POROUS COMPOSITE MATERIALS WITH POLYAMIDE REINFORCEMENT AND SILOXANE MATRIX WITH NANO-HYDROXYAPATITE AS BIOMATERIALS

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ABSTRACT

Composite materials based on a polyamide fabric and a polydymethylsiloxane matrix were designed for application in bone surgery. In order to increase the bioactivity, 2, 5, 10, 15, 20 and 25 vol. % of nano/micro hydroxyapatite (HA) and tricalcium phosphate (TCP) were added. The effect of the additives on the mechanical properties was studied. Simultaneously, changes in the inner structure of the composites were investigated by means of image analysis. The effect of the additives on the biocompatibility was determined by both in vitro and in vivo tests. It appears that in comparison with the micro particles, the nano additives have a more favourable effect on mechanical properties. From the point of view of the final application of the composites as substitutes for hard tissues, 10 – 15 vol. % of nano additives is an optimum amount: in this case both the optimization of the toughness and the increase in the ultimate strength in bending occur without any changes in the inner structure of the composite. The results of biological evaluations are in an agreement with the results of mechanical tests.

KEYWORDS: aramid fibers, polymer-matrix composites, mechanical properties, matrix modifications, hydroxyapatite, tricalcium phosphate

1. INTRODUCTION

Artificial substitutes for parts of the human body based on a relatively broad spectrum of materials are investigated. Prostheses of bones made of metals and their alloys, ceramics, bioglass, polymers and various composites, in the form of both fibers and particles, always exhibit certain disadvantages. There is often high toughness, fragility, corrosion, low strength in bending, particle release and insufficient bioactivity (for the given requirements) (Mata, 2002; Rose, 2004; Bačáková, 2001; Suchanek, 1998; Springer, 2001; Kawai, 2004; Chavarria, 2004). Several polymers (for review see (Ramakrishna, 2004; Manikandan, 2001)) are considered as biocompatible and biostable materials within the body, and they are widely applied. However, their specific disadvantage is their low mechanical strength and above all their low Young modulus (Ramakrishna, 2004).

A successful product of tissue engineering must necessarily result from combining several disciplines dealing with mechanical properties, the interaction of the implant with the surrounding tissue, and also practical clinical experience. With composites consisting of a polymer reinforcement and a polymer matrix with the possibility of selecting the volume ratio of the fiber reinforcement to the matrix and also a suitable orientation, mechanical properties identical with those of human bone can be obtained (Ramakrishna, 2004). The reason for their wide use in various medical applications is mainly the availability of materials with various properties in various forms and compositions as well as the fact that they can be hardened directly into the required shape or structure with the most suitable fiber orientation, e.g., with respect to the direction of the acting load. Their biocompatibility and mechanical properties can also be enhanced by inserting a bioactive component into the matrix. Our study reported in (Suchý, 2007) dealt with preparing fiber composites based on an aliphatic or aromatic polyamide and on a polysiloxane as the matrix to which HA particles 20 – 70 nm in size were added. We also reported on their mechanical properties and moreover their biocompatibility.

Polyamide fabrics were chosen because of their mechanical stability and bioactivity. Polyamide monofilaments were used for constructing a non-resorbable, long-lasting and stress-absorbent reinforcement for designing articular disc substitutes (Springer, 2001). In Springer's study, polyamide also promoted the adhesion of human or porcine fibrocartilage cells in cultures derived from the temporomandibular joint, and showed no toxicity to these cells. In addition, poly(hexamethylene adipamide), i.e., a polyamide containing carboxyl and amide groups similar to collagen, was successfully used for preparing a biomimetic composite with nano-hydroxyapatite, matching well the mechanical properties of the natural bone (Wang, 2002).

Although siloxane materials are hydrophobic, they generally allowed the adhesion, growth and differentiation of osteoblasts. Their osteoinductive behavior was further enhanced when they were rendered hydrophilic by exposure to an oxygen plasma treatment or by microtexturing their surface (Mata, 2002; McFarland, 2000; Liao, 2003; Walboomers, 2004; Balík, 2007). Composites based on polymethylphenyl siloxane resins (produced by Lučební závody, Kolín, Czech Republic) promoted their colonization with human osteoblasts of the line hFOB 1.19 (Gumula, 2004). Another siloxane, i.e. 3-(glycidoxypropyl) trimethoxysiloxane, was used for constructing a bioactive composite with gelatin and Ca^{2+} ions, which stimulated the proliferation and differentiation of mouse osteogenic MC3T3-E1 in vitro. When these reinforcements were soaked in a simulated body fluid, apatite was formed by the reaction of a hydrated silica gel surface (Si-OH groups) and Ca^{2+} ions (Ren, 2002).

