EFFECT OF NANO/MICRO PARTICLES OF CALCIUM PHOSPHATES ON THE MECHANICAL PROPERTIES OF COMPOSITES BASED ON POLYSILOXANE MATRIX REINFORCED BY POLYAMIDE

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Composite materials based on a polyamide fabric and a polysiloxane matrix were designed for application in bone surgery. In order to increase the bioactivity, 2, 5, 10, 15, 20 and 25 vol. % (powder/matrix) 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. 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.

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) [1-7]. Several polymers (for illustration see Table 1) 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 [8]. Table 1 compares some mechanical properties of various types of hard tissues and of some of the polymers mentioned above. The presented values show in the first place the low modulus of elasticity and the low tensile strength of the polymer materials. Only PEEK exhibits mechanical strength comparable with that of human bone.

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 [8]. 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 [10] 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 [5]. 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 [11].

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 [1, 12-15]. 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 [16]. 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 [17].

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 [18]. HA-containing materials act as sources of calcium ions, which are known to stimulate osteoblast proliferation and differentiation [17]. 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 [19, 20]. However, HA by itself has insufficient mechanical properties, 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 [21]. 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).

The aim of our study was to perform an effect of matrix powder additives commonly used to increase the bioactivity on the behaviour of the composite potentially suitable for application as a filling material or as a substitute element in the human body. We are looking for a compromise between the optimum of amount fillers of the resulting composite and suitable mechanical properties. This study was focused just on description of 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 preparation of samples for subsequent in vitro and in vivo biocompatibility testing (cytotoxicity, bioactivity testing).

EXPERIMENTAL

A composite material based on fabric reinforcement (Aramid balanced fabric, based on aromatic polyamide fibers HM 215, Hexcel, FR) and a polysiloxane matrix M130 (Lučební závody Kolín, CR) was prepared, see Figure 1.

HA and/or TCP powder, average particle size 100 nm and/or 100 m, was inserted into the matrix before impregnation in the amount of 0, 2, 5, 10, 15, 20 and 25 vol.% (powder/matrix), see Table 2 and Figure 2.

Table 1. Mechanical properties of some biomaterials [8, 9].

	Materials	Modulus (GPa)	Tensile strength (MPa)
Hard tissues	Cortical bone (longitudinal direction)	17.7	133.0
	Cortical bone (transverse direction)	12.8	52.0
	Cancelous bone	0.4	7.4
	Enamel	84.3	10.0
	Dentine	11.0	39.3
Typical polymeric biomaterials	Polyethylene (PE)	0.88	35.0
	Polyurethane (PU)	0.02	35.0
	Polytetrafluoroethylene (PTFE)	0.5	27.5
	Polyacetal (PA)	2.1	67.0
	Polymethylmethacrylate (PMMA)	2.55	59.0
	Polyethylene terepthalate (PET)	2.85	61.0
	Polyetheretherketon (PEEK)	8.3	139.0
	Silicone rubber (SR)	0.008	7.6
	Polystyrene (PS)	0.39	34.9
	Polysulfone (PSU)	2.65	75.0

