples crosslinked for 40 minutes. Also thermal stability of samples was improved by an average of 21 °C.

In conclusion, chemical crosslinking of biodegradable triblock PLGA–PEG–PLGA copolymers modified by itaconic anhydride have positive influence on the swelling behavior and hydrolytic stability. Prepared chemically crosslinked samples will be able to found potential use in medical applications e.g. for wet coverage of burns or as drugs and cells carriers in tissue engineering of bone and cartilage.

[LV-5] VOJTOVÁ, L.; MICHLOVSKÁ, L.; OBORNÁ, J.; JANČÁŘ, J., VÁVROVÁ, M.: Biodegradabilní hydrogely s řízenou dobou života. Czech Intellectual Property Prague, PUV 2014-29121, 2014-03-03. CZ utility.

2.2 DEGRADABLE THERMOSETS FOR INDUSTRIAL APPLICATIONS

Novel thermo- and bio- degradable polyurethanes for furniture or automotive industry synthesized via addition polymerization and modified either by specially synthesized thermo-labile azo-compound or by natural cellulose or starch derivatives, were published in 7 followed papers[LV-6 – 12].

2.2.1 Biodegradable polyurethanes

PU foams are very common waste occurring in non-biotic components of the environment such as water and soil. Due to the recycling complications of PU thermoset materials they are commonly discarded after being used representing serious contamination problems due to their difficult disintegration and incorporation to the environment. Because of the high quantities of PU produced worldwide, whose main part consists of resistant polyether PU, waste PU materials represent a serious environmental problem. For instance, the half-life of polyether PU foam in the environment was predicted to be 400 years[3]. Enhancement of the biodegradability of polyether PU materials is a strategy which could help reduce this environmental problem, in agreement with the recent trends in the management of residual municipal solid waste[4]. Preparation of biodegradable PU foams (BIO-PU) including natural materials seems to be advantageous in order to avoid environmental contamination. PU filled with natural polymers having active –OH groups suitable for urethane linkages and ensuring good biodegradability may be used as –OH providers to modify the PU properties and structure.

Starch and cellulose modified polyurethane foams

In 2007 we reported on the modification of PU foams by bio-polyols based on cellulose or starch derivatives[LV-6], where the PU biodegradability was evaluated by using thermophilic bacteria and the samples were additionally ecotoxicologically tested[LV-7]. Flexible BIO-PU foams were prepared by one-shot process using commercially available co-reactants and modified by substituting biodegradable additive based on the acetylated starch (AS), acetylcellulose (AC), 2-hydroxyethylcellulose (HEC) and carboxymethylcellulose sodium salt (CMC) for 5 or 10 wt% of commercial polyether polyol. The BIO-PU foams were characterized by ATR-FTIR, TGA and light microscopy. Ecotoxicity of BIO-PU foams freshwater extracts was evaluated using alternative crustacean toxicity test Thamnotoxkit[FTM]. Values of toxicity were expressed as percentage mortality of the instars II--III larvae of freshwater fairy shrimps *Thamnocephalus platyurus* dependence on the effect criterion of the respective assay. The leaches of BIO-PU foams modified with 5 or 10 wt% of HEC showed higher toxicity then other BIO-PU foams, whereas leaches of BIO-PU with 5wt% of AS and 10wt% of AC were even less toxic than REF. PU foam.

The biodegradation of flexible biopolymer PU foams modified with HEC, AS and AC we have evaluated by thermophilic bacteria *Thermophillus sp*. and yeast strain *Aureobasidium pullulans* that produce a large amount of hydrolytic enzymes capable of degrading polymeric materials. Each week 0.5 g of lactose was added into cultivation medium and in regular intervals the concentration of biomass was determined (g of cells/l of medium). After cultivation, surface light microscopy of polyurethanes was tested (Figure 4).

[LV-6] VOJTOVÁ, L., VÁVROVÁ, M., BEDNAŘÍK, K., ŠUCMAN, E., DAVID, J., JANČÁŘ, J.: Preparation and ecotoxicity assessment of new biodegradable polyurethane foams. Journal of environmental science and health - part a toxic/hazardous substances and environmental engineering. 2007, 42 (5), 677-683.

[LV-7] DAVID, J., VOJTOVÁ, L., BEDNAŘÍK, K., KUČERÍK, J., VÁVROVÁ, M., JANČÁŘ, J.: Development of novel environmental friendly polyurethane flaks. Environmental chemistry letters. 2010, 8 (4), 381-385.

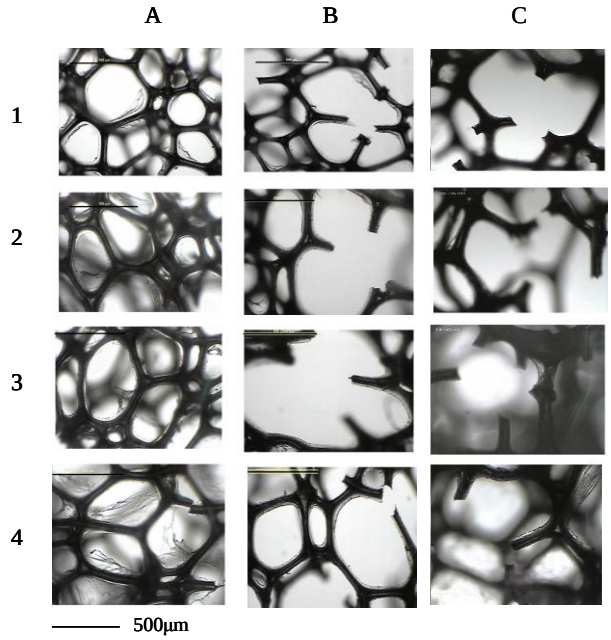


Figure 4 Light micrographs of BIO-PU foams (1) ref. PUR, (2) 10% HEC, (3) 10% AS, (4) 10%AC before biodegradation (A) and after biodegradation by *Thermophillus sp.* (B) and *Aureobasidium pullulans* (C).

The highest degree of the degradation was found in BIO-PU modified by 10% of acetylated starch (AS) with about four fold lower biomass in comparison with the reference culture (without polyurethane). High degree of biodegradation exhibited also the sample modified by 10% of 2-hydroxyethyl cellulose (HEC). On the other hand, the BIO-PU modified by 10% of acetylcellulose (AC) was seemed to be the most stable structure showing minimal growth changes. We presupposed that the biodegradation of PU foam modified by AC bio-polyol insoluble in common polyether polyol proceeded from „outside" the polymer determining very slow progress, while the biodegradation of BIO-PU foams modified by soluble AS and HEC proceeded from „inside" the PU network implicating the higher rate of the degradation.

Polyhydroxybutyrate modified PU elastomers

In this paper[LV-8], new elastomeric BIO-PUs having specific mechanical as well as biological properties with a built-in novel polyhydroxybutyrate (PHB) filler were prepared by a one-shot solvent-free process. Commercial non-degradable polyether polyol was partly substituted by biodegradable PHB powder also named as poly[(R)-3-hydroxybutyric acid], which belongs to a group of poly(hydroxyalcanoates) having both hydroxyl and carboxylic acid end groups. Particle-based PHB powder was chosen for two reasons: due to our experience with the incorporation of powder-like bio-polyols (e.g., cellulose or starch derivatives) into PUs' network and secondly, and more interestingly, due to PHB biosynthetic pathways investigated by our colleagues[5]. PHB is with advantage produced by microorganisms (*Alcaligenes eutrophus, Bacillus megaterium*) having physico-mechanical properties comparable to those of synthetic polypropylene (e.g., tensile strength of PHB of about 40 MPa). However, PHB is readily environmentally degradable while the products of its decomposition are CO_2 and H_2O[6]. Therefore, PHB can be used as commodity plastics or in specialty polymers for medical applications.

