J Mater Sci: Mater Med (2007) 18:501–506
DOI 10.1007/s10856-007-2010-4

Preparation and bioactivity of novel multiblock thermoplastic elastomer/tricalcium phosphate composites

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Received: 16 May 2005 / Accepted: 24 October 2005

Abstract There is a recognized need for improved treatment of osteoarthritis of the finger joints disease. Joint fusions are commonly used for treating the pain and potential deformity of arthritis. At severe deformity, artificial joint replacement is required. The most widely used are space-filler type joints made of high performance silicone rubber. One of the problems that occurs with these artificial replacements is that they can fail, because silicone elastomer used for their fabrication is relatively weak material and show to break apart and fragment. We have recently developed novel poly(aliphatic/aromatic-ester) (PED) material of sufficient mechanical properties and excellent flexibility. To enhance the bioactivity of these polymers (PED) and bone-bonding properties, PED/β-TCP composites were prepared. The ceramic particles were homogeneously distributed during conventional blending and showed good adhesion to the polymer matrix. The thermal characteristics and mechanical properties of the composites were investigated as a function of β-TCP content. The Young's modulus and the yield strength of the composites increased with the increase in β-TCP volume while the tensile strength and fracture strain decreased. *In vitro* investigations demonstrated an increase in cytocompatibility with increasing amount of β-TCP up to 20 vol%.

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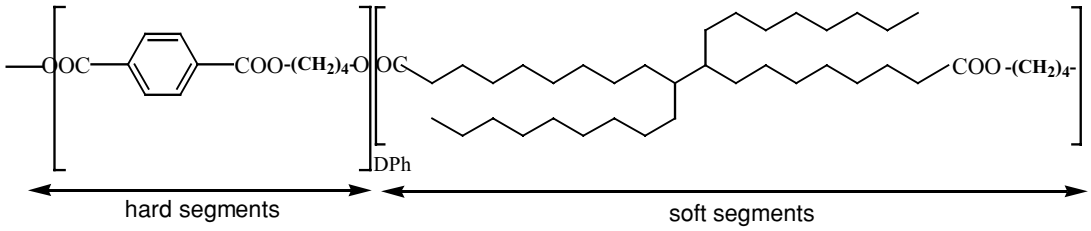
1 Introduction

Several million people (over 16 million in USA) suffer from severe joint pain and related dysfunction, such as loss of motion, as a result of injury or osteoarthritis [1, 2]. In painful rheumatoid arthritis a variety of deformities and deviations of the metacarpophalangeal joints occur. The ability to use the fingers for daily activities is greatly affected. The replacement of the destroyed joints may be the most practical means of relieving pain, realigning the fingers and improving function [3]. Swanson silicone implant is still the "gold standard" of metacarpophalangeal joint reconstruction in rheumatic arthritis patients. Till now the fixation to bone is realized by squeezing it into the bone. Among the complications occurring after implantation are fracture, silicone-induced synovitis, dislocation of the implant, infection and recurrent deformation [4]. The silicone implants fail because of weak fatigue properties [5]. Therefore, there is a need for implants of enhanced mechanical (fatigue) properties. Patients could benefit from a novel design of an implant with peripheral parts contacting with a bone that show bioactivity and bone bonding properties.

Materials of good mechanical properties and high flexibility, comparable to silicone rubber are multiblock thermoplastic elastomers (TPE), such as poly(ether-urethanes) or poly(ester-ether)s which are widely utilized as biomedical materials with tailored structure and properties [6, 7]. Their specific physico-chemical and mechanical properties combined with high biocompatibility are a consequence of chemical structure, and especially of a matrix-domain morphology (appearance of hard and soft segments as a result of microphase separation).

Novel poly(aliphatic/aromatic-ester)s PED of segmented (multiblock) structure belong to the group of thermoplastic elastomers and are composed of



DPh – degree of polycondensation of hard segments (= 1.2)

Fig. 1 Chemical structure of PED copolymer.

semicrystalline poly(butylene terephthalate) (PBT) (hard segments) and dimer of fatty acid, namely hydrogenated dilinoleic acid (DLA) (soft segments) [8–10]. The resulting nanostructured morphology contributes to different mechanical properties, locating these new PED materials between commercially available thermoplastic poly(urethane-ether) and poly(ester-ether) elastomers. Their long-term mechanical properties (fatigue) are superior compared to medical grade polyurethanes or silicone rubber [11] what makes them as very interesting materials for soft tissue reconstruction. PED copolymers have been evaluated as candidate materials for preparation of tempory tendon prothesis [9–11]. PEDs are synthesized without thermal, and often irritant thermal stabilizers due to excellent oxygen and thermal stability. This feature is especially important if material is used for biomedical applications. Sterilization does not affect the structure and the properties of the polymer [8]. PED copolymers are biocompatible *in vitro* and *in vivo* [9], and when specially modified with active molecules, they show antibacterial properties [10].