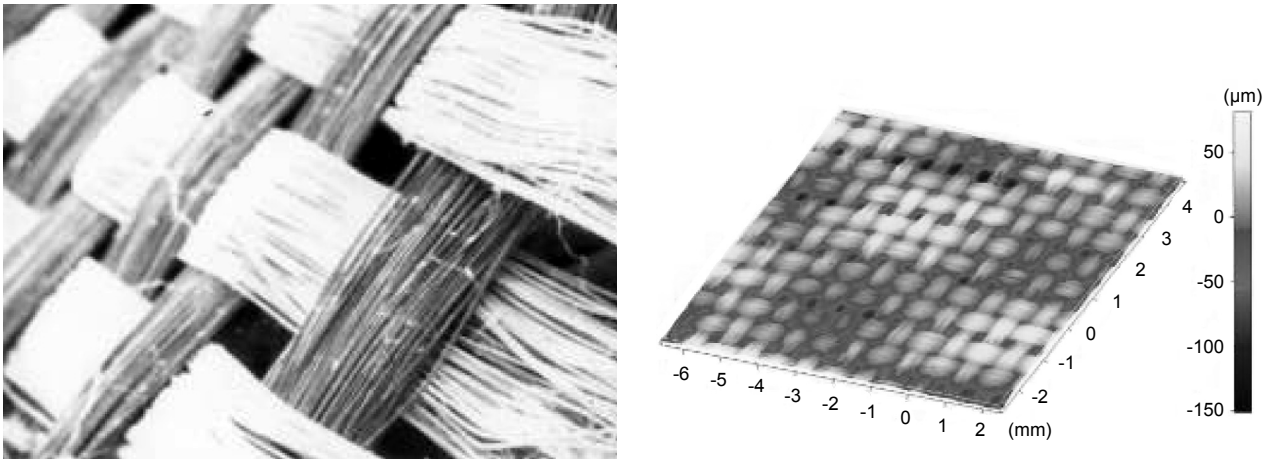
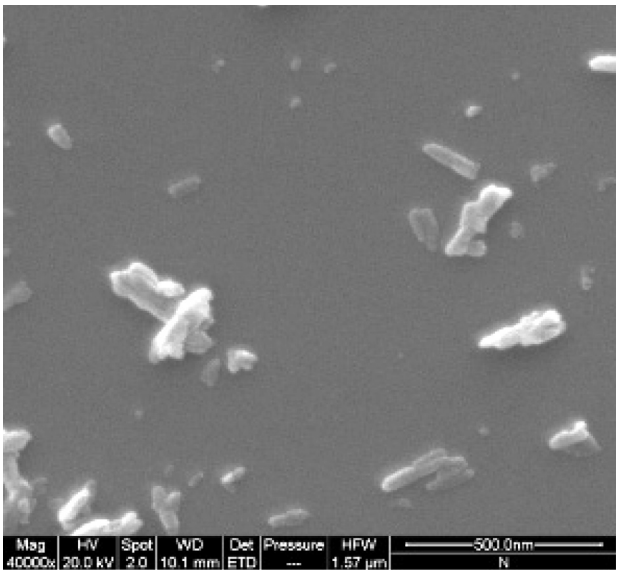
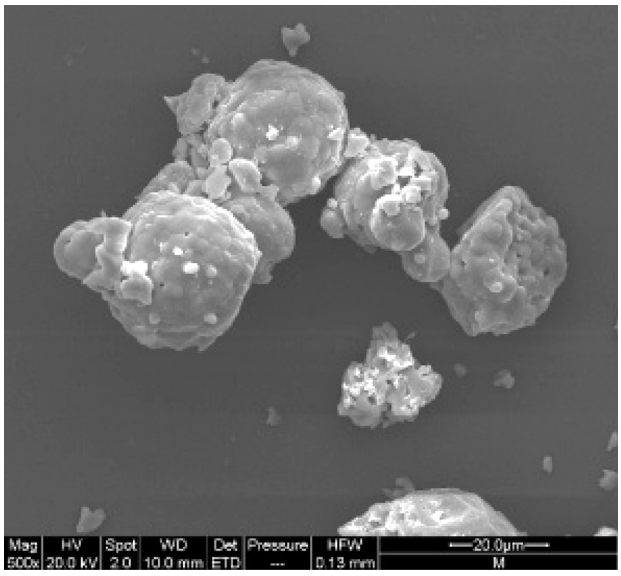


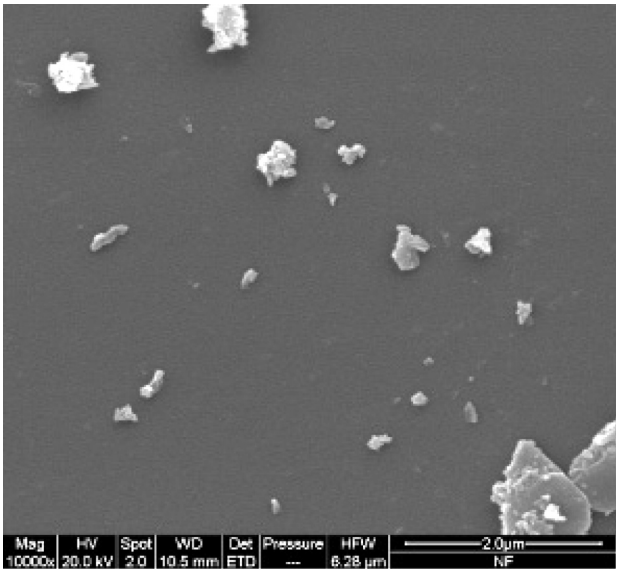
Figure 1. SEM micrograph and 3D scan (MarSurf TS 50/4 optical equipment, Mahr, Germany) of Aramide fabric.