In the proposed work, commercial non-degradable polyether polyol (PEP) derived from petrochemical feed stocks was partly (in the amount of 1, 5 and 10 wt % marked as PHB1, PHB5

[LV-8] VOJTOVÁ, L., KUPKA, V., ŽÍDEK, J., WASSERBAUER, J., SEDLÁČEK, P., JANČÁŘ, J.: Biodegradable polyhydroxybutyrate as a polyol for elastomeric polyurethanes. Chemical papers. 2012, 66(9), 869-874.

and PHB10, respectively) substituted by the biodegradable PHB (see photos in Figure 5A). Due to the insolubility of PHB in PU compounds, the PHB additive makes BIO-PUs samples more white starting from the transparent reference (REF) sample.

Morphology of elastomeric PU composites was evaluated by scanning electron microscopy and mechanical properties of the prepared samples were obtained by both tensile measurements and prediction via the Mooney–Rivlin equation. Electron microscopy proved that the prepared materials have the character of a particle filled composite material, where PHB particles are regular with their size of about 1–2 μm in diameter (Figure 5B).

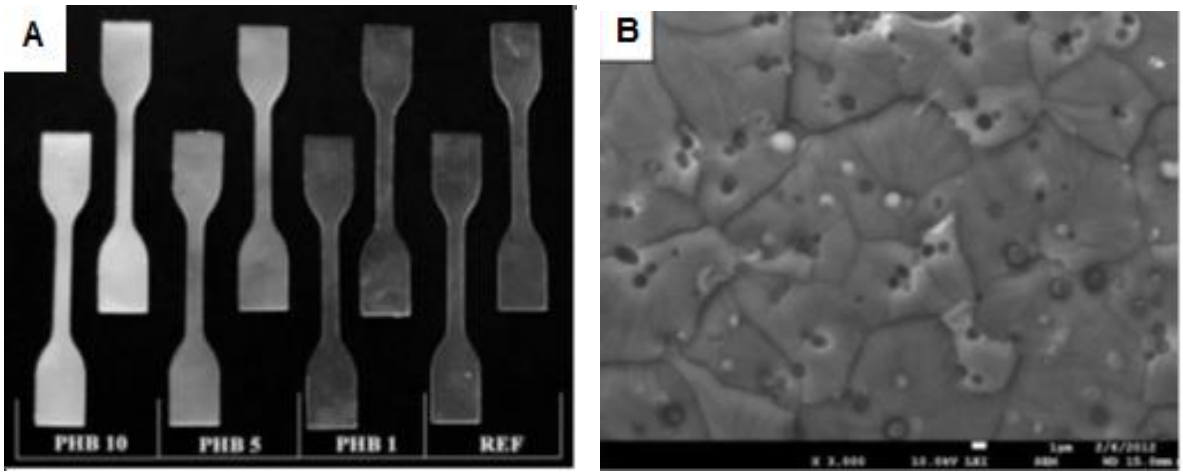


Figure 5 Prepared samples of PU (REF) and bio-PU (PHB1, PHB5, and PHB10) elastomers (A), SEM pictures of PHB10 sample fracture broken in liquid nitrogen.

Tensile measurements demonstrated that the Young's modulus (Figure 6), tensile stress at break, and tensile strain at break of each sample increase with enhancing the increase of the volume fraction of the filler.

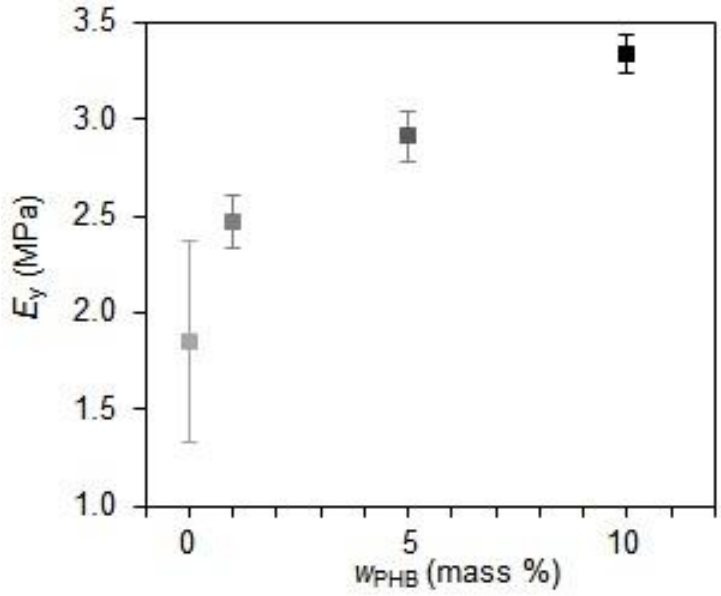


Figure 6 Young's modulus of prepared PU specimens.

From the measured stress-strain data, the first and the second term of the Mooney–Rivlin equation were calculated. The obtained constants were applied to recalculate the stress-strain

curves. It was found that the Mooney–Rivlin equation corresponds well with the stress-strain behavior of the prepared specimens (Figure 7).

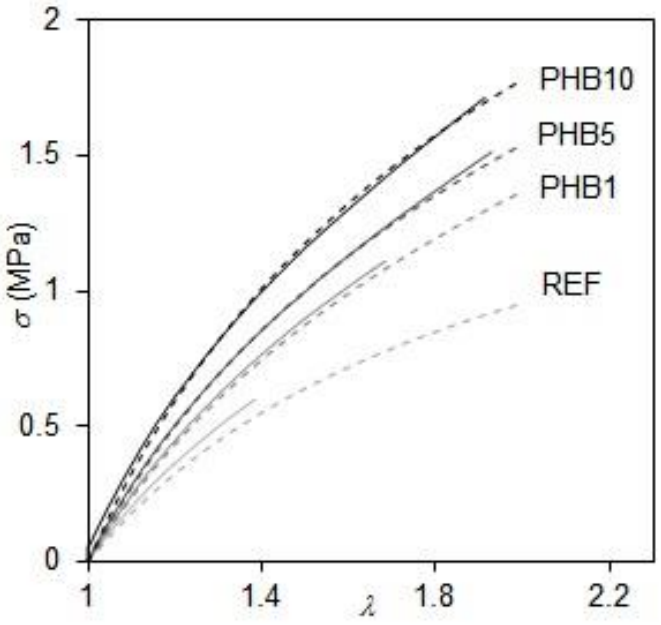


Figure 7 Comparison of measured stress–strain curves for REF PU and bio-PU filled with PHB (full lines) and stress–strain curves calculated by the Mooney–Rivlin equation (dashed lines).

The proposed work shows that the PHB biodegradable filler, replacing a part of the polyol component, can improve mechanical properties of the final product and possibly enhance the biodegradability of commercial PU. Biodegradation study of new PHB filled bio-PU elastomers is under investigation.

Starch and cellulose modified polyurethane elastomers

In the followed paper[LV-9], new elastomeric PUs with specific mechanical as well as biological properties were prepared using the bio-polyols based on cellulose or starch derivatives. AS, AC, CMC and HEC were used for substitution of common PEP in PU matrix in an amount from 1, 5 and 10 wt % (see Figure 8).

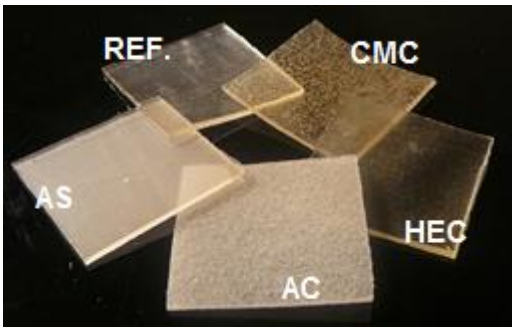


Figure 8 Photos of PU matrix (REF.) filled with CMC, HEC, AC and AS.

Based on the SEM pictures of PU specimens it was evident that all added fillers were not dissolved in polyol and prepared samples have character of particle-reinforced composite materials. The best adhesions show both AC and AS even if their particle sizes and shapes are

[LV-9] VOJTOVÁ, L., NOVOTNÝ, M., OBRUČA, S., PIECHOVÁ, J., MÁROVÁ, I., & JANČÁŘ, J.: Polysaccharides modified elastomeric polyurethane. Chemické Listy, 2008, 102, 1279–1281.

different. However, the morphology does not say anything about the mechanical properties of resulted polymer composite materials, which were evaluated by tensile measurement.

