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Degradable phosphate glass fiber reinforced polymer matrices: mechanical properties and cell response

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Abstract The development of biodegradable materials for internal fracture fixation is of great interest, as they would both eliminate the problem of stress shielding and obviate the need for a second operation to remove fixation devices. Preliminary investigations for the production of degradable fiber reinforced polymer composite materials are detailed. Composites were produced of phosphate invert glass fibers of the glass system P_2O_5–CaO–MgO–Na_2O–TiO_2, which showed a low solubility in previous work. The fibers were embedded into a matrix of a degradable organic polymer network based on methacrylate-modified oligolactide. Fracture behavior, bending strength and elastic modulus were evaluated during 3-point bending tests and the fracture surface of the composites was investigated using a scanning electron microscope. Short-term biocompatibility was tested in an FDA/EtBr viability assay using MC3T3-E1 murine pre-osteoblast cells and showed a good cell compatibility of the composite materials. Results suggested that these composite materials are biocompatible and show mechanical properties which are of interest for the production of degradable bone fixation devices.

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Introduction

Metals and alloys are commonly used for internal fracture fixation to promote bone union at the fracture site. However, the elastic moduli of cortical bone, which range from 17 to 24 GPa [1], and of commonly used metallic fixation devices (100 to 200 GPa [2]) differ considerably, resulting in relative motion between the implant and bone upon loading as well as high stress concentrations at bone-implant junctions. As healing progresses, rigid fixation can cause bone atrophy which can result in loss of bone mass and osteoporosis [3, 4].

Degradable fixation devices provide an alternative to metal implants. They obviate the need for a second surgery to remove hardware, and they allow for the gradual transfer of stress to the healing bone, thereby eliminating the problem of stress shielding. Poly (α-hydroxy esters), especially poly lactic acid (PLA), are among the few synthetic polymers approved for human clinical uses [5, 6]. However, their lower stiffness (elastic modulus of PLA screws is about 3 GPa [7]) in comparison to metal devices may allow too much bone motion for satisfactory healing. Reinforcement therefore is essential for the development of degradable fracture fixation materials. This can be obtained on the one hand by self-reinforcement [5, 8, 9] or by fabrication of polymer matrix composites. Composite materials consist of at least two materials, which are different in composition, structure, and properties, defining a continuous phase (matrix) and at least one reinforcing phase. Currently, bioactive fillers, such as hydroxyapatite, tricalcium phosphate, and bioactive glasses, are studied extensively. Embedding particles of these materials into the polymer matrix is known to promote bone-bonding properties and increase both the elastic modulus and the strength of the resulting composite. Additionally, the

ceramic phase can act as a hydrolysis barrier, delaying the degradation of the polymer [10–15].

For completely biodegradable composites, both the continuous phase and the reinforcement should be degradable. Therefore, the use of phosphate glasses is of special interest. They are water-soluble and the degradation rate can be adjusted by altering their composition [16–21]. It was shown that degradation rates of the composite could be adjusted by changing the glass composition [22–24]. Furthermore, incorporation of glass particles into the polymer considerably increased both the compressive modulus and the compressive strength [25].

Statistically homogeneous but anisotropic media, e.g., fiber reinforced polymers, represent an important class of composite materials as they offer superior strength and stiffness in comparison with isotropic ones. Recent research included both short [26, 27] and continuous fibers [28–31] as fillers. However, the use of continuous fibers is preferable as short fiber composites do not allow to obtain as high stiffness and strength as continuous fiber composites [6, 32].

Aim of this work was the development of a completely degradable continuous phosphate glass fiber reinforced composite for use in fracture fixation. Fibers of a phosphate invert glass in the system P_2O_5–CaO–MgO–Na_2O–TiO_2 were produced using a preform technique. Fibers were embedded into a matrix of a degradable organic polymer network based on methacrylate-modified oligolactide [33]. Both the glass investigated and composite materials based on the glass and the polymer were shown to be biocompatible in previous work. Our hypothesis was, that it would be possible to obtain a fiber reinforced composite material which degrades completely, shows adequate mechanical properties for bone fixation and is biocompatible. Mechanical strength of the fiber composites was assessed in bending tests, and preliminary biocompatibility studies were performed using murine MC3T3-E1 pre-osteoblast cell line.