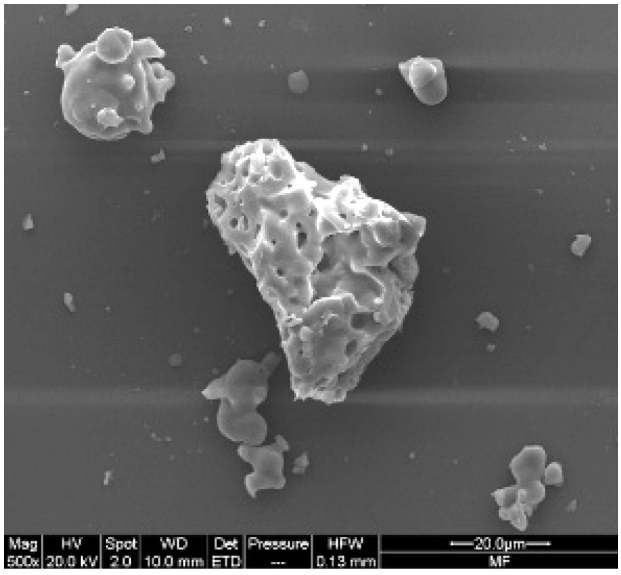
a) n-HA



b) m-HA



c) n-TCP



d) m-TCP

Figure 2. SEM micrographs of HA/TCP additives used.

Table 2. Nano and micro particles used for composite matrix modifications (Berkeley Advanced Biomaterials Inc., San Leandro, CA, USA).

Sample designation	Particle size	Material	Designation according to producer
n-HA	100 ± 50 nm	Hydroxyapatite; $Ca10(PO_4)_6(OH)_2$	BABI-HAP-N100
m-HA	100 ± 50 μm	Hydroxyapatite; $Ca10(PO_4)_6(OH)_2$	BABI-HAP-HS2
n-TCP	100 ± 50 nm	Tri-Calcium Phosphate; $Ca_3(PO_4)_2$	BABI-TCP-N100
m-TCP	100 ± 50 μm	Tri-Calcium Phosphate; $Ca_3(PO_4)_2$	BABI-TCP-G2

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 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 TEM examinations, for an illustration, see Figure 3. This 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 square pieces with dimensions of 60×7 mm 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 4-point bending mechanical tests (see below).

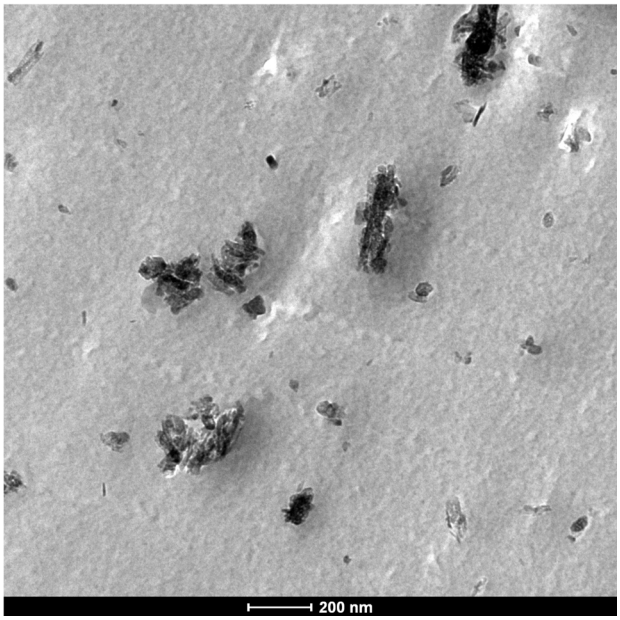


Figure 3. TEM micrograph of n-HA dispersed in cured matrix.

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 60×7×2.2 mm (length × width × thickness) were applied.

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 LUCIA software, ver. 4.8 (Laboratory Imaging, Inc., Czech Republic).

RESULTS AND DISCUSSIONS

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 3. The open porosity of the composites 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.