Based on the stress-strain curves all prepared specimens behaved like tough materials without the yield point. Reaching the elasticity limit was followed up by a long extension ended with the break. Better elongation at break then reference PU sample embodied only specimens having 1 and 5 wt.% of CMC and all PU elastomers filed with hyperbranched AS polysaccharide. Figure 9 shows that the addition of the bio-filler increased the Young's modulus in all cases except the specimens filled with 1 wt. % of HEC, AC and AS, which should not be taken into deep account due to the relatively high standard deviation of Ey.

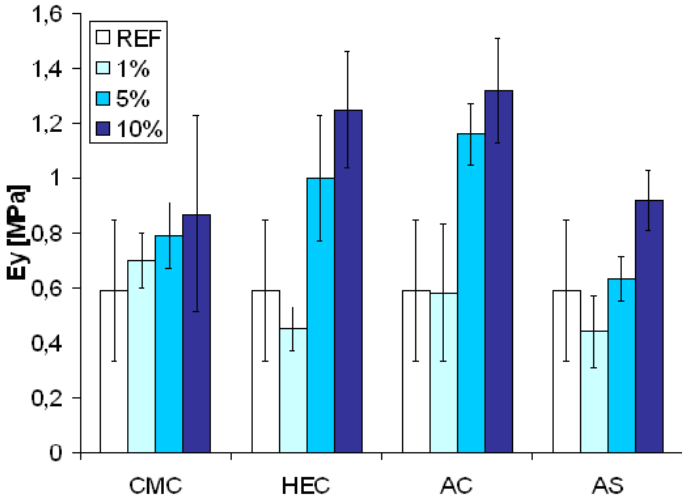


Figure 9 Young's moduli of prepared specimens.

Moreover, the biodegradability was evaluated by thermophilic bacteria[LV-10]. We observed that simple replacement of 10 wt% of PEP with biopolymer, which is of renewable origin, results in a significantly reduced lag phase of the bacterial growth dependent on the type of modifying agent. It can be expected that the toxic effect of PU material is reduced. So, the partial substitution of PEP by biopolymers not only enhances its biodegradability but, moreover, also reduces its toxicity. Nevertheless, the chemical substance responsible for the unusual lag phase should be identified in order to understand the mechanism of its formation and its impact on bacterial cells.

2.2.2 Thermodegradable polyurethane foams

For the development of novel thermodegradable polyurethane foams we were recently granted by European[LV-11] and Czech patent[LV-12]. The proposed work was focused on the investigation of a new life-time controlled thermodegradable flexible polyurethane (TD-PU) foams that maintain useful structural properties at working conditions (up to 160 °C) but deliberately degraded in a controlled fashion by heating at predefined temperature (at around 180 °C). To control and enhance the thermodegradation of common PU foams at the temperatures lower than 240 °C (the initial degradation temperature of the urethane bonds) modification of the polymer network

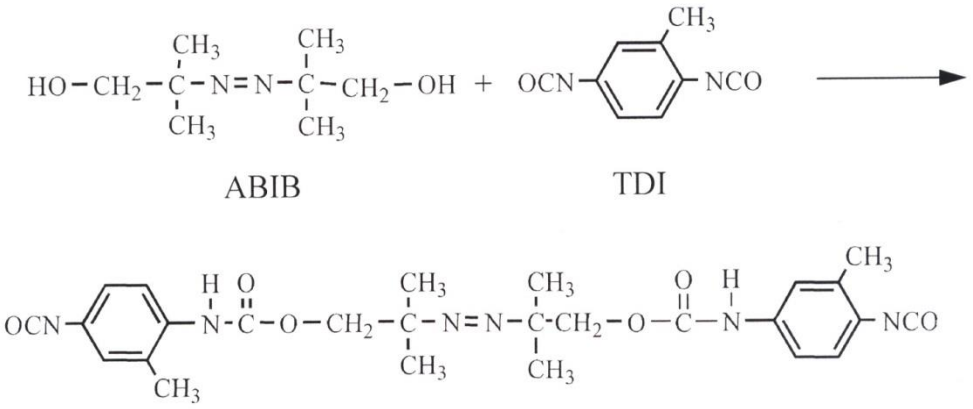
[LV-10] OBRUCA, S., MAROVA, I., VOJTOVA, L.: Biodegradation of polyether-polyol-based polyurethane elastomeric films: influence of partial replacement of polyether polyol by biopolymers of renewable origin. *Environmental Technology*, 2011, 32, 1043–1052.

[LV-11] VOJTOVÁ, L.; JANČÁŘ, J.: Method of preparation of thermodegradable polyurethane foams., Appl. VUT v Brne, European Patent Office, Germany, EP 2370487 (B1), 2013-03-06. EU patent.

[LV-12] VOJTOVÁ, L.; JANČÁŘ, J.: Způsob přípravy termodegradabilních polyurethanových pěn. Appl. VUT v Brne, Czech Intellectual Property Prague, CZ303835 (B6), 2013-22-05. CZ patent.

preferably by a thermosensitive prodegradant is required. The most commonly used thermal initiator for radical polymerization is azobisisobutyronitrile (AIBN), because its decomposition rate is not dependent on the medium and therefore it is exactly known. Unfortunately, the AIBN decomposition rate at the temperatures above 80 °C is too fast for decomposition kinetic experiments. Moreover, some polymers have to be thermally stable up to 160 °C (including also polyurethanes), that is why already in 1967 Mortimer[7] synthesized azobisisobutanol (ABIB) as a compound providing free radicals applicable for kinetic studies at the temperatures in the range of 150 to 200 °C. At these temperatures, ABIB undergoes a homolytic bond cleavage by disproportionation to free tertiary radicals, which do not recombine, and its decomposition rate follows first-order kinetics.

In the proposed work, synthesized ABIB was used as the thermoprodegradant of flexible PU foam at around 180 °C in order to control the thermodegradation of modified TD-PU foam at predefined temperature (Scheme 4).



Scheme 4 Azoisocyanate precursor synthesis by reacting of ABIB with toluenediisocyanate (TDI).

Both thermal stability and the thermodegradation kinetics of TD-PU foam modified with 1.6; 7.8; 12.6 and 18.3 wt% of ABIB were studied. The thermodegradation of the resulting TD-PU foams has been studied by the thermogravimetric analysis and differential scanning calorimetry.

Addition of the thermolabile ABIB in the form of azo-TDI precursor into the PU foam affected the thermal stability of the resulting thermodegradable PU foam significantly. It was found that the thermodegradable PU foam containing an incorporated azo-compound ABIB has the initial degradation temperature by about 62.3 °C lower (177.7 °C) in comparison with the original commercial foam (240.9 °C). In order to evaluate the degradation rate after incorporating ABIB to PU foams, the REF.PU sample together with TD-PU foam samples containing 7.8 and 18.3 % w/w of ABIB was subjected to thermodegradation at 200 °C for the period of 6 hours (isothermal mode). Results of individual degradation curves in Figure 23 show the direct dependence between the enhancing degradation rate of TD-PU and the increasing amount of thermolabile ABIB in TD-PU. The degradation rate constants (k) of each TD-PU foam sample in Figure 10 were calculated from 1/t1 in accordance with the equation (1) that fits the kinetic data:

$$y = A1.\exp(-kx/t1) + y0 \quad (1)$$

More than 3 fold enhancement of the thermodegradation rate (from 0.003 to 0.01 s^{-1}) was calculated for sample with 18.3 w% of ABIB. In this case, the weight loss after 6 hours at 200 °C increased almost three times from 6.8 % to 20.1 % in comparison with the unmodified PUR foam.