HA and TCP additives were chosen because they can mimic the crystalline mineral component of the bone. Inclusion of HA nanofibers in a beta-tricalcium phosphate (β-TCP) matrix significantly improved the mechanical properties of this material, especially its strength and toughness (Ramay, 2004). HA-containing materials act as sources of calcium ions, which are known to stimulate osteoblast proliferation and differentiation (Ren, 2002). In addition, hydroxyapatite crystals can serve for nanopatterning the pore walls in order to enhance the osteoinductive activity of our newly constructed materials, as mentioned above (Woo, 2003; Wei, 2004). However, HA by itself has an insufficient mechanical property, especially low mechanical strength and increased brittleness. It is mainly applied in the form of bone fillers of several shapes for unloaded implants and in the form of a coating material on metallic prostheses, dental or maxillofacial applications (Fazan, 2005). Application of HA as composite matrix additives should overcome these problems. The rate of the interaction between the body and the artificial particles depends on their microstructure, morphology and size (e.g. nano/micro size).

2. PROJECT OBJECTIVES

The aim of our study was to develop an advanced porous composite material with suitable mechanical properties for potential use in the bone tissue engineering, hierarchically organized at both micrometer and nanometer scale. The composite consist of polyamide fabrics embedded in polydymethylsiloxane matrix with HA/TCP micro- or nanocrystals. At the same time, the nanocrystals homogeneously dispersed within the matrix protrude on the pore walls and create a bioactive nanopattern on the wall surface. We were looking for a compromise between the optimum of amount fillers of the resulting composite and suitable mechanical properties. The aim of this study was to describe the mechanical behaviour and the changes in the structure of the composite (by image analysis and measurements of open porosity and density), and also essentially the in vitro and in vivo biocompatibility testing (cytotoxicity, bioactivity testing).

3. CONCEPTIONS, MATERIALS, METHODS AND RESULTS

3.1. COMPOSITE MATERIALS PREPARATION

A composite material based on fabric reinforcement (Aramid balanced fabric, based on aromatic polyamide fibers HM 215, Hexcel, FR) and a polydymethylsiloxane matrix M130 (Lučební závody Kolín, CR) was prepared. HA ($Ca_{10}(PO_4)_6(OH)_2$) and/or TCP ($Ca_3(PO_4)_2$) powders (Berkeley Advanced Biomaterials Inc., San Leandro, CA, USA), average particle size 100±50 nm and/or 100±50 μm, were inserted into the matrix before impregnation in the amount of 0, 2, 5, 10, 15, 20 and 25 vol. % (powder/matrix). For this purpose, the DI 18 Basic homogenizer (IKA Werke GmbH) was used. A weighed amount of additive was gradually inserted into a weighed amount of polysiloxane
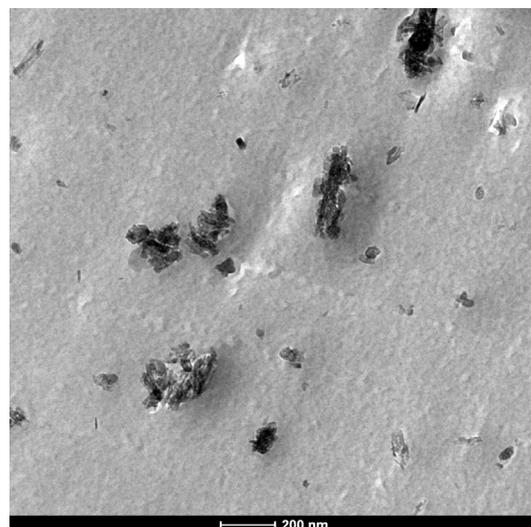

Fig. 1 SEM micrograph of n-HA dispersed in cured matrix.

Table 1 Porosity and bulk density of the examined composites (composite with 0% of additives: porosity 14.5 %, bulk density 1.18 g/cm^3).

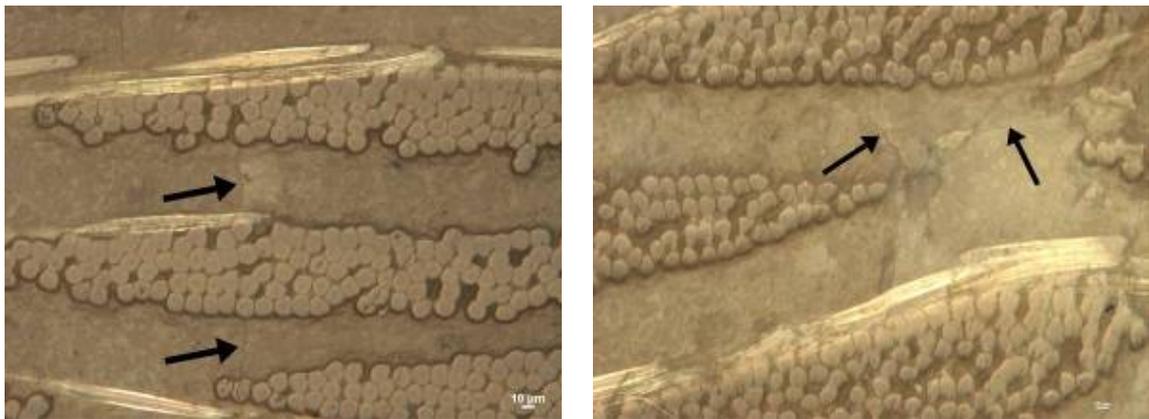|	vol.% (additives/matrix)	porosity [%]	bulk density [g/cm^3]		vol. % (additives/matrix)	porosity [%]	bulk density [g/cm^3]
ARAMID+M130+ n-HA	2	10.5	1.24	ARAMID+M130+ n-TCP	2	14.5	1.17
	5	9.4	1.27		5	14.3	1.19
	10	10.1	1.29		10	10.0	1.29
	15	6.1	1.3		15	9.8	1.33
	20	8.0	1.43		20	10.9	1.39
	25	8.3	1.48		25	11.2	1.46
ARAMID+M130+ m-HA	2	7.9	1.14	ARAMID+M130+ m-TCP	2	14.1	1.19
	5	8.7	1.2		5	11.1	1.26
	10	8.7	1.3		10	11.5	1.29
	15	12.0	1.38		15	10.3	1.36
	20	13.8	1.46		20	11.5	1.38
	25	10.3	1.57		25	10.8	1.45