To enhance the bioactivity of biodegradable and non-biodegradable polymers, and in consequence bone-bonding properties, different composites are prepared using bioactive glass and glass ceramics, hydroxyapatite (HAP) or tricalcium phosphate (β-TCP) ceramics [12–21]. Such composites represent a new group of biomaterials for tissue engineering scaffolds and biomedical implants. The first commercial polymer/calcium phosphate composite consisting of HAP mixed with HDPE (HAPEX®) was developed by Bonfield [21]. An advantage of polymer composites is the tailored Young's modulus, matching well to that of natural bone (3–25 GPa) [18].

β-TCP is used in cements, bone grafts and various polymer-ceramic combinations providing biodegradation and osteoconductivity [18, 19, 22]. TCP enhances cell differentiation by releasing Ca^{2+} and PO_4^{3-} ions as well as adjusts pH of surrounding body fluid in the case of composite scaffolds based of resorbable polyesters or polylactones such as polylactides and their copolymers [23–29], polyhydroxybutyrate (PHB) [30], poly(propylene fumarate) (PPF) [31], or CPLA/TCP [32, 33]. In earlier work we showed that low concentration of hard segments in PED copolymers favours SBF crystal adhesion to polymer surface [34]. By adding β-TCP the apatite layer formation in SBF was favoured in that areas where ceramic particles appeared on the surface [35].

The aim of this work was to obtain novel elastomeric polyester/ceramic composite materials for innovatory finger joint solution. Their thermal and mechanical properties as well the *in vitro* cytocompatibility were characterized.

2 Experimental

2.1 Materials and methods

The synthesis method of poly(aliphatic/aromatic-ester) (PED) copolymer, involving transesterification and polycondensation from the melt, was described in previous publications [8–10]. PED copolymer (Fig. 1) containing a very high amount of soft segments (74 wt.%) was used as an elastomeric matrix where β-TCP ($d_{50} = 3.2\ \mu$m, Budenheim) has been incorporated.

Composite hybrid material containing 5, 10, 20 and 30 vol% β-TCP was prepared in a single screw extruder at the temperature of 150°C. The total time for plasticizing and extruding polymer blend was 20 min to assure a good blending. The extruded strands were subsequently dried and palletized. After preparation, material was compression moulded at the temperature higher than the melting point of respective composite. Samples were prepared by press compaction according ASTM D 1897-77 and then stamped for mechanical testing (0.5 mm think micro-dumbbells) and for thermal analysis (5 mm diameter discs).

2.2 Analysis

2.2.1 Thermal properties

Differential scanning calorimetry (DSC) scans were performed with a DuPont apparatus. The samples were dried in vacuum at 70°C, and then kept in desiccator. The process was carried out in a triple cycle: first heating, then cooling, and second heating in the temperature ranges from −120°C to the temperature which was 30°C higher than the melting

point of each material. The rate of heating and cooling was 10 K min^{-1}. The glass transition temperature (T_g) was determined from the temperature diagrams as the mid point of the inflection on the curve. The DSC results were averaged from two heating-cooling scans.

2.2.2 Microscopy

Scanning electron microscopy (SEM) micrographs of the fracture surfaces of hybrid materials were acquired with a JEOL-JSB-6400 microscope. All samples were fractured in liquid nitrogen, vacuum dried and coated with gold prior to scanning. Fracture surfaces after tensile testing were also examined.

2.2.3 Tensile testing

The quasi-static tensile data were collected at room temperature with an Instron TM-M tensile tester equipped with a 500 N load cell employing a crosshead speed of 100 mm/min. The strain was measured using the clamp displacement according DIN 53 455. The starting clamp distance was 25 mm. The obtained results were averaged from 6 specimens with cross section of 0.5×4 mm. Tensile properties were determined in the dry state and in the wet state, after immersion in distilled water at 37°C for 3 days.