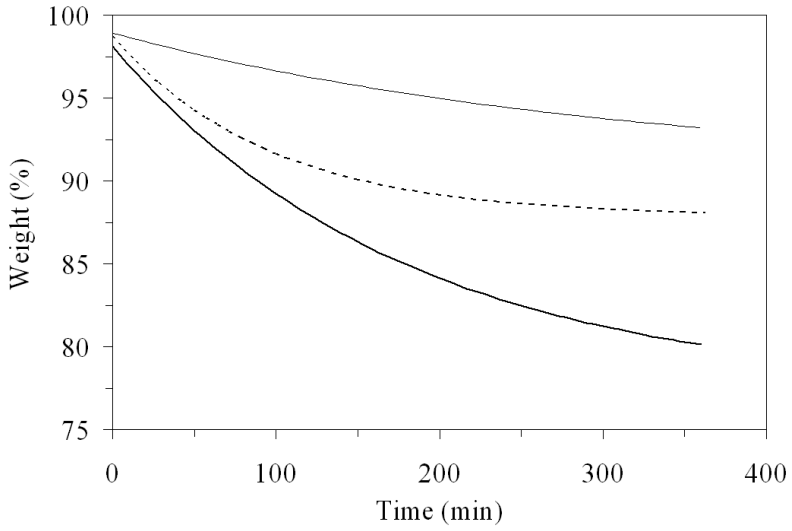


Figure 10 Thermodegradation of TD-PU at 200 °C: REF. PU (thin line), TD-PU with 7.8 % of ABIB (dashed line) and TD-PU with 18.3% and of ABIB (thick line).

The degradation of 3D-network is going along with molecular weight decrease, which might positively affect the solubility of the degradation products useful for recycling.

3 MODIFIED NATURAL POLYMERS

Unique biodegradable scaffolds for tissue engineering based on collagen and other natural polymers and fillers are described in followed 8 papers[LV-13-20]. Materials used in tissue engineering as scaffolds must be biocompatible, promote cell adhesion and growth[8]. Over time, as the cells produce their own matrix, the scaffold should degrade into nontoxic components that can be eliminated from the body[9]. Several scaffolds have been developed by our group and tested for tissue engineering. Scaffolds vary with respect to material chemistry (e.g. collagen, or synthetic materials), geometry (e.g. fibrous meshes, porous sponges), structure (e.g. porosity, pore size, pore distribution, orientation and connectivity), mechanical properties (e.g. tension, compression, resistant to shear and permeability) and the sensitivity to and rate of degradation[10].

The most used material is collagen due to its non-antigenetic, non-toxic, hemostatic, biocompatible, biodegradable and bioresorbable properties. In order to increase collagen's mechanical properties and hydrolytical stability especially when used *in vivo*, combination with other biomaterials or with cross-linking agents is needful. The most used ingredients are hyaluronic acid, chitosan[11], hydroxyapatite or β -tricalcium phosphate[12] and demineralized bone powder[13].

3.1 POROUS COLLAGEN SCAFFOLDS FOR TISSUE ENGINEERING

In 2008[LV-13] we firstly published preparation and characterization of collagenous scaffolds with interconnected pores via lyophilization of frozen hydrogels (Figure 11B) modified with chitosan nano-fibers, hyaluronic acid, PLGA–PEG–PLGA copolymers and hydroxyapatite nano-particles. The modified collagen compositions were cross-linked using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) with N-Hydroxysuccimide (NHS) system in a water solution.

[LV-13] SLOVIKOVÁ, A., VOJTOVÁ, L., JANČÁŘ, J.: Preparation and modification of collagen-based porous scaffold for tissue engineering. Chem Pap. 2008, 62 (4), 417-422.

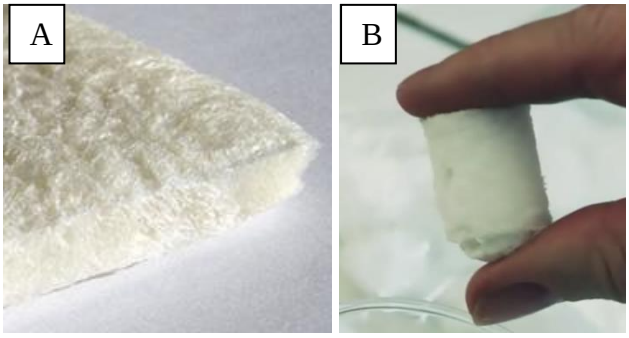


Figure 11 Pure lyophilized 100% collagen (A) and collagen scaffold (B)

Basic physico-chemical and mechanical properties were measured and an attempt was made to relate these properties to the molecular and supermolecular structure of the modified collagen compositions (see morphology of selected scaffold in Figure 12).

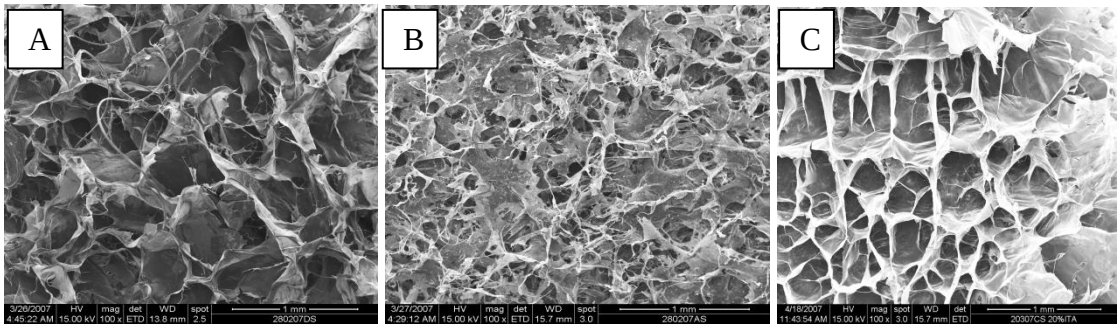


Figure 12 Morphology of cross-linked collagen sponges modified with 20 wt% of additive: chitosan (A), hydroxyapatite (B) and PLGA–PEG–PLGA (C) observed by SEM.

The fastest degradation rate was observed for synthetic copolymer containing scaffolds, which are generally soluble in water. The results showed that the addition of hydroxyapatite or hyaluronic acid to the collagen matrix increases the rigidity in comparison with collagen/chitosan scaffold. Collagen scaffold modified with hyaluronic acid reduced the deformation at break, while hydroxyapatite enhanced the deformation under tensile loading. The tensile elastic modulus of chitosan nanofibers collagen scaffold was the lowest but closest to articular cartilage, on the other hand, the strength and deformation to failure increased up to 200 %.

As conclusion, all scaffolds prepared in this work might be suitable for tissue engineering. The collagen sponges involving chitosan fibers could be used as scaffolds for growing cells in systems with low mechanical loading, on the other hand, collagen-hydroxyapatite scaffolds might be suitable for systems which require higher mechanical resistant. The collagen-copolymers sponges showed partly hydrophobic character and high porosity thus they could be used as scaffolds witch involve antibiotics solute in hydrophobic solutions. The hyaluronic acid based scaffolds did not show unique properties in that case, but its healing or lubricant properties have not been measured yet.

3.1.1 Hydroxyapatite modified collagen scaffolds for bone regeneration

Since we have got preliminary results how to prepare functional scaffolds with controllable pore size, we were focused more on the effect of scaffold composition and pore size on the *in vitro* and *in vivo* testing related to bone tissue regeneration. A promising novel approach seems to be bone tissue engineering based on combination of suitable materials for scaffold with autologous mesenchymal stem cells (MSCs) and/or signaling molecules (growth factors) (Figure 13)[14].