Mechanical properties

The ultimate strength in bending (modulus of elasticity in bending)/HA (TCP) volume fraction relationships were determined (see Figure 4, 5). 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. %.

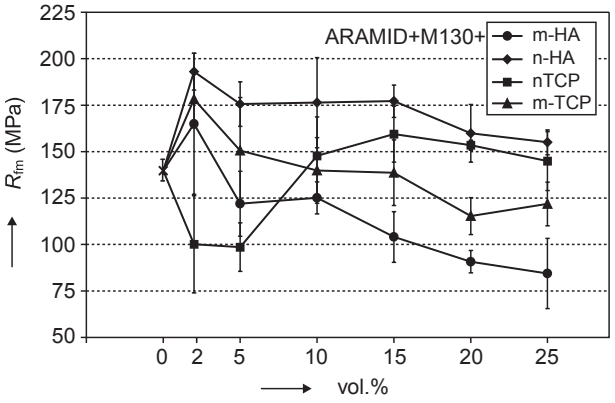


Figure 4. Effect of nano and micro additives upon the ultimate strength in bending (R_{fm}).

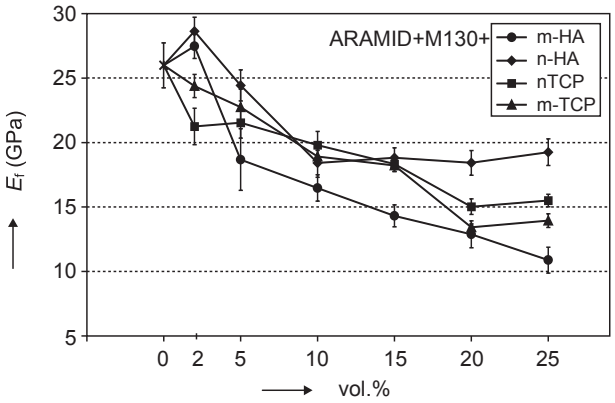


Figure 5. Effect of nano and micro additives upon the modulus of elasticity in bending (E_f).

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

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

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 Figures 6 and 7). A greater number of cracks can be observed on polished sections of composites added with micro powders (see Figure 7). It seems that micro powders form aggregates in the matrix of the composites (see Figures 8 and 9). 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 Figures 8 and 9) 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.

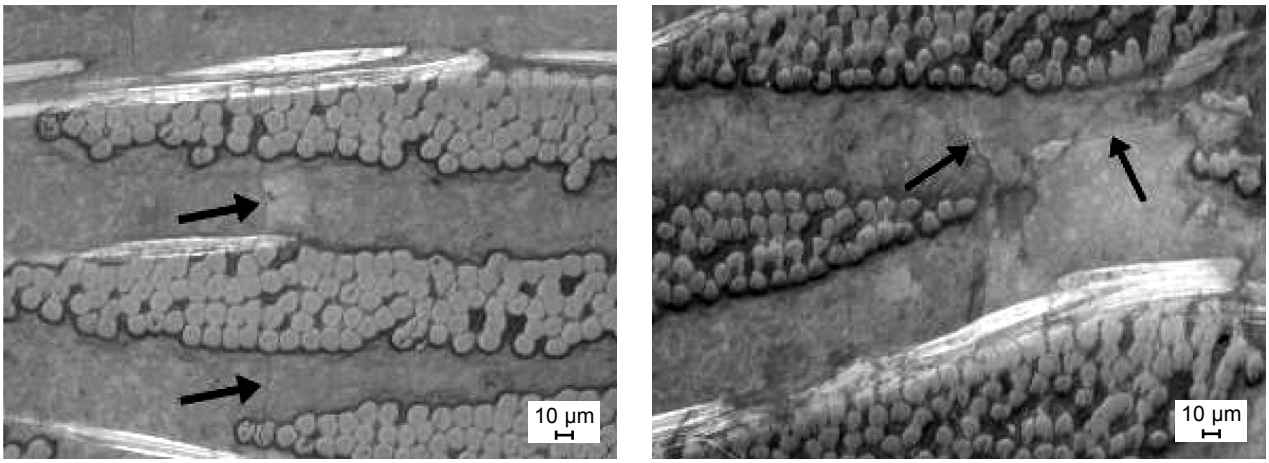


Figure 6. Micrographs of polished sections of composite ARAMID+M130 added with nano powders (left: n-HA, 25 vol.%, right: n-TCP, 25 vol.%).