2.2.4 In vitro cytocompatibility

The *in vitro* cytocompatibility investigations were carried out according ISO 10 993-5. The short–term test represents a pure toxicity investigation. Polymer films were stamped into round disks of 15 mm diameter, sterilized by the ethylene oxide (EtO) and placed in 24-well plates (Greiner). Polystyrene of the 24-well culture plates served as positive control. L-929 mouse fibroblasts (DSMZ, Braunschweig) were seeded at 1.0×10^5 cells/ml for the 24 h test in 90% RPMI 1640 medium (GIBCO) supplemented with 10% fetal bovine serum (GIBCO) and 1% Penstrep (GIBCO). Cells were cultured at 37°C in a humidified atmosphere (RH = 95%) and 5% CO_2. The WST-1 test assay was used to estimate the mitochondrial activity of the cells. Samples were washed with phosphate buffered saline PBS without Ca^{2+}, Mg^{2+} (1×) (Biochrom) and incubated with 10 μl WST-1 in 750 μl 90% RPMI 1640 and 10% FBS at 37°C for one hour. The cells were enzymatically detached by trypsination (0.25% trypsin in EDTA, Sigma) and counted with a cell counter (Beckman, Coulter Z2). To investigate a possible damage of the cytoplasmic cell membrane and therefore the release of cytoplasmic components into the culture medium, the Resazurin-assay (Sigma) was introduced. Added resazurin is transformed to resofurin, which was detected using an ELISA reader (Polarstar, Optima).

Four measurements on each material were performed. The test results were evaluated as percentage of the values received with polystyrene.

Results are presented as means ± SEM. Analysis of variance (one-way ANOVA) was performed and differences considered highly significant at $0.001 > p$.

Changes in cell structure caused by the influence of the cytotoxical potential of the material were examined by using a scanning electron microscope (JSB 6400, JEOL). Cells were fixed with glutaraldehyde (Sigma), treated with paraformaldehyde (Sigma) and 0.1 M sodium cacodylic acid (Sigma), dehydrated in acetone, critical-point dried and finally gold-coated.

3 Results and discussion

3.1 DSC

Table 1 summarizes characteristic temperature transitions of a neat PED copolymer and composite materials containing variable amount of β-TCP (from 5 to 30 vol%). Analysis of DSC scans indicated that addition of variable amount of ceramic particles has no significant influence on glass transition temperature. The lowest T_g has been found for material containing 20 vol% TCP. The crystallization temperature of composite materials also decreased, showing a drop from 12°C for PED copolymer to 7°C for materials containing 30 vol% of ceramic. The melting peaks were very broad

Table 1 Characteristic temperature transitions of the neat PED copolymer and composite materials containing β-TCP

Sample	Temperature transitions					
	T_g (°C)	T_c (°C)	ΔH_c (J/g)	T_m (°C)	ΔH_m (J/g)	$W_{c,h}$ (%)
PED	−53.3	12.0	5.3	117.1	6.2	4.3
5 vol% β-TCP	−53.7	8.5	3.5	107.2	6.6	4.6
10 vol% β-TCP	−52.6	8.3	2.8	103.2	5.5	3.8
20 vol% β-TCP	−55.1	7.6	4.6	101.1	4.3	3.0
30 vol% β-TCP	−53.2	7.2	4.4	98.5	3.8	2.6

T_g—glass transition of the soft segments; T_m, T_c—melting and crystallization temperatures, respectively, of the hard segments; ΔHc, ΔH_m—crystallization and melting enthalpy of the hard segments, respectively; $W_{c,h}$—mass content of PBT crystallites in the material.