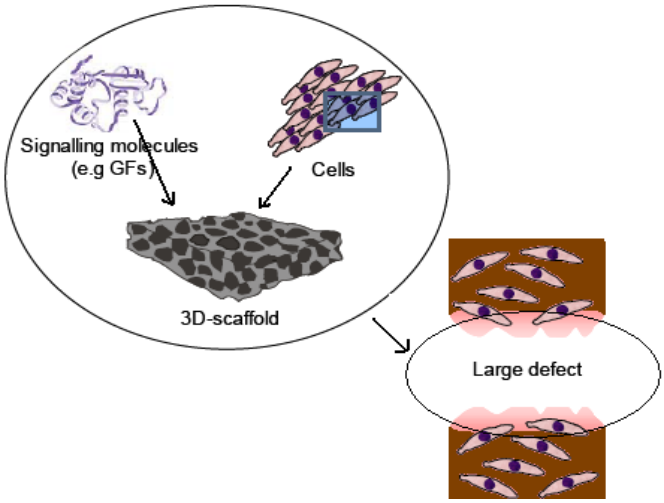


Figure 13 Cells and growth factors are seeded on 3D-scaffold and then incorporated into the faulted tissue area.

Our study[LV-14] confirmed that a higher collagen content (concentration of collagen solution raised from 0.5 up to 2 wt%) increased scaffold stiffness (Figure 14) but that a greater stiffness was not sufficient for bone tissue formation, a complex process is evidently also dependent on scaffold porosity. The mechanical stiffness of the composite scaffold increased with the amount of collagen and decreased with the pore size. On the other hand, the minimum pore size for optimal cell proliferation was determined to be 400 μm (Figure 15). Thus, the optimal collagen/HA mixture to induce the osteogenic differentiation of MSCs was found to be between 40 – 50% HA with 0.5% collagen (Col 0.5/HA 40 and Col 0.5/HA 50).

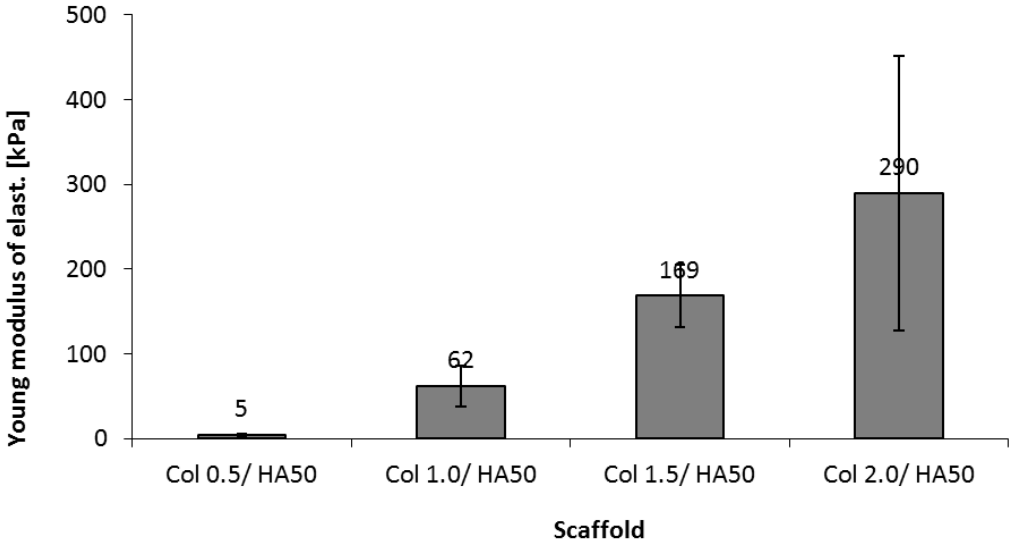


Figure 14 Young modulus of elasticity dependence on the concentration of collagen in the collagen/HA scaffold

[LV-14] PROSECKA, E; RAMPICHOVA, M; VOJTOVA, TVRDIK, D; MELCAKOVA, S; JUHASOVA, J; PLENCNER, M; JAKUBOVA, R; JANCAR, J; NECAS, A; KOCHOVA, KLEPACEK, J; TONAR, Z; AMLER, E: Optimized conditions for mesenchymal stem cells to differentiate into osteoblasts on a collagen/hydroxyapatite matrix. J Biomed Mat Res, Part A. 2011, 99(A), 2, 307-315.

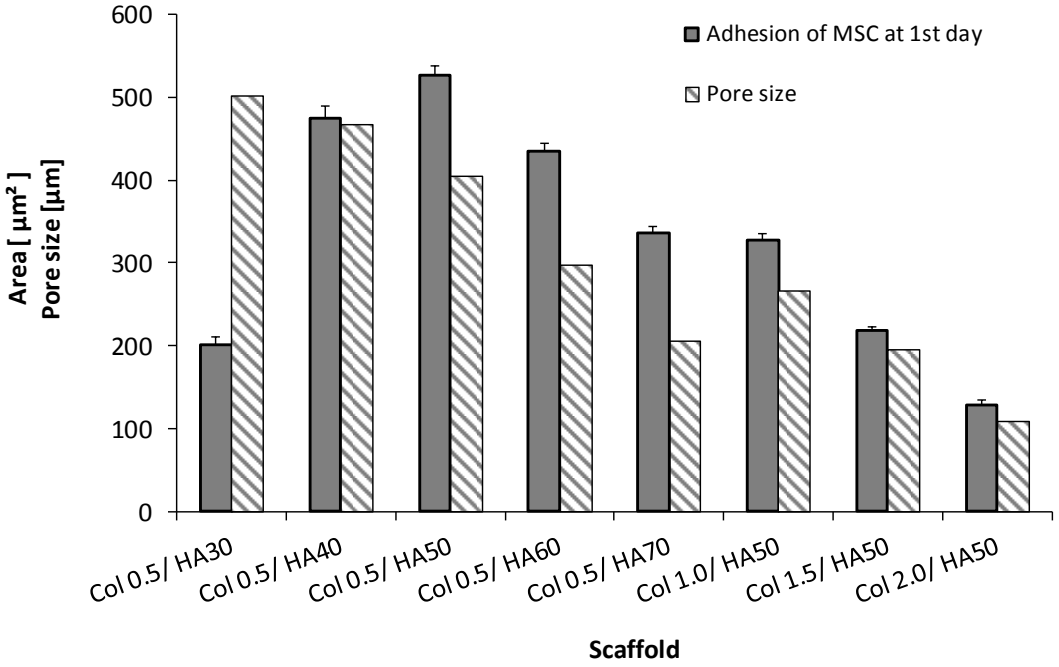


Figure 15 Dependence of MSCs adhesion area and pore size on the collagen/HA composition.

After these *in vitro* tests the selected best Col 0.5/HA 50 scaffold was used for *in viv*o testing on minipigs[15,LV-15,LV-16]. This study evaluates the healing of segmental femoral defects in miniature pigs (Figure 16A and B) based on the radiological determination of the callus: cortex ratio at 16 weeks after ostectomy (Figure 16C).

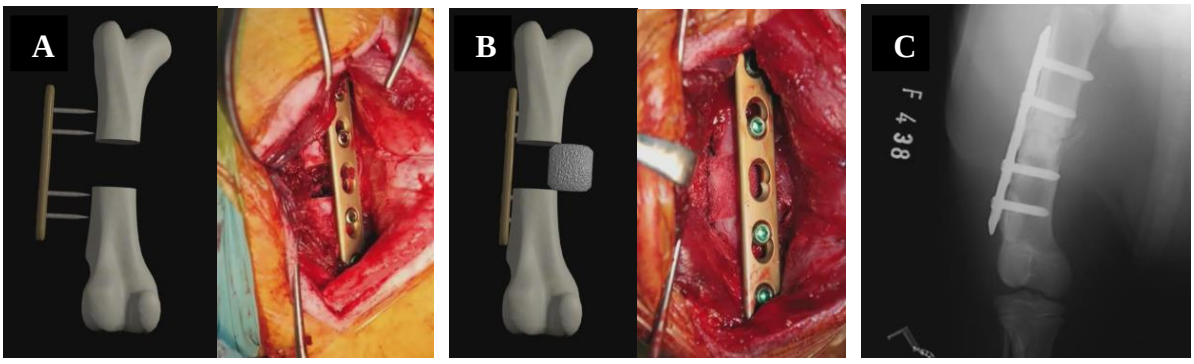


Figure 16 Animation and real photos of segmental femoral defect fixed with LCP plate in a miniature pig (A) transplantation of biocompatible scaffold seeded with MSCs into the defect (B) X-ray of newly formed bone in 16 weeks after transplantation (C).