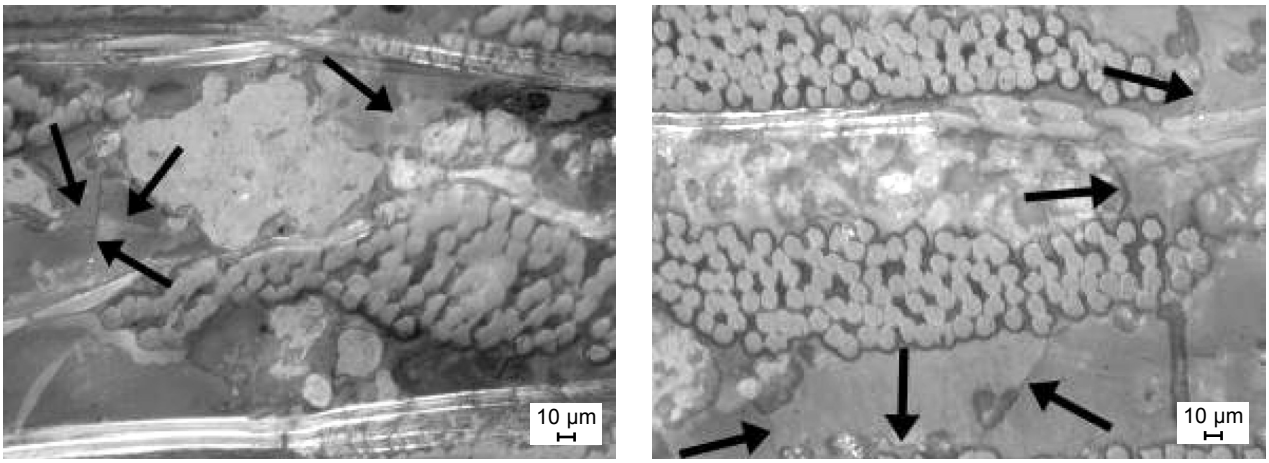


Figure 7. Micrographs of polished sections of composite ARAMID+M130 added with micro powders (left: m-HA, 25 vol.%, right: m-TCP, 25 vol.%).

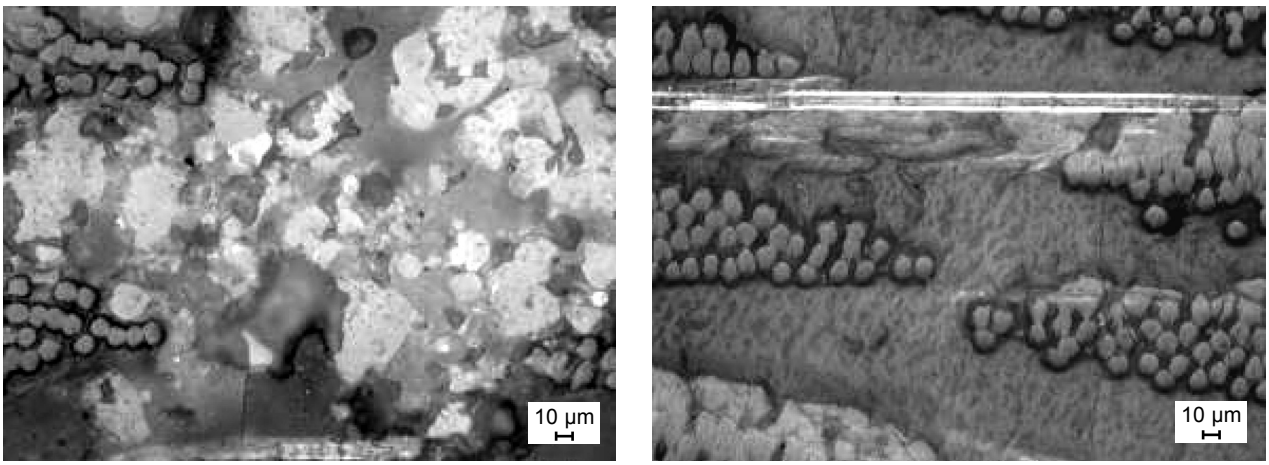


Figure 8. Micrographs of polished sections of composite ARAMID+M130 added with micro and nano powders (left: m-HA, 20 vol. %, right: n-HA, 20 vol. %). The micro particles of hydroxyapatite are not homogeneously dispersed in the matrix, in contrast to the nano powder.