Fig. 2   Micrographs of polished sections of composite ARAMID+M130 added with nano powders (left: n-HA, 25 vol. %, right: n-TCP, 25 vol. %).

matrix, so that uniform dispersion of the additive filler in the matrix was achieved (running speed of the homogenizer 17 500 min^{-1}, dispersion time 6 hours). A successful dispersion of additives in the matrix was then verified by SEM examinations, for an illustration, see Fig. 1. This second homogenization was followed by kneading in a HAAKE machine (Thermo Electron Corporation, USA), at RT and at a rotation speed of 50 min^{-1} for 24 hours.

After these procedures, the fabric was impregnated with the matrix/additives blend following by cuting into pieces with desired dimensions after 24 hours. Eleven impregnated layers were placed into the curing mould, taking into account the axis of the fibers (each layer has the same orientation of the warp, with ply direction (0°) and the fill weft, with ply direction (90°)). The green composite was heated in a mould at a temperature of 135 °C for two hours and then cured under the pressure of 1.1 MPa at 225 °C in the air atmosphere for 4.5 hours and finally cured without applying pressure at the temperature of 250 °C for 4 hours. Cured plates were cut by a diamond disc to an appropriate size according to further mechanical and biocompatibility tests.

3.2. PHYSICAL PROPERTIES

The open porosity and the apparent density of all composite samples were measured according to ASTM C-373. An image analysis of the polished sections was performed using NIS-Elements AR software, ver. 2.3 (Laboratory Imaging Inc., CR).

3.2.1. POROSITY AND BULK DENSITY

Twenty six pieces of the samples with various ratio of HA/TCP were examined. The open porosity and the apparent density of all composite samples are listed in Table 1. The open porosity of the composites
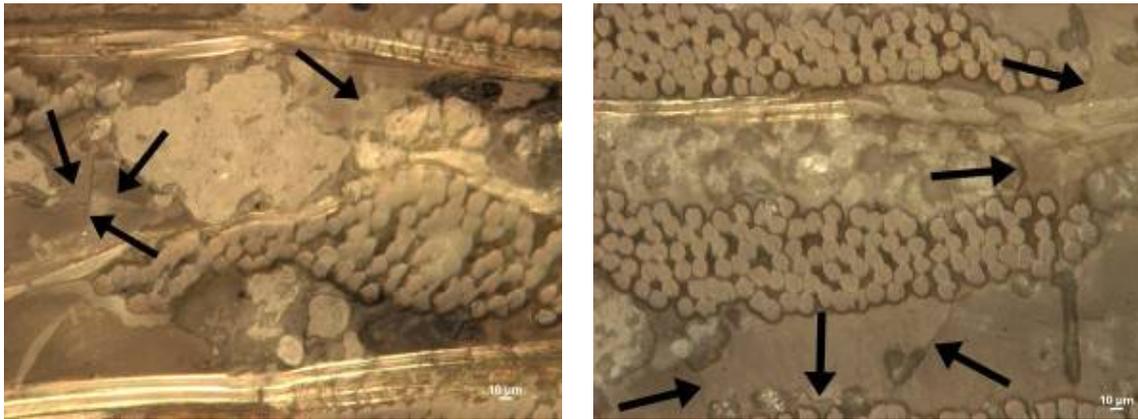
Fig. 3 Micrographs of polished sections of composite ARAMID+M130 added with micro powders (left: m-HA, 25vol. %, right: m-TCP, 25 vol. %).

decreases with the amount of nano powders. This tendency could be explained by the more homogenous distribution of nano fillers. Compared to this fact, in the case of micro fillers it is difficult to establish an explicit tendency. It can indicate inhomogeneous distribution of micro fillers in the matrix and possibly also creation of aggregates as is shown further in micrographs findings.

3.2.2. LAMINATE MORPHOLOGY

The following conclusions can be drawn from the image analysis of all added composite samples. With the composites with both types of added powders, cracks (both horizontal and vertical) appear with volumes higher than 20 and especially 25 % (see Figs. 2 and 3). A greater number of cracks can be observed on polished sections of composites added with micro powders (see Fig. 3). It seems that micro powders form aggregates in the matrix of the composites (see Fig. 3). These findings are ilustrated by the decrease of mechanical properties, especialy in the case of bending strength. Nano powders exhibit better dispersion with less frequent formation of aggregates ("maps") (see Fig. 2) leading in increase of bending strength. From the prepared polished sections we can draw the conclusion that the nano powders (both HA and TCP), with their better dispersion, are in closer proximity to the fibers. In general, we can state that the image analysis shows no distinct difference between the HA and TCP fillers: differences are visible only on micrographs with a different particle size of the fillers.