Materials and methods

Glass synthesis

Glass composition was 35 P_2O_5–27.5 CaO–9.5 MgO–22.5 Na_2O–5.5 TiO_2 (in mol%). The glass was prepared by melting mixtures of carbonates and metaphosphates of calcium, sodium and magnesium as well as titanium dioxide in a silica crucible at 1300°C using an electrically heated furnace. After quenching, the glass was remelted in a platinum crucible for 30 min. Melting times were kept short in order to minimize losses through evaporation. The glass melt was cast into a pre-heated rod shaped graphite mold and annealed at temperatures above transition temperature (500°C). The resulting glass rod was about 13 cm in length and had a diameter of 10 mm.

Fabrication of fibers

Fibers were produced using a preform technique. The fibers were drawn from the rod shaped preform described above at temperatures between 600 and 620°C at a rate of 6 m/min, sized and wound up on a rotating drum. As sizing, the oligomer/HEMA mixture described below was used.

Organic polymer-forming component

A dianhydro-D-glucitol bis[di(lactoyl)methacrylate] macromer was used as polymer network forming component. Macromer synthesis was performed in a two-step process as described earlier [34, 35]. Briefly, in the first step, ring-opening oligomerization of L-lactide in the presence of dianhydro-D-glucitol and stannous ethylhexanoate at 150°C for 2 h afforded the corresponding oligolactide which was purified by repeated precipitation from dichloromethane into heptane. In the second step, the obtained oligolactide was acrylated with methacryloyl chloride in the presence of triethylamine and dichloromethane as solvent. The reaction mixture was extracted several times with 1 M HCl, saturated aqueous solution of $NaHCO_3$, and distilled water, dried over Na_2SO_4, and treated with silica gel to remove coloured impurities. *p*-Methoxyphenol was added as stabilizer. After removal of the solvent and drying, the macromer shown in Fig. 1 was obtained as a yellow viscous oil.

Using the macromer and methacrylic acid 2-hydroxyethylester (HEMA) as a co-monomer (10 wt%), polymeric coating systems were produced. For polymerization, dibenzoyl peroxide was used as initiator and the mixture was cured for one hour at 100°C.

Fabrication of composites

For fabrication of fiber reinforced composites, the fibers were sized with macromer/HEMA mixture without starter directly after drawing before winding up on a rotating drum. Later, the fibers were cut into shorter pieces of about

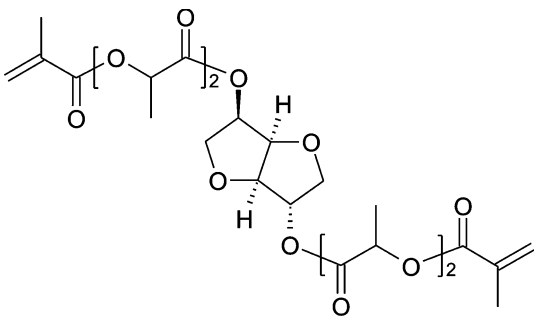

Fig. 1 Polymer network forming dianhydro-D-glucitol bis[di(lactoyl)methacrylate] macromer

50 cm in length, bunched, soaked in macromer/HEMA/dibenzoyl peroxide mixture and cured as described above.

Mechanical properties

Mechanical properties and fracture behavior of the fiber composites were evaluated in 3-point and 4-point bending tests using a hydraulic testing machine (UPM 1445, Zwick GmbH, Germany). The number of samples tested was 11 in 3-point bending and 9 in 4-point bending. The fracture surface of the composites was investigated under a scanning electron microscope (DSM 940 A, Zeiss AG, Oberkochen, Germany). Fiber composites were clamped into a small 3-point bending device in which a screw with a Teflon® tip was used for bending the sample. The composite was bent, carbon sputter-coated and the fracture was investigated using the SEM.

Cell experiments

MC3T3-E1 cells (DSMZ No. ACC 210, German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany) were cultured in alpha medium with 2 mM L-alanyl-L-glutamine (Biochrom AG, Berlin, Germany), 50 U/mL penicillin, 0.05 mg/mL streptomycin, and 10% fetal calf serum at 37 °C under 5% CO_2 atmosphere. Cell suspensions were obtained after trypsination by standard protocol.