Implanted cylindrical scaffolds support callus formation directly in the center of original bone column in segmental femoral ostectomy, and can be successfully used in the treatment of large bone defects.

[LV-15] NEČAS, A., PROKS, P., URBANOVÁ, L., SRNEC, R., STEHLÍK, L., CRHA, M., RAUŠER, P., PLÁNKA, L., AMLER, E., VOJTOVÁ, L., JANČÁŘ, J.: Radiographic assessment of implant failures of titanium 3.5 LCP vs. 4.5 LCP used for flexible bridging osteosynthesis of large segmental femoral diaphyseal defects in a miniature pig model. Acta Veterinaria Brno. 2010, 79 (4), 599-606.

[LV-16] NEČAS, A., PROKS, P., URBANOVÁ, L., SRNEC, R., STEHLÍK, L., CRHA, M., RAUŠER, P., PLÁNKA, L., JANOVEC, J., DVOŘÁK, M., AMLER, E., VOJTOVÁ, L., JANČÁŘ, J.: Healing of large segmental bone defect after implantation of autogenous cancellous bone graft in comparison to hydroxyapatite and 0.5% collagen scaffold combined with mesenchymal stem cells. Acta Veterinaria Brno. 2010, 79 (4), 607-612.

Morphology by micro-computed tomography

In addition to the chemical composition of the biopolymer scaffold based on modified collagen, the scaffold morphology is a very important characteristic of the 3D porous structure playing a significant role in tissue regeneration. The morphology of the porous scaffold is commonly observed using scanning electron microscopy (SEM), unfortunately, the image of SEM (Figure 17A) is only two-dimensional and does not determine the morphology of the entire volume of the sample, but only a small part of the cut. In addition, the sample must be adjusted prior to observation (cut and coated). For these reasons, we newly applied analysis using X-ray micro-computed tomography (μCT), when the sample of scaffold is nondestructively evaluated throughout its volume. Figure 17B shows the 3D image of the same μCT of collagen/HA scaffold, frame C is only a perpendicular cut. Bright maps show the collagen fibrils coated by microparticles of HA agglomerates, the dark parts represent pores.

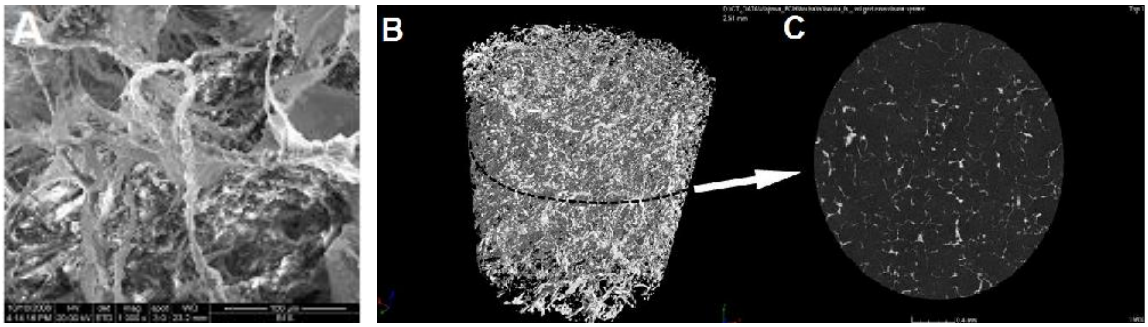


Figure 17 SEM of collagen scaffold modified by micro-hydroxyapatite (50/50 w/w) (A), μCT of the entire 3D collagen/HA scaffold (B) and perpendicular scaffold cut (C)

Subsequently, the obtained three-dimensional map of pores by watershed segmentation method was derived from the binarized 3D scaffold structure (Figure 18).

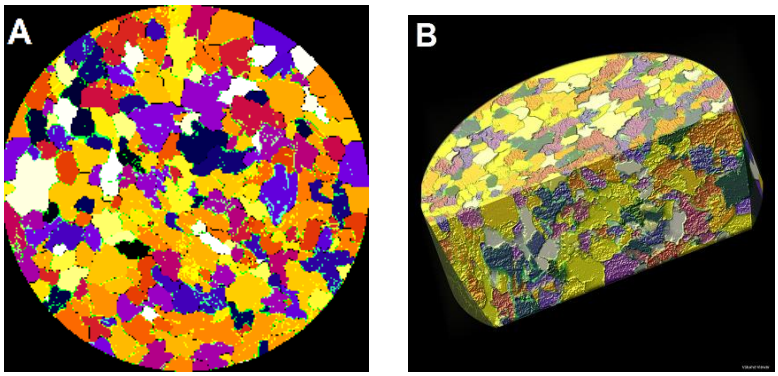


Figure 18 Cutted scaffold segmentation (A) and visualization of 3D scaffolds (B)

Subsequently, the calculated porosity (88%) and total pore interconnection with each other (48%, the remaining 52% of the pores do not affect the second pore walls but collagen). The pore size from μCT was determined by fully automated method (216 ± 86 μm). Moreover, by the fully automated method the average pore volume (8.106 ± 1.107 μm3) was calculated.

This new technology will help us to better understand the methods of seeding the scaffold by cells, the behavior of cells in the scaffold (proliferation and differentiation in different time intervals), or changes in the morphology of the scaffold after some time exposed to the cells, which is essential in the modern tissue engineering for tissue regeneration.

3.1.2 Polycaprolactone nanofiber modified collagen scaffolds

Recently, cell-based therapy studies demonstrated that Platelet Rich Plasma (PRP), which contains a cocktail of multiple growth factors (GFs) stimulate Mesenchymal Stem Cels (MSCs) in bone marrow to proliferate and differentiate[LV-17]. There is an increasing interest in a possibility of using bone-marrow stimulation based on scaffolds allowing precise spatial and time release of growth factors and other bioactive substances. The use of porous bionanocomposite scaffolds with engineered pore size and distribution based on biodegradable polymer and inorganic nanofiller allowing controlled release of plasma-rich solution seems to provide a viable alternative to MSCs containing scaffolds. In a recently submitted review[LV-18], we critically described existing literature on the effects of the osteoinductive bionanocomposite scaffold preparation technique, morphology and composition on cell proliferation and differentiation in both *in vitro* and *in vivo* bone tissue engineering procedures using either MSCs and/or blood derivatives.

The 3D scaffold from type I Collagen and Hydroxyapatite (Coll/HA) enriched with polycaprolactone nanofibres (Coll/HA/PCL), autologous mesenchymal stem cells in osteogenic media (MSCs) and Trombocyte Rich Solution (TRS) has been found as an optimal implant for bone regeneration in vivo tested on white rabbits (Figure 19)[LV-17].

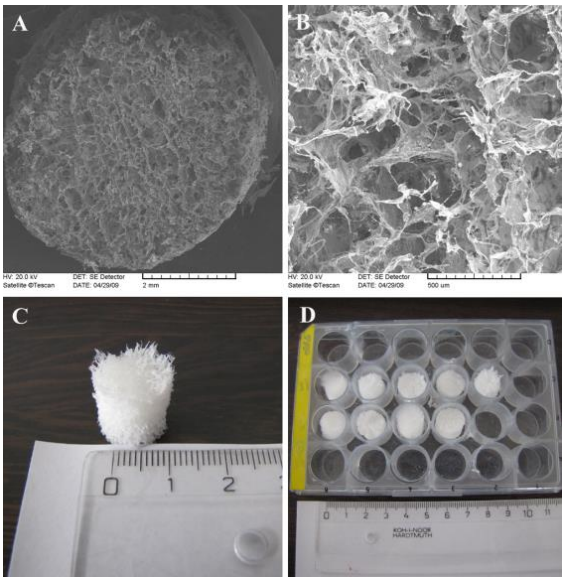


Figure 19 Morphology of a coll/HA scaffold enriched with PCL nanofibers (Coll/HA/PCL). Scaffold morphology was analyzed using scanning electron microscopy (A and B) and by macroscopic evaluation (C and D)

Nanofibers (made using Nanospider machine by electrospinning) optimized viscoelastic properties of Coll/HA scaffold for bone regeneration . Their size and shape can be fashioned to match those of cells and extracellular components. Moreover, the high surface area to volume ratio of nanofibers allows for good adsorption and high immobilization of cells.