3.3. MECHANICAL PROPERTIES

The ultimate strength in bending (R_{fM}) and the modulus of elasticity in bending (E_f) in the direction of the fiber axis were determined by a four-point bending set-up with the Inspekt 100 HT material tester (Hagewald & Peschke, Germany), in accordance with ISO 14125. Six samples from each group with dimensions of 60x7x2.2mm (length x width x thickness) were applied.

3.3.1. MECHANICAL PROPERTIES - RESULTS

The ultimate strength in bending (modulus of elasticity in bending)/HA (TCP) volume fraction relationships were determined (see Figs. 4, 5). Statistical analysis was carried out via nonparametric analysis of variance, at the significant level of 0.05 (Kruskal-Wallis test, Mann-Whitney as post hoc test). Additions of nano powders in the range of 2 – 5 vol. % increase the strength in bending by 20 – 30 %. With further additions above 15 vol. % the strength in bending decreases slightly, and with 20 – 25 vol. % distinct cracks appear in the matrix. A similar course (with lower values of strength in bending) is observed when micro powders are added. It seems that the optimum amount of additives with both fillings is in the range of 10 – 15 vol. %.

3.4. IN VITRO TESTS

In vitro tests were carried out on the human osteoblast-like cell line MG-63 (European Collection of Cell Cultures, Salisbury, UK), currently used for studies of cell-material interaction and retaining markers of osteoblastic differentiation, and on the osteogenic cells, including bone marrow progenitor cells, prepared from the calvarias and long bones of newborn rats. Cells on the material samples were cultured using conventional static systems as well as a rotating cell reactor, better allowing the colonization of inner parts of the material. The cells were incubated in standard or modified media supplemented with the factors promoting osteoblastic differentiation. The potential release of microparticles from the material and their phagocytosis by cells were also monitored (by densitometry of the medium sediment or microscopically).
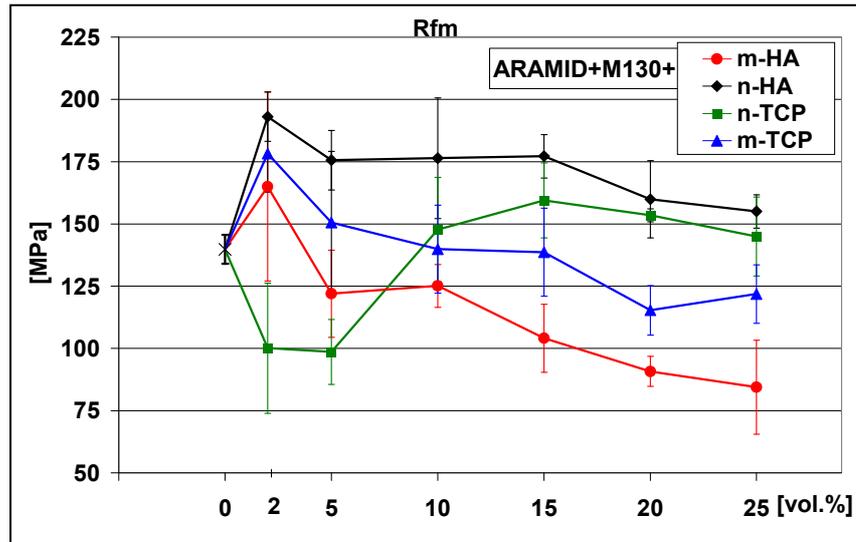
Fig. 4   Effect of nano and micro additives upon the ultimate strength in bending (R_{fm}).
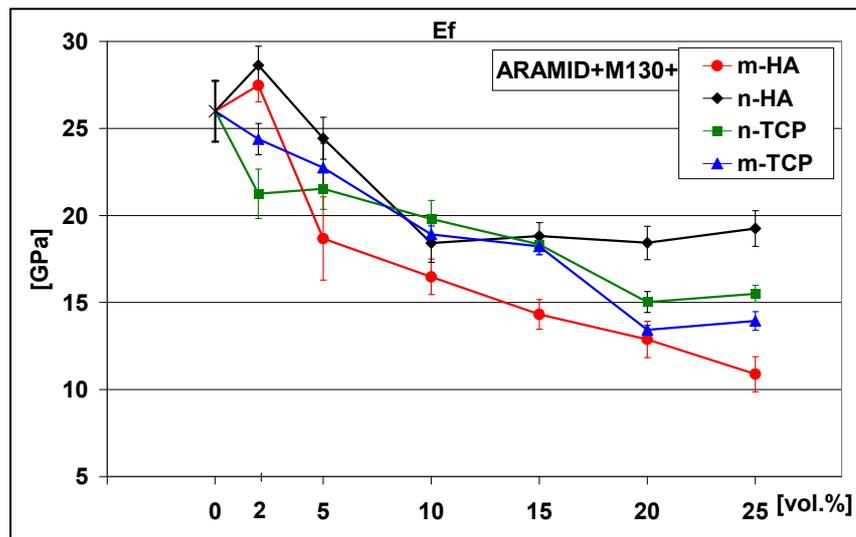

Fig. 5   Effect of nano and micro additives upon the modulus of elasticity in bending (E_f).