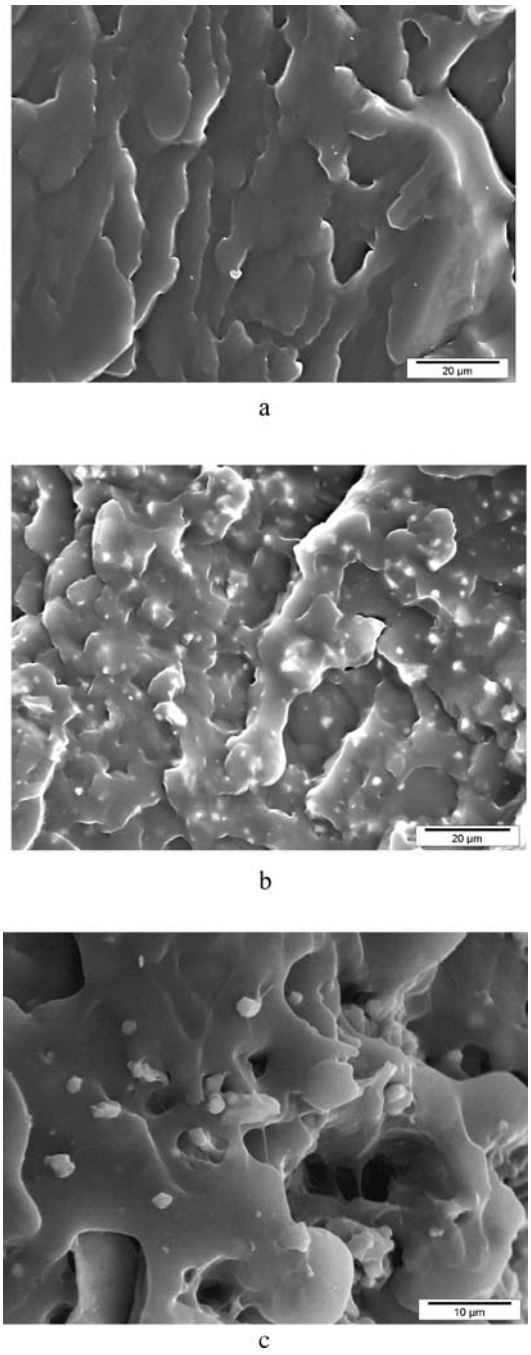


Fig. 2 SEM pictures of fracture surfaces (liquid nitrogen) of (a) PED copolymer, and (b) composite materials containing β-TCP in amount of 20 vol% and (c) after tensile fracture (PED/βTCP 20 vol%).

and melting transitions were found in a range from 117°C to 98°C. Broad and flat melting transitions were reflected by low values of melting enthalpy, ΔH_m. The mass content of PBT crystallites in the materials, as calculated from the melting enthalpy, indicated very low values from 4.3% (neat PED) to 2.6% (30 vol% β-TCP). This indicates that adding ceramic component such as β-TCP, the total crystallinity of copolymer is reduced. Ceramic component seems to disturb crystallization of semicrystalline poly(butylene terephthalate) sequences (hard segments) of a PED copolymer by possible increase of amorphous phase.

3.2 SEM

Morphology of obtained composites was visualized in the SEM photographs shown in Fig. 2. SEM micrographs prepared from samples prepared by fracture in liquid nitrogen show homogeneous distribution of the β-TCP particles in the polymer matrix. It can also be seen that β-TCP particles are covered with polymer, which is an indication of a good filler/matrix interface (Fig. 2b). The same results can be observed examining fracture surface of PED/β-TCP 20 vol% after tensile testing (Fig. 2c).

3.3 Mechanical properties

Tensile testing was performed to evaluate mechanical properties of hybrid materials. Non-modified PED copolymer exhibits excellent elastomeric properties with $780 \pm 20\%$ elongation at break and an ultimate tensile strength of 4.4 ± 0.2 MPa. Results presented in Table 2 show a significant improvement of Young's modulus (from 6.2 to 16.8 MPa) and yield strength (from 2.5 to 4.1 MPa) indicating that composite effect is achieved. After comparison of a data measured in a dry state (Table 2) and in a wet state (Table 3), the lower values of E_{mod} was observed for samples in a wet state. This can be explained by the swelling of the samples and the plasticizing effect of the water resulted in weakening of the polymer-filler interface. A typical behaviour of particle

Table 2 Mechanical properties of the neat PED copolymer and composite materials in a dry state

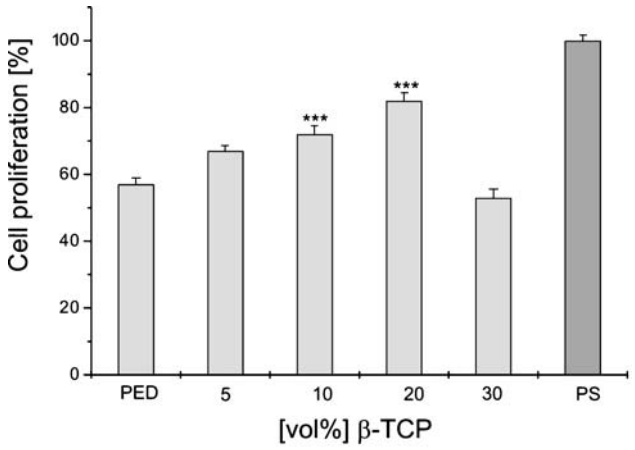
Sample	E (MPa)	σ (MPa)	σ_e (MPa)	ε (%)
PED	6.2 ± 0.4	4.4 ± 0.2	2.5 ± 0.2	780 ± 20
PED+5% β-TCP	5.9 ± 0.3	3.8 ± 0.1	2.4 ± 0.2	680 ± 20
PED+10% β-TCP	10.5 ± 0.2	3.7 ± 0.2	2.7 ± 0.1	510 ± 15
PED+20% β-TCP	14.3 ± 0.2	3.5 ± 0.1	3.3 ± 0.1	440 ± 30
PED+30% β-TCP	16.8 ± 0.3	3.9 ± 0.1	4.1 ± 0.2	340 ± 20