Three groups of the Coll/HA/PCL scaffolds were prepared, namely the MSCs seeded scaffold, the TRS enriched scaffold and the scaffold enriched with both MSCs and TRS. These scaffolds

[LV-17] PROSECKÁ, E., RAMPICHOVÁ, M., LITVINEC, A., TONAR, Z., KRÁLÍČKOVÁ, M., VOJTOVÁ, L., KOCHOVÁ, P., PLENCNER, M., BUZGO, M., MÍČKOVÁ, A., JANČÁŘ, J., AMLER, E.: Collagen/hydroxyapatite scaffold enriched with polycaprolactone nanofibers, thrombocyte-rich solution and mesenchymal stem cells promotes regeneration in large bone defect in vivo. J Biomed Mat Res - Part A. 2014, (in press available on-line).

[LV-18] VOJTOVÁ, L., ABDEL-MOHSEN, A. M., BUZGO, M., PROSECKÁ, E., PLENCNER, M., RAMPICHOVÁ, M., AMLER, E., JANČÁŘ, J.: Effects of Composition and Preparation Protocol On the Osteoinductivity of Bionanocomposite Scaffolds for Bone Regeneration: A Review. Biotech Adv, 2014, 1-37. Submitted 2014-31-08.

were implanted into the rabbits femoral condyle defects sized 6 mm in diameter and 10 mm in depth. Non-treated defects were used as for control (Figure 20).

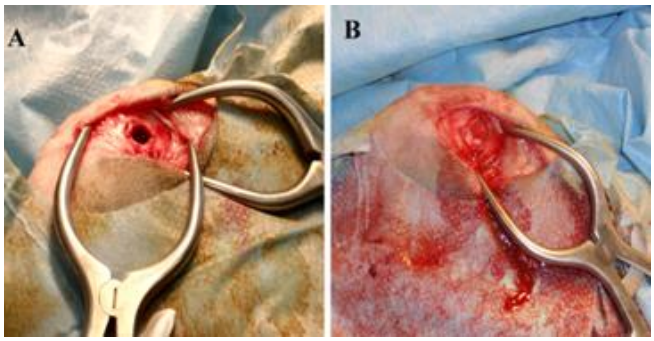


Figure 20 Surgical procedure. Critical size defects were made in femoral condyles using a 3.2-mm drill gradually expanded to obtain defects 6 mm in diameter and 10±5 mm deep (A) and filled with scaffold (B)

The macroscopic and histological analysis of the regenerated tissue from all groups was performed 12 weeks after the implantation. The highest volume and the most uniform distribution of newly formed bone were found in bone defects treated with scaffolds enriched with both MSCs and TRS when as compared with bone defects treated with scaffolds enriched by addition of alone TRS only or MSCs only. The moduli of elasticity under compressive test were significantly higher in the Coll/HA/PCL scaffold compared to the scaffold without nanofibers. Thus, this composite collagen scaffold enriched with PCL nanofibers, MSCs and TRS seems to be a novel tool for bone defect treatment (Figure 21). However, in order to replace MSCs due to the translational difficulty, TRS might be used alone. The volume of newly formed bone is comparable with the scaffold seeded only with MSCs and the standard deviation is even lower meaning that using only TRS exhibits better reproducibility in comparison with MSCs.

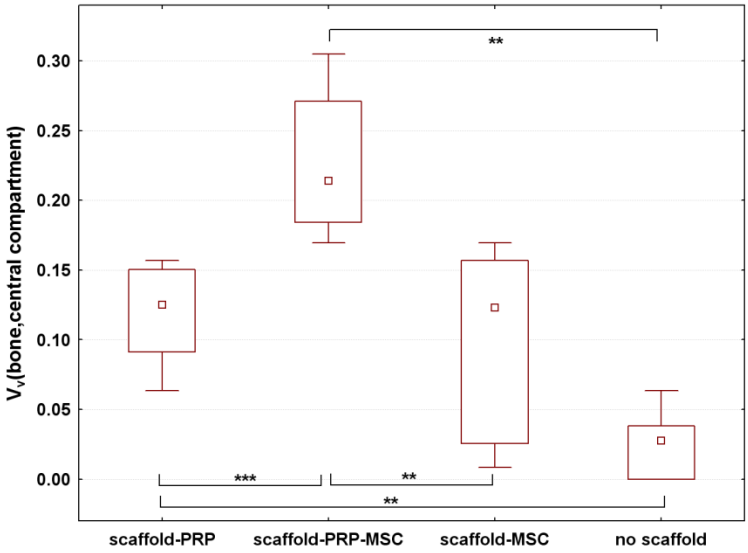


Figure 21 Bone quantity was expressed as volume fraction (V_v) of bone tissue within the central volume of the defect (A) or within the outer (B), middle (C), and central (D) compartments. Data are presented as medians.

In order to slow down rapid release of GFs from porous scaffold, it has been already confirmed, that thrombocytes immobilized on PCL nanofibers effectively enhanced chondrocyte proliferation due to time-dependent degradation of thrombocytes and release of their GFs[16]. So the next study is directed to the PRP immobilization in a porous PCL nanofibers to improve and well-control GFs release *in vivo*.

3.1.3 Chitosan microfiber modified collagen scaffolds for cartilage regeneration

Based on our previous research[LV-13], chitosan-modified collagenous scaffold was prepared for hyaline cartilage regeneration via tissue engineering. Chitosan alone has good mucoadhesive properties due to its positive charge, which increases the adhesion to mucosa and so the time of contact for drug penetration. Its hemostatic and antibacterial properties also limit the risk of infection[17]. Figure 22 shows morphology of both unmodified (A) and chitosan microfiber modified collagen scaffolds (B). The chitosan additive slightly increase the collagen scaffold porosity (78 vs. 69) while the pore size diameter was kept almost same (355 μm vs. 405 μm). Also the half-life time of collagen scaffold was increased by chitosan additive from approx. 6 days up to 8 days proving the incorporation of chitosan microfibers into the collagen network during the chemical cross-linking.

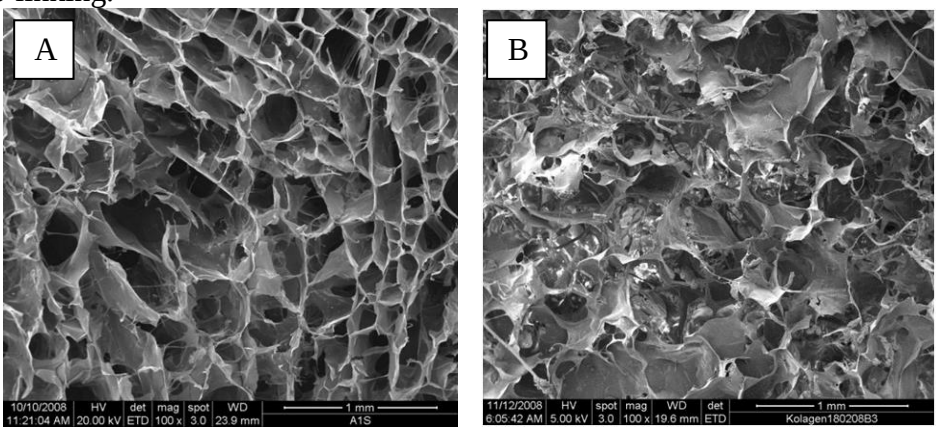


Figure 22 SEM pictures of unmodified collagen scaffold (A) and chitosan microfiber modified scaffold (B)

Iatrogenic defect of the surface of articular cartilage on femur condyles in miniature pigs were treated by transplantation of mesenchymal stem cells in a composite scaffold based on collagen I containing chitosan microfibers (Figure 23A)[LV-19]. Newly formed hyaline-like cartilage in 16 weeks after transplantation is shown in Figure 23B.