Initial adhesion and morphology of cells were determined. The cells were visualized by fluorescence staining or scanning electron microscopy, counted, and the size of cell spreading area will be measured using image analysis. Cell number was also determined in haemocytometer after trypsinization, by measurement of DNA content in cell lysates, as well as using MTT test.

Molecules mediating cell-substrate adhesion was studied. The adhesion of cells to artificial materials is mediated by the adsorption of extracellular matrix molecules. Thus, we will follow the adsorption of collagen, an important component of bone extracellular matrix, and vitronectin, selectively promoting the adhesion of osteoblasts, to the cell-free material surface from the serum of the culture media or pure solutions of these proteins. The proteins were visualized by their conjugation with fluorescence labels or immunocytochemical staining. Also the integrin receptors for these proteins on the cell surface (integrins $\alpha_1\beta_1$ and $\alpha_2\beta_1$ for collagen, and $\alpha_v\beta_3$ for vitronectin), their recruitment into focal adhesion plaques and association with talin, vinculin and actin cytoskeleton, were investigated by the immuno-fluorescence staining, enzyme-linked immunosorbent assay (ELISA), flow cytometry, electrophoresis, immunoblotting and immunoprecipitation. Also the confocal microscopy, able to perform optical sections through the material in order to visualize cells adhering to the pore bottom and walls, was applied.

Cell proliferation was studied. Proliferation kinetics was evaluated by construction of growth curves, calculation of cell population doubling time. Bromodeoxyuridine (BrdU) incorporation into the newly synthesized DNA and expression of some proliferation antigens (PCNA, Ki-67) was also determined by immunocytochemical and flow-cytophotometric approaches.

Differentiation of osteogenic cells was assessed by the activity of alkaline phosphatase and production of collagen I as well as non-colagenous calcium-binding exracellular matrix proteins osteocalcin and osteopontin. These markers were detected by immunofluorescence, ELISA, electrophoresis and Western blotting in cells as well as in the cultivation media.

The possible immune activation of cells growing on the material was also tested by monitoring the expression of immunoglobulin and selectin adhesion molecules such as ICAM-1 or ELAM-1, and production of cytokines (i.e., interleukins, tumor necrosis factors). These molecules were detected by immunofluorescence, electrophoresis, Western blotting and ELISA.

3.4.1. CULTIVATION OF CELLS ON BASIC COMPOSITE COMPONENTS

Before the preparation proper of the composites the behavior of the cells on the component parts of these composites (i.e. Aramid fabric, polydimethyl-siloxane matrix) was investigated systematically.

We can resume that Aramid fabric supported the colonization by osteogenic cells to an extent comparable to standard cultivation materials. Aramide fabric with a small distance between the fibers the cells even managed to bridge these gaps and to form a continuous layer of cells (see Fig. 6). A relatively high colonization by osteogenic cells was attained also with a polydymethylsiloxane matrix although this material is relatively highly hydrophobic (it is well known that hydrophobic materials do not support the adsorption of proteins which mediate the adhesion of the cells in a suitable geometrical configuration which makes possible to attain specific aminoacid sequences in the molecule by the adhesion receptors in the molecule).

3.4.2. CULTIVATION OF CELLS ON FABRIC REINFORCED COMPOSITES WITH A MATRIX ENRICHED WITH HA/TCP ADDITIVES

For testing modified composites in cell cultures, a series of samples with a varying content in the additives in the matrix, i.e. 2, 5, 10, 15, 20, 25 vol. % of HA or TCP were prepared. The surface of the tested samples was modified in order to obtain optimum open pores with the size of 0.4-0.6 mm. The samples were sterilized in an autoclave, inserted into polystyrene cultivation cells (TPP, Transadigen, Switzerland) and inoculated with human osteogenic cells of the line. With the cells the adhesion, the
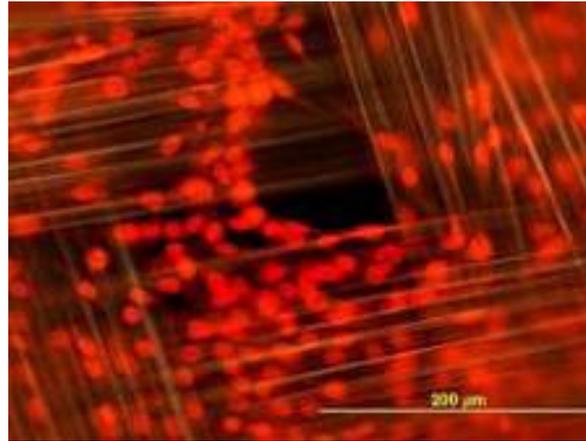

Fig. 6 Osteogenic cells on Aramide fabric the seventh day after the inoculation, stained with propidium iodide.