E—Young's modulus; σ—tensile strength; σ_e—yield strength; ε—fracture strain.

Table 3 Mechanical properties of the neat PED copolymer and composite materials in a wet state

Sample	E (MPa)	σ (MPa)	σ_e (MPa)	ε (%)
PED	6.2 ± 0.4	4.4 ± 0.2	2.5 ± 0.2	780 ± 20
PED+5% β-TCP	6.1 ± 0.3	4.0 ± 0.1	2.5 ± 0.2	600 ± 20
PED+10% β-TCP	12.1 ± 0.2	3.7 ± 0.2	2.6 ± 0.1	520 ± 20
PED+20% β-TCP	12.3 ± 0.2	3.7 ± 0.2	3.0 ± 0.1	470 ± 30
PED+30% β-TCP	15.2 ± 0.3	3.8 ± 0.1	3.5 ± 0.2	490 ± 30

E—Young's modulus; σ—tensile strength; σ_e—yield strength; ε—fracture strain.



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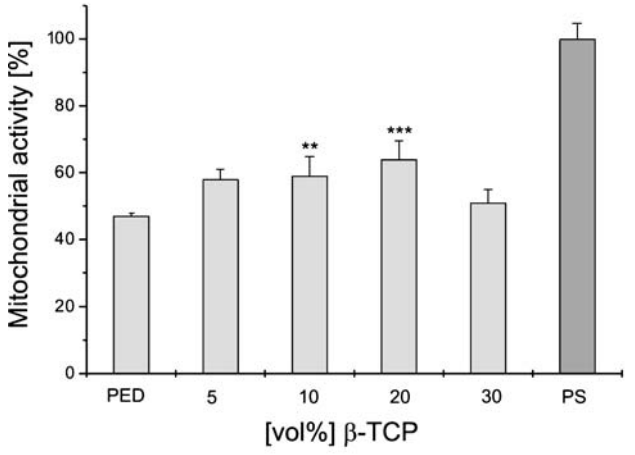

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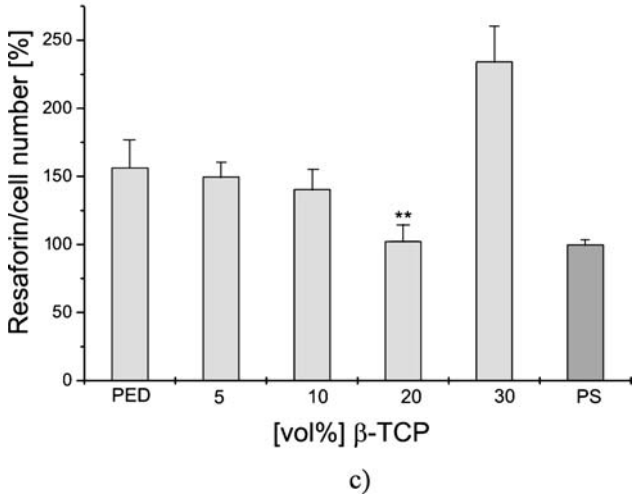
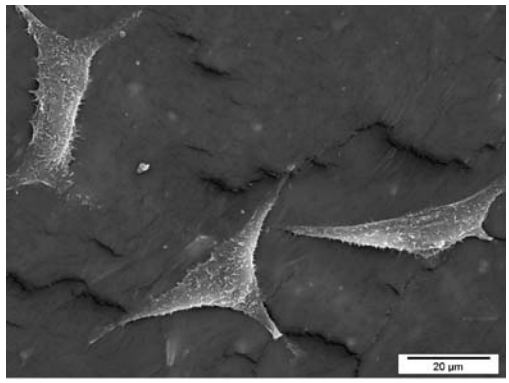


c)