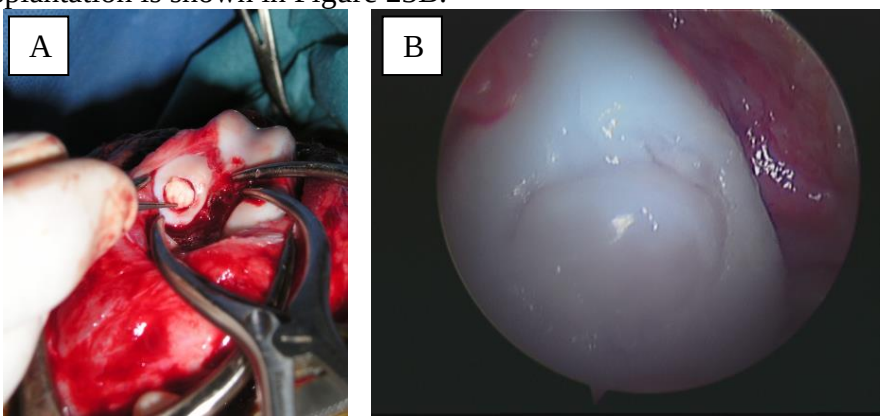


Figure 23 Real photos of scaffold seeded with MSCs transplantation into the iatrogenic lesion of articular cartilage on femur condyles in mini pigs (A) and newly formed hyaline-like cartilage in 16 weeks after transplantation (B)

Histological evaluation of the newly formed tissue is displayed in Figure 24. Iatrogenic lesions of the surface of articular cartilage treated by implantation of scaffold alone (A) or by micropicking (B) were filled with fibrous cartilage (between red arrows) whereas treatment of lesions by collagen/chitosan composite scaffold seeded with MSCs resulted in filling tissue

[LV-19] NEČAS, A., PLÁNKA, L., SRNEC, R., CRHA, M., HLUČILOVÁ, J., KLÍMA, J., STARÝ, D., KŘEN, L., AMLER, E., VOJTOVÁ, L., JANČÁŘ, J., GÁL, P.: Quality of newly formed cartilaginous tissue in defects of articular surface after transplantation of mesenchymal stem cells in a composite scaffold based on collagen I with chitosan micro- and nanofibres. Physiol Res. 2010, 59 (4), 605-614.

defects in a hyaline-like cartilage with inferior macroscopic, histological and immunohistochemical parameters[LV-20].

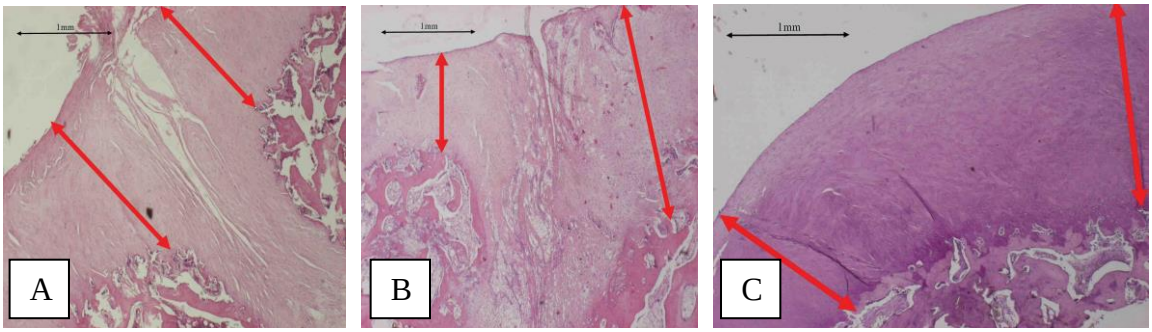


Figure 24 Articular cartilage defect of Group B healed with fibrous cartilage (arrows) after transplantation of scaffold without MSCs (A). Cartilage defect (arrows) of Group C healed with fibrous cartilage after micropicking (C) Hyaline-like cartilage of Group A in the healed articular surface defect (arrows) after transplantation of scaffold seeded with MSCs (C).

4 SUMMARY

The presented habilitation thesis deals with design, synthesis, functionalization and characterization of new advanced polymeric materials and composites directed mainly to applications in medicine and automotive industry. To understand the relation between the modified polymeric structure and related properties the materials were studied in terms of a wide range of physico-chemical as well as biological methods. The selected papers show how the required properties meeting specific applications can be tailored by a design and functionalization/modification of suitable polymeric materials.

To be pointed out, the controlled degradation or recycling of petrol-derived polymers after the end of their life-time has been dealt with by our group as a key factor of environmental protection. Moreover, we have shown how the functionalization by either functional groups or cross-linking can affect the hydrolytical degradation and mechanical properties of polymeric materials suitable for both drug delivery and tissue engineering of bones and cartilages.

To date, no single material in mucosceletal regeneration can suffice and that is the reason of the new materials and technologies development. A promising material seems to be hierarchical, multifunctionalized bionanocomposite prepared by the advanced self-assembly method to mimic the “bottom-up” process used by the nature.

[LV-20] PLÁNKA, L.; NEČAS, A.; CRHA, M.; PROKS, P.; VOJTOVÁ, L.; GÁL, P.: Léčba kostního můstku transplantací mezenchymových kmenových buněk a chondrocytů v kompozitním nosiči u prasat - experimentální studie. Acta chirurgie orthopaedicae et traumatologie Čechoslovaca, 2011, 78(6), 526-536.

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ABSTRACT

Submitted habilitation thesis summarizes the essential results of applicant activity selected from 20 scientific works included patents and utility model published from 2003 to 2014. After a brief introduction the first part is focused on the development of novel functionalized synthetic polymer materials, which are sensitive to light, temperature, or specific bacteria. Due to these new features their mechanical properties, biological activity or life-time can be controlled. These materials were characterized mainly by physico-chemical methods. Biological properties of selected materials were investigated in terms of ecotoxicity and biodegradation by thermophilic bacteria. The aim was to describe the effect of functionalization of these new materials on their end point with regard to targeted applications in medicine and industry.

Second part deals with the modification of natural polymers such as collagen as a suitable substrate for cell and growth factors incorporation useful in tissue engineering of bone and cartilage. The effect of the modifier compounds (inorganic nanoparticles, organic nano (micro) fibers, both natural and synthetic polymers) of prepared degradable implants on the physico-chemical properties, morphology, hydrolytical stability and bioactivity both *in vitro* and *in vivo* were investigated.

ABSTRAKT

Předložená habilitační práce shrnuje podstatné výsledky činnosti předkladatele vybrané z 20-ti vědeckých prací včetně patentů a užitného vzoru publikovaných od roku 2003 do roku 2014. Po krátkém úvodu se práce v první části zaměřuje na vývoj nových funkcionalizovaných syntetických polymerních materiálů, které jsou díky novým vlastnostem citlivé na světelné záření, teplotu nebo specifické bakterie a díky těmto novým vlastnostem lze řídit jejich mechanické vlastnosti, biologickou aktivitu nebo i dobu života. Tyto materiály byly charakterizovány převážně fyzikálně-chemickými metodami. Biologické vlastnosti vybraných materiálů byly zkoumány z hlediska ekotoxicity či biodegradace termofilními bakteriemi. Cílem bylo popsat vliv funkcionalizace těchto nových materiálů na jejich sledované vlastnosti s ohledem na cílenou aplikaci v medicíně či průmyslu.

Další část práce se zabývá modifikací přírodních polymerů, především kolagenu jako vhodného substrátu pro ukotvení buněk a růstových faktorů s využitím v tkáňovém inženýrství kostí a chrupavek. Byl sledován vliv modifikačních látek (anorganických nanočástic, organických nano(mikro) vláken, přírodních i syntetických polymerů) na fyzikálně-chemické vlastnosti, morfologii, hydrolytickou stabilitu a bioaktivitu jak *in vitro* tak i *in vivo* připravených degradabilních implantátů.