growth and the osteogenic differentiation were determined. As an adhesion indicator the number of cells after 24 hours after the inoculation, the surface of their spreading on the material and the formation of the focal adhesion plaques with the content of integrin adhesion receptors and the associated proteins talin and vinculin was used. Further, the formation of beta-actin cytoskeleton in the course of the spreading of the cells was investigated by immunofluorescence. The proliferation of the cells was indicated by the changes in the number of cells in three time intervals after the inoculation, i.e. after 1, 3 and 7 days, as well as by the course, the shape and the slope of the proliferation curves and the cell population doubling time. The osteogenic differentiation was indicated by the production of osteocalcin and osteopontin, glycoproteins of the extracellular matrix which bind calcium. At the same time the viability of cells was followed by using a commercially available kit LIVE/DEAD (Invitrogen) based on the detection of the activity of esterases in living cells and on the penetration of the ethidium homodimer dye into the dead cells as well as by staining with trypan blue when counting the cells with the apparatus ViCell Analyser (similarly as ethidium homodimer this dye penetrates through the membrane of damaged or dead cells).

3.4.2.1. CULTIVATION OF CELLS ON COMPOSITES WITH ADDITIONS OF HA

It was found that the adhesion of the MG 63 cells in one day after inoculation on the investigated composite materials was similar to that on the reference surface represented by a polystyrene cultivation dish (TPP, Trasadingen, Switzerland). The lowest average number of adhering cells was found on the composite with 25 vol. % of micro-HA. The viability of cells was relatively high (> 81.5 %) on all investigated materials and after 3 days after inoculation it rose up to 86-99 % which indicates the initial

damaging of the cells in the course of their trypsinisation and the manipulation during the inoculation rather than their subsequent damaging caused by the material.

The third day after the inoculation the highest numbers of cells were obtained on composites with 2-15 vol. % of nano-HA where these numbers became equal to the reference values on the polystyrene dishes. With the other types of composites the numbers of cells stayed on values statistically significantly lower than with polystyrene, especially with materials containing 20 and 25 vol. % of micro-HA, where the numbers of cells attained only about one half of the reference value. Nevertheless, even with these samples the viability of cells attained 92-94 %.

However, at the end of the experiment, i.e. after 7 days of cultivation, the highest population densities of cells (comparable with those on polystyrene dishes) were attained on surfaces with micro-HA in the concentration of 5-15 vol. %. Contrary to that a very high concentration of micro-HA, i.e. 20 and 25 vol. %, did not support the growth of the cells and was even cytotoxic as it was indicated by low viability values (21-31 %) of the cells. Also on composites with nano-HA the cell growth was slower than on the reference polystyrene. However, on composites with the concentration of 5-15 vol. % of nano-HA the concentration of cells per cm^2 was statistically significantly higher in comparision with the pure composite without HA. HA also enhanced the formation of focal adhesive plaques containing talin and vinculin, most with the content of 5-15 vol. %. The cells on all samples exhibited a pronounced immunofluorescence of osteocalcin and osteopontin, i.e. of markers of the osteogenic differentiation.

3.4.2.2. CULTIVATION OF CELLS ON COMPOSITES WITH ADDITIONS OF TCP

Similarly as with composites with HA, the number of initially adhered human bone cells MG 63 after 1 day after the inoculation exhibited no statistically significant difference between the individual composites with additives of TCP. However, the number of cells after 1 day after the inoculation on the composite was lower than on the reference surface represented by a microscope cover glass. Also after 3 and 7 days of cultivation the highest population density of MG 63 cells was attained on glass. As far as the composites are concerned, the highest numbers of cells were observed on materials with 20 vol. % micro-TCP and 15 vol. % nano-TCP. On all surfaces MG 63 cells had a well developed beta-actin cytoskeleton. The focal adhesion plaques, visualized by immunofluorescence staining of vinculin (i.e. of the protein associated with the integrin adhesion receptors) were well formed on all surfaces studied. On composites with 15-25 vol. % of TCP the cells exhibited a more intense colouring of

osteocalcin, i.e. the glycoprotein of the extracellular matrix which binds calcium and is an important indicator of the osteogenic differentiation.

3.5. IN VIVO TESTS

These tests which are performed by the test method applied by the research team in the previous years aimed at the assessment of the influence of modification of the matrix as well as the effect of additional surface treatements of the samples. The research concerned the samples of the original unmodified composite, of the composite enriched with HA or TCP (in both cases nano as well as micro), further of an enriched composite with modified open porosity, where the size of the open pores formed was determined on the basis of the results of the investigations performed by the research team in the previous years (fraction 0.4-0.6 mm).

The tests in vivo were performed with laboratory animals (rabbit; breed Belgium Giant, age 1 year). The cylindrical samples were implanted under the proximal condyle of the femur of the right hindlimb. The hole is made with a special drill under general anaesthesia and under sterile conditions – see Fig. 7. In contrast to the originally implanted samples the dimensions of the hole were reduced and the shape was changed to a cylindrical one in order to reduce the lesion of the bone during the operation. The samples were in the implanted position for eight weeks, after their removal they were together with the surrounding tissue embedded in acrylate, cut to slices, stained with hematoxylin-eosin and histologically evaluated.

Fig. 7 Implanted sample of the modified composite.