For cell experiments, fiber composites were embedded in epoxy resin, cut and polished to expose the cross-sectional area.

Cytocompatibility was tested using a fluorescein diacetate (FDA)/ethidium bromide (EtBr) viability assay. Cell viability of MC3T3-E1 cells was assayed after 1, 4 and 8 days of culture on the fiber composites; tests were performed in triplicates. Tissue culture polystyrene (TCPS) was used as control (not shown). Scaffold slices of about 10 mm in diameter and 3 mm in height were transferred each into a separate well of a 24 well culture plate. After disinfection with 1 mL of 70% ethanol for 1 h, scaffolds were washed three times with phosphate buffered saline (PBS) and stored in complete cell culture medium for at least 2 h. The medium was changed and 50,000 cells suspended in 1 mL of culture medium were seeded into each well onto the scaffolds. After 1, 4 and 8 days, respectively, the culture medium was replaced by PBS, the scaffolds were placed onto microscopic slides, overlayed with 0.05 mL of two-fold concentrated staining solution (2×: 0.030 mg/mL fluorescein diacetate, 0.008 mg/mL ethidium bromide in PBS), covered with a cover slide and evaluated microscopically. Green and red fluorescence were monitored after 1 min using an Axiotech microscope (Zeiss AG, Jena, Germany) with filter sets 09 and 14. Photomicrographs were recorded using a CCD fluorescence imager microscope (MP 5000, Intas, Göttingen, Germany). Imaging was supported by Image-ProPlus software (Media Cybernetics, Silver Spring, MD, USA). The percentage of cells was calculated from the ratio of orange-fluorescent nuclei of dead cells and green-fluorescent living cells. Cell densities were calculated as cell numbers per area from cell numbers counted on photomicrographs mapping known areas. At each time point three independent cross sections were evaluated and counted.

Results

Glass fibers

Glass fibers had a diameter of 125 μm and a total length of about 100 m. A micrograph of the fibers is shown in Fig. 2. Polarization microscope investigations of fibers and remaining parts of the preform showed no signs of crystallization (results not shown).

Composites

Glass fibers were bunched, soaked with macromer and cured. The resulting fiber composites showed an elliptical profile of about 2 mm in height and 3 mm in width. Fig. 3 shows an SEM micrograph of a section of the fiber composite. The polymer/glass ratio in the composite was about 1:3.5 by volume.

Mechanical properties

Mechanical properties of the composites were evaluated using 3-point and 4-point bending tests. During 3-point bending tests, the fiber reinforced composite material showed bending strengths of about 115 MPa (cf. Table 1); the mean elastic modulus was 16 GPa.

Curves of 4-point bending tests (Fig. 4, top) exhibit linear behavior at the early stages of loading and then transform to non-linear, before a maximum load is reached. This is followed by a drop in load corresponding with beginning failure of the composite.

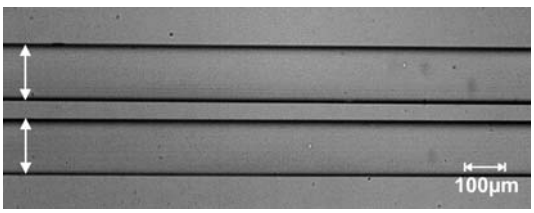

Fig. 2 LM micrograph of the glass fibers

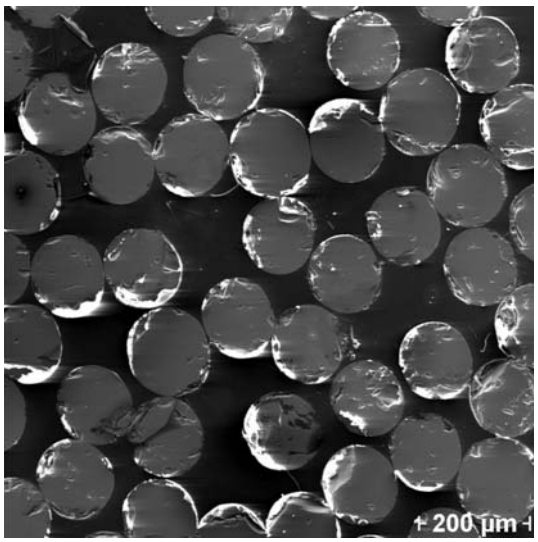

Fig. 3 SEM micrograph of a fiber composite cross section

Curves of 3-point bending tests (Fig. 4, bottom) show a fibrous fracture mode. The composites did not break evenly but by degrees when loaded. When the outer fibers broke, inner fibers still provided stability of the composite. This was confirmed by SEM micrographs of the fracture (Fig. 5), which show broken outer fibers but intact inner fibers. However, the curves show several unstable "stick-slips" during crack propagation. In this case, the load drops suddenly at a certain strain and the crack is arrested. Then the load increases steadily until the crack grows again.