Fig. 3 Cell proliferation (a), mitochondrial viability (b) and cell membrane permeability (c) of fibroblasts (L-929, 24 h) on the neat PED copolymer and composite materials containing various amount of β-TCP. (*** highly significant difference between the neat PED material and composite material, ANOVA: $0.001 > p$,** significant difference between the neat PED material and composite material, ANOVA: $0.01 > p \geq 0.001$).

filled composites is manifested by decreasing elongation at break with increasing amount of β-TCP. At a very high concentration of ceramic particles (20 vol%), hybrid materials shows very high elongation at break of about ~470%. These values indicate that PED +20% β-TCP hybrid material still shows good mechanical properties comparable to silicone rubber ($\sigma = 3 - 9$ MPa, $\varepsilon = 350 - 1500\%$). This indicate that such composite materials can be targeted on small joints thus showing comparable properties to silicone rubber.



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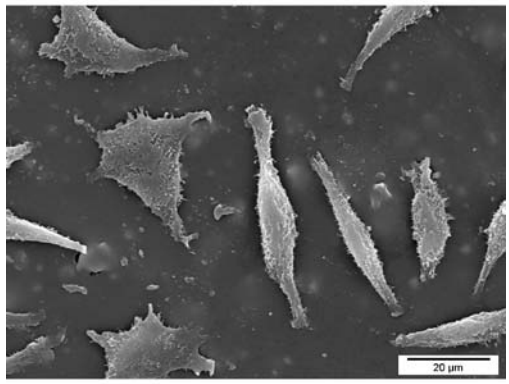

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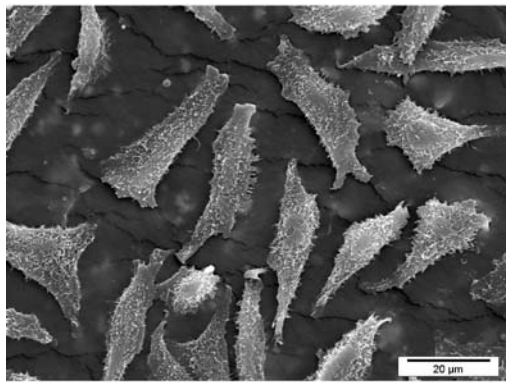

c)

Fig. 4 SEM pictures of cells on materials surface: (a) the neat PED copolymer, (b) PED+10 vol% β-TCP, (c) PED+20 vol% β-TCP.

3.4 *in vitro* cytocompatibility

By adding β-TCP the cell number and the cell viability is further improved up to an optimum at 20 vol% β-TCP. The differences in cell proliferation and mitochondrial activity between hybrid material containing 20 vol% β-TCP and the neat PED material (Fig. 3) are highly significant. Significantly reduced cell membrane permeability was determined on the composite PED +20 vol% β-TCP. The same low cell membrane permeability value like for polystyrene is reached for cells, cultured in direct contact on PED + 20 vol% β-TCP.

SEM images reflected the *in vitro* test results as indicated in Fig. 4. After an incubation time of 24 h, more and healthy fibroblasts were detected on the hybrid material. The cells are spread and show a normal surface structure. Furthermore, no sign of cell damage or cell death was observed by scanning electron microscopy.

4 Conclusions

Multiblock polyester copolymer (PED)/β-TCP composite materials containing up to 30 vol% TCP were obtained by common low cost extrusion technology. It has been shown that a composite effect and good filler/matrix interaction are achieved. The Young's modulus and the yield strength increase with volume fraction of β-TCP. Especially, very good mechanical properties, which are comparable to silicone rubber, and the demonstrated improved *in vitro* cytocompatibility of the PED/20 vol% β-TCP make this composite material interesting for orthopaedic applications, e.g. as novel solution for joints replacement. Swanson design of finger joints made from hydrophobic PED material modified on both ends (stems) by bioactive components can offer a stable implant-hard tissue interface during physiological loading. In addition to that feature, incorporation of ceramic particles into polymer matrix should induce composite's bioactivity thus overcoming common complications (fracture and buckling) associated with use of conventional silicone rubber joint implants.

Further work will focus on investigation of osteoblasts behaviour cultured on these composites as well as their fatigue properties will be evaluated.

Acknowledgments We thank T. Mummert, Friedrich-Baur-Research Institute for Biomaterials, University of Bayreuth for *in vitro* cytocompatibility investigations.

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