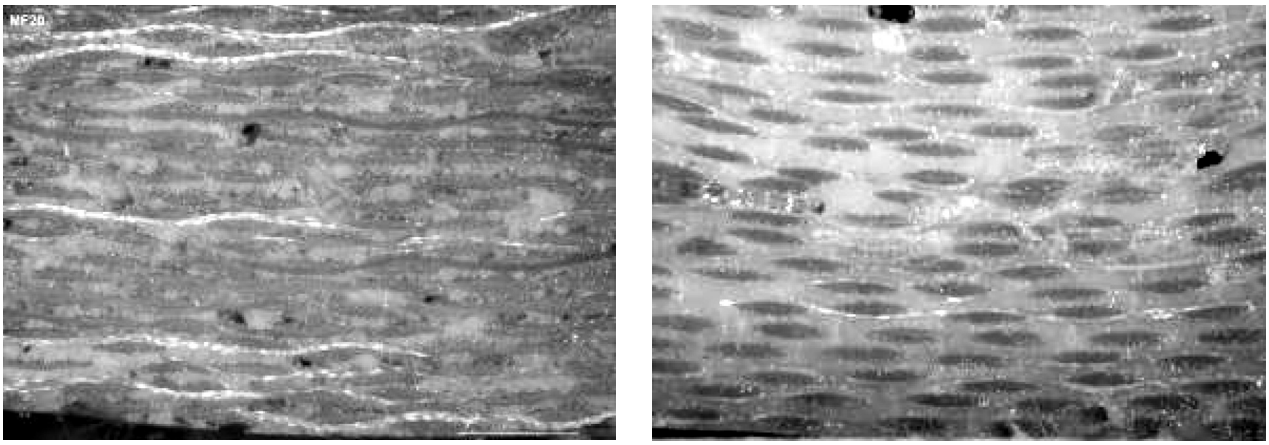


Figure 9. Micrographs of the inner structure of composite ARAMID+M130 added with micro and nano powders (left: m-TCP, 20 vol.%, right: n-TCP, 20 vol.%) illustrate the more homogeneous dispersion of the latter powder in the matrix.

CONCLUSIONS

This paper has investigated the effect of micro and nano particles on the mechanical properties of a fiber composite designed for applications in bone surgery. The aim was to find and verify a suitable ratio of additives to optimize mechanical properties of composites to be comparable with that of the human bone. It has been shown that in general both the micro and nano fillers reduce the modulus of elasticity in bending. Bending strength increased by the addition of nano powders. Micro particles tend to produce a negative effect of bending strength decrease, which is probably due to their non-uniform dispersion in the composite matrix or due to the formulation of the aggregates. Addition of nano powders results in the possitive effect on the mechanical properties compare to micro particles. From the point of view of mechanical properties, the addition of 10-15 vol.% of nano particles appears to be the optimum amount with which a suitable optimization of the mechanical properties is achieved without any changes in the inner structure of the composite. The formation of cracks and aggregates with additive volumes above 20 vol.% could have a negative effect on the long-term properties of the composite, especially on the further propagation of cracks and on the fatigue strength. An analysis of the mechanical properties, and also the image analysis, show no fundamental difference between the HA and TCP fillers. In this paper, two different particle sizes (micro and nano) of the powder fillers were investigated with regard to their effect on the mechanical properties; further studies will deal with the effect of their size and quantity on their interaction with the bone tissue. It will be necessary to verify the effects of the composite material, its porosity and its individual components on the adhesion, growth, maturation, viability and potential immune activation of osteogenic cells in vitro. The influence of long-term storage in simulated body fluid on the chemical composition and mechanical properties of the composite will also be studied.

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VLIV NANO/MIKRO KALCIUM FOSFÁTOVÝCH PLNIV NA MECHANICKÉ VLASTNOSTI KOMPOZITŮ NA BÁZI POLYSILOXANOVÉ MATRICE VYZTUŽENÉ POLYAMIDEM

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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.% (plnivo/ /matrice). Byl ověřován vliv aditiv na mechanické vlastnosti a současně byly pomocí obrazové analýzy studovány změny ve vnitřní struktuře kompozitů. 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.