The SEM micrographs (Fig. 5) also show delamination, branching cracks and fiber pull-out, which acted fracture energy consuming. Debonding of the matrix from the fibers as well as bare fibers were observed at the fracture site, indicating that the fiber-matrix bonding is not ideal.

Cell experiments

Cell viability was assayed on glass fiber reinforced polymer samples. Results of the FDA/EtBr viability assay showed that within the observation period the percentage of dead cells was less than 5% on all samples. Fluorescence micrographs (Fig. 6) showed that the cells did not only adhere on the sample surface but had grown into a continuous cell layer. Cells were evenly distributed over the cross section of fibers and polymer matrix. Cell density was similar on both materials of the composite (glass and polymer), hence, no difference between the materials could be observed regarding the adherence of cells. No signs of cytotoxicity were found; more than 95% of the cells were viable on both fiber cross sections and control cultures (control not shown). Moreover, cell proliferation on the specimens showed that the material served as suitable support for pre-osteoblast cells. The cell number per cm^2 on the cross sections developed from 17.740 ± 1.370 (standard deviation) at 1 day of culture to 152.810 ± 20.770 (SD) at 8 days of culture corresponding to an 8.6 fold increase within 7 days.

Table 1 Mechanical properties of fiber reinforced polymer composite: bending strength (σ_{max}) and elastic modulus (E) ± standard deviation

σ_{max} in MPa	E in GPa
115.4 ± 11.9	16.0 ± 2.4

Discussion

The ideal implant material for fracture fixation would be biocompatible, chemically related to the surrounding tissue, and would degrade at the same rate at which the bone healed. The rate of resorption should not exceed the rate of bone formation, and the rate at which the implant weakens should closely match the increase in tissue strength to ensure a gradual stress transfer. Furthermore, fracture fixation materials must show adequate mechanical properties and be able to withstand the process of implantation. Fiber reinforcement increases both bending strength and elastic modulus in comparison with polymer alone and it also leads to more ductile behavior [36, 37]; increasing amounts of fibers result in an increase in strength [38]. Completely degradable phosphate glass fiber reinforced polymers show potential for use as temporary fixation devices. They combine the mechanical strength of glass with the pliability of a plastic component and their strength and rigidity decrease with time as the materials degrade [26, 30, 37].

In this work, we performed preliminary studies to develop and characterize completely degradable glass fiber reinforced polymer matrices for use as fracture fixation devices. In previous work [16], we showed that titanium dioxide stabilized phosphate invert glasses gave the best results in proliferation assays with murine pre-osteoblast cells. This was attributed to the lower solubility, which affected initial cell adhesion and subsequently cell numbers on the glasses. In addition, the incorporation of titanium dioxide into the invert glass structure stabilized the network against crystallization to allow for fiber production from a preform. These phosphate glass fibers were incorporated into a matrix of a degradable organic polymer network based on methacrylate-modified oligolactide, which exhibited a good biocompatibility in previous *in vitro* studies [39].

Mechanical properties of the specimens were evaluated in 3-point and 4-point bending tests. Results showed elastic moduli of 16 GPa and bending strengths of around