3.5.1. HISTOLOGICAL FINDINGS

All samples were examined histologically. From the histological investigation it follows that all implants were incorporated into the bone, in the adjoining adherent tissue no morphological indications of the cell degeneration, inflammation or necrosis were observed. The fact that between the bone and the implant no layer of fibrous connective
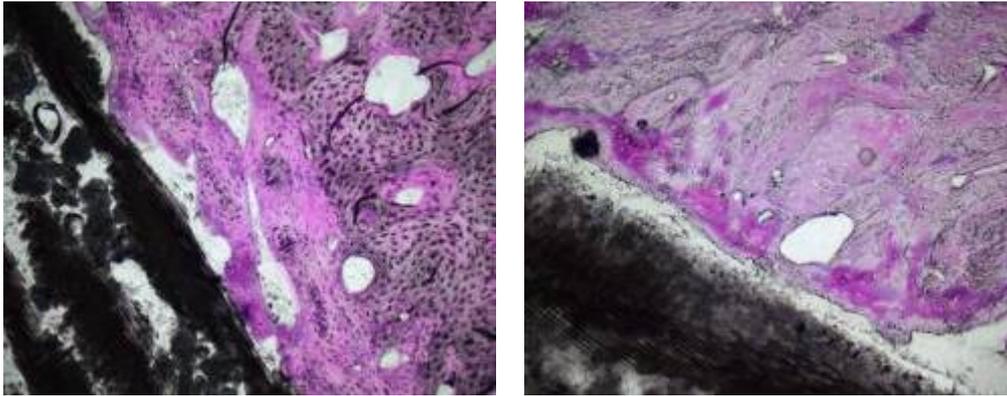
Fig. 8 Histological microsections of composite implants added with micro-HA (on the left) and nano-HA (on the right).

tissue was formed represents a positive feature. The applied implant material is non-toxic. The original bone tissue is completed by a new more eosinophilic layer. With implants with TCP additives the healing quality seems to be better than with unmodified samples. No difference between the particle sizes of the TCP additives was observed. In the case of samples with HA additives a more exuberant character of newly formed bone tissue is evident, again without traces of inflammation or necrosis. Among all investigated implants just in this case the highest degree of osteointegration is attained. Around the whole implant a homogeneously thick layer of newly formed bone tissue was formed which adheres closely to the surface layer of the implant material (Fig. 8).

4. EXPLOITATION OF RESULTS

The mechanical properties: In this project the effect of micro and nano powder additives on the mechanical properties of the fiber composite determined for the applications in bone surgery was investigated. The aim was to verify and find a suitable content in powder additives employed for increasing the biocompatibility, aimed at optimum mechanical properties for the planned final application in the form of substitutes for hard tissues especially with regard to the rigidity and the bending characteristics. It appears that in general both the micro and the nano fillers reduce the bending elasticity modulus what seems to be useful in the case of comparison of the values with the cortical bone. The bending strength is increased by adding nano powders whereas the microparticles exhibit a rather negative influence which is probably caused by the nonuniform dispersion in the composite matrix or by the formation of agglomerates as it has been shown by image analysis. In general it can be stated that nano powders have a more favourable effect on mechanical properties than micro fillers. From the point of view of mechanical properties the optimum additive appears to be that of 10-15 vol. %. In this case a suitable optimization of the mechanical properties without any changes in the internal

structure of the composite takes place. The formation of cracs with additive contents surpassing 20 vol. % - in the case of micro fillers – also of agglomerates could have a negative effect on long-time properties of the composite, mainly on the propagation of cracs and on the fatigue strength.

The results of the in vitro and in vivo tests are in a surprising agreement with the results of the investigation of the mechanical properties. Also in these cases adhesion, growth, differentiation and viability of cells had an optimum value in the range of 10-15 vol. % of the additives, whereas with higher concentrations there was the danger of a toxication of the cells. The best results were in both cases attained when nano HA/TCP additives were applied.

REFERENCES

Bačáková, L., Starý, V., Kofroňová, O. and Lisá, V.: 2001, Polishing and coating carbon fibre-reinforced carbon composites with a carbon-titanium layer enhances adhesion and growth of osteoblast-like MG63 cells and vascular smooth muscle cells in vitro, J. Biomed. Mater. Res., Vol. 54, 567–578.

Balík, K., Sochor, M., Hulejová, H., Suchý, T. and Černý, M.: 2007, The influence of a ahort-term tissue culture medium storage on the mechanical properties of composites based on glass fibers and polysiloxane, Ceramics − Silikáty, Vol. 51, 198–201.

Chavarria, F. and Paul, D.R.: 2004, Comparison of nanocomposites based on nylon 6 and nylon 66, Polymer, Vol. 45, 8501–8515.

Fazan, F.: 2005, Bone Grafts and Bone Substitutites. Basic Science and Clinical Applications, 1st ed., World Scientific Publishing, London, 489–500.

Gumula, T. and Blazewicz, S.: 2004, Study on polysiloxane resin-based composites for bone surgery application, Polim. Med., Vol. 34, No. 3, 49–54.

Kawai, T., Ohtsuki, C., Kamitakahara, M., Miyazaki, T., Tanihara, M., Sakaguchi, Y. and Konagaya, S.: 2004, Coating of an apatite layer on polyamide films containing sulfonic groups by a biomimetic process, Biomaterials, Vol. 25, 4529–4534.