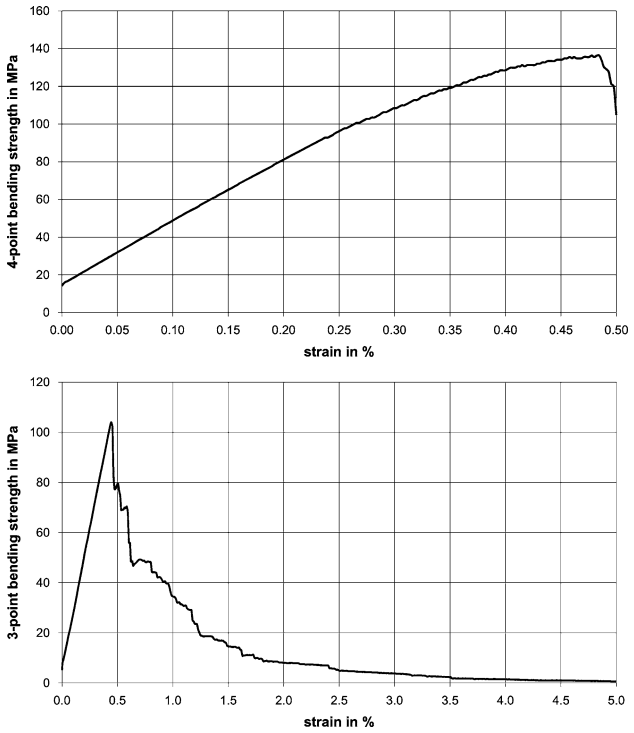

Fig. 4 Exemplary graphs of 4-point (top) and 3-point (bottom) bending tests

115 MPa. Elastic moduli of the composites were in the range of elastic moduli of cortical bone. Hence, complications due to stress shielding are not to be expected. However, a slightly higher elastic modulus might be desirable to prevent bone motion during the healing process. This could possibly be obtained by production of composites, which contain a larger number of fibers with smaller diameter.

Investigations of the fracture behavior showed a fibrous fracture mode, which is desired as sudden failure of implants for internal fixation can result in fatal consequences for the patient. The curves of the bending tests showed that the combination of fibers and polymer positively affected the overall stability of the material. While glass fibers alone show a brittle fracture mode, the combination with polymer assures that the material does not break evenly but by degrees. However, the curves show several unstable "stick-slips" during crack propagation.

SEM micrographs of the fracture site show broken outer fibers but intact inner fibers which still provided partial stability of the composite. SEM micrographs also show delamination, branching cracks and fiber pull-out, which acted fracture energy consuming. Debonding of the matrix from the fibers as well as bare fibers can be observed at the fracture site, indicating that the fiber-matrix bonding is not ideal and needs to be improved. Fiber breakage occurs by the development of delamination during loading. The main crack then grows through fractures of both fiber and polymer matrix.

The strength of fiber composites depends on the evolution of damage, which is a combination of fiber fracture, matrix cracking, debonding, fiber pull-out, and inelastic matrix deformation throughout the application of loading [40, 41]. These consume the main parts of fracture energy thus improving fracture toughness [36]. However, one of the key parameters in controlling the successful design of polymer matrix composites is the control of the interface properties between the matrix (i.e. polymer) and the filler (glass fibers). The interface can be improved by, either chemical bonding or physical interlocking between the matrix and the reinforcement. The goal is to obtain a good transfer of load from the continuous phase to the filler. Since glass fibers act as a barrier, cracks cannot pass easily. In this case, the cracks should either move towards the interface or break the fiber. At poor adhesion, the dominant mechanism is moving towards the interface leading to debonding and delamination. At strong adhesion, fiber breakage will be the dominant mechanism.

In the case of completely degradable fiber composites, adhesion between matrix and filler is also of special importance to maintain implant performance in an aqueous environment. Completely degradable fiber reinforced