Liao, H., Andersson, A.S., Sutherland, D., Petronis, S., Kasemo, B. and Thomsen, P.: 2003, Response of rat osteoblast-like cells to microstructured model surfaces in vitro, Biomaterials, Vol. 24, No. 4, 649–54.

Manikandan, M., Naira, K.C., Thomasa, S. and Groeninck, G.: 2001, Thermal and dynamic mechanical analysis of polystyrene composites reinforced with short sisal fibres, Comp. Sci. Tech., Vol. 61, 2519–2529.

Mata, A., Boehm, C., Fleischman, A.J., Muschler, G. and Roy, S.: 2002, Growth of connective tissue progenitor cells on microtextured polydimethylsiloxane surfaces, J. Biomed. Mater. Res., Vol. 62, No. 4, 499–506.

McFarland, C.D., Thomas, C.H., DeFilippis, C., Steele, J.G. and Healy, K.E.: 2000, Protein adsorption and cell attachment to patterned surfaces, J. Biomed. Mater. Res., Vol. 49, No. 2, 200-210.

Ramakrishna, S., Huang, Z.M., Kumar, G.V., Batchelor, A.W. and Mayer, J.: 2004, An Introduction to Biocomposites, Vol.1, 1st ed., Imperial College Press, London.

Ramay, H.R.R. and Zhang, M.: 2004, Biphasic calcium phosphate nanocomposite porous scaffolds for load-bearing bone tissue engineering, Biomaterials, Vol. 25, 5171–5180.

Ren, L., Tsuru, K., Hayakawa, S. and Osaka, A.: 2002, Novel approach to fabricate porous gelatin-siloxane hybrids for bone tissue engineering, Biomaterials, Vol. 23, No. 24, 4765–4773.

Rose, F.R., Cyster, L.A., Grant, D.M., Scotchford, C.A., Howdle, S.M. and Shakesheff, K.M.: 2004, In vitro assessment of cell penetration into porous hydroxapatite scaffolds with central aligned channel, Biomaterials, Vol. 25, 5507–5514.

Springer, I.N.G., Fleiner, B., Jepsen, S. and Açil, Y.: 2001, Culture of cells gained from temporomandibular joint cartilage on non-absorbable scaffolds, Biomaterials, Vol. 22, 2569–2577.

Suchanek, W. and Yoshimura, M.: 1998, Processing and properties of hydroxyapatite-based biomaterials for use as hard tissue replacement implants, J. Mater. Res., Vol. 13, 94–117.

Suchý, T., Balík, K., Sucharda, Z., Černý, M. and Sochor, M.: 2007, Influence of nanoparticles additivies on mechanical properties of fabric reinforced composites, Eng. Biomater., Vol. 69-72, 1–3.

Walboomers, X.F., Habraken, W.J., Feddes, B., Winter, L.C., Bumgardner, J.D. and Jansen, J.A.: 2004, Stretch-mediated responses of osteoblast-like cells cultured on titanium-coated substrates in vitro. J. Biomed. Mater. Res., Vol. 69, No. 1, 131–139.

Wang, X., Li, Y., Wei, J. and de Groot, K.: 2002, Development of biomimetic nano-hydroxy-apatite/poly(hexamethylene adipamide) composites, Biomaterials, Vol. 23, 4787–4791.

Wei, G. and Ma, P.X.: 2004, Structure and properties of nano-hydroxyapatite/polymer composite scaffolds for bone tissue engineering, Biomaterials, Vol. 25, 4749–4757.

Woo, K.M., Chen, V.J. and Ma, P.X.: 2003, Nano-fibrous scaffolding architecture selectively enhances protein adsorption contributing to cell attachment, J. Biomed. Mater. Res., Vol. 67, 531–537.

PORÉZNÍ KOMPOZITNÍ MATERIÁLY S POLYAMIDOVOU VÝZTUŽÍ A SILOXANOVOU MATRICÍ S NANO-HYDROXYAPATITEM JAKO BIOMATERIÁLY

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ABSTRAKT:

Byly navrženy kompozitní materiály na bázi polyamidové tkaniny a polysiloxanové matrice pro aplikace v kostní chirurgii. Do matrice kompozitních vzorků byl pro zvýšení bioaktivity přidán nano/mikro hydroxyapatit (HA) a fosforečnan vápenatý (TCP) v množství 2, 5, 10, 15, 20 a 25 obj. %. Byl ověřován vliv aditiv na mechanické vlastnosti a současně byly pomocí obrazové analýzy studovány změny ve vnitřní struktuře kompozitů. Pomocí testů in vitro a in vivo byl určen vliv aditiv na biokompatibilitu. Ukazuje se, že nano příměsi mají na mechanické vlastnosti, oproti mikro částicím, příznivější vliv. Jako optimální se z hlediska konečné aplikace ve formě náhrad tvrdých tkání jeví obsah nano aditiv 10-15 obj. %, při jejichž přidání dochází jednak k optimalizaci tuhosti kompozitu, tak ke zvýšení ohybové pevnosti, a to bez změn ve vnitřní struktuře kompozitu. Výsledky biologických hodnocení jsou ve shodě s výsledky mechanických testů.