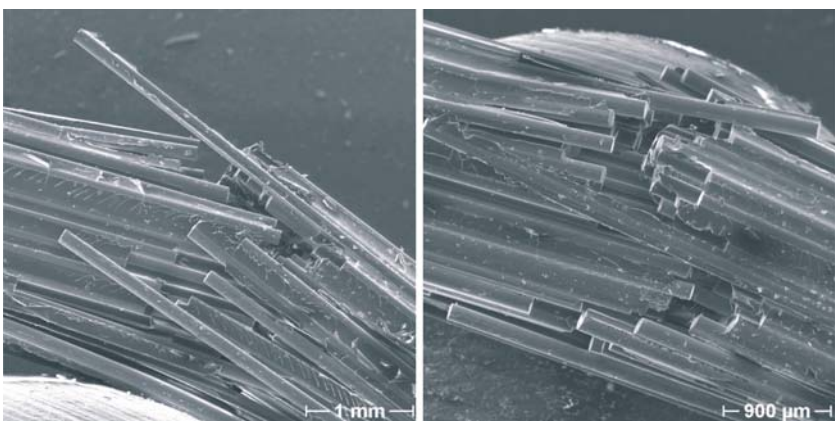

Fig. 5 SEM micrograph of fiber composite: fracture during 3-point bending

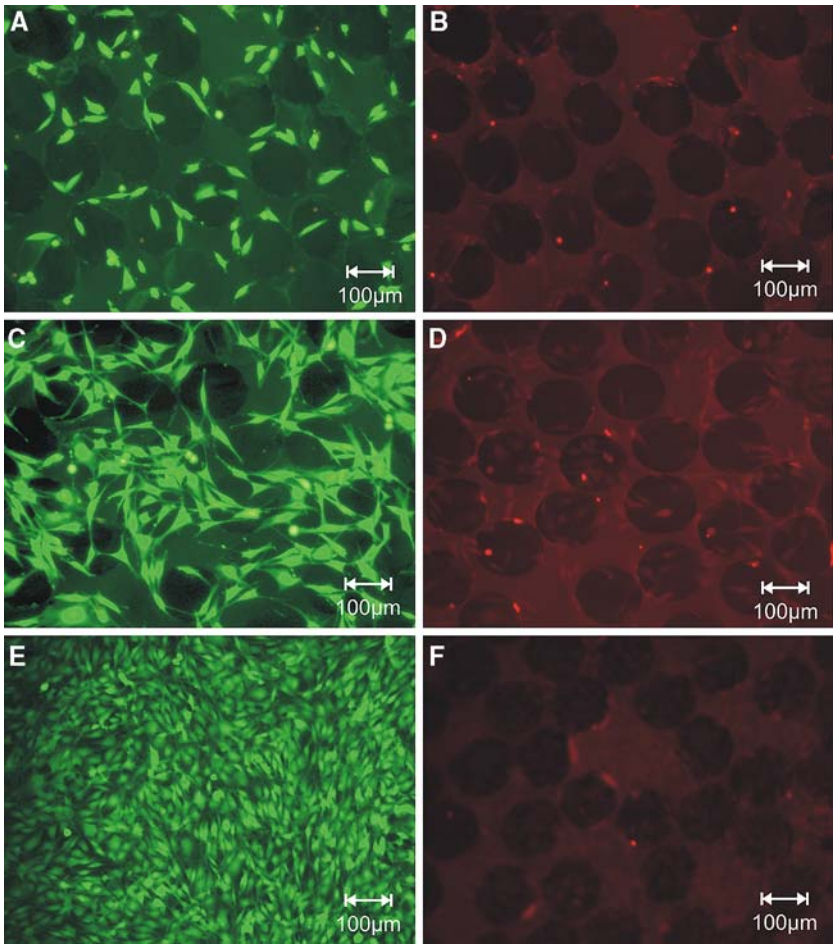

Fig. 6 Fluorescence micrographs of fiber composite cross sections settled with MC3T3-E1 cells after 1 day (top), 4 days (middle), and 8 days (bottom); left column: detection of green fluorescent viable cells; right column: detection of orange fluorescent nuclei of dead cells (same area as on the left), partly detached from substrate, with single fiber cross sections visible in this view

polymers described in literature often degraded too rapidly resulting in deterioration of mechanical properties. This was attributed to inadequate bonding between the glass fibers and the matrix causing water absorption and subsequent delamination [15, 37].

Initial biocompatibility studies on the fiber composites were performed using murine MC3T3-E1 pre-osteoblast cells and an FDA/EtBr assay. Cell viability was assessed after 1, 4 and 8 days *in vitro*. The specimens showed no signs of cytotoxicity; on both the fiber composites and tissue culture polystyrene control samples more than 95% of the cells were viable. Moreover, cells adhered on the composite surface, proliferated, and grew into a continuous cell layer. Hence, the material served as suitable support for pre-osteoblast cells.

Conclusion

Phosphate glasses are an interesting group of materials for polymer reinforcement. As their solubility can be adjusted by changing their composition, they are especially interesting as reinforcement for degradable implants for internal fixation.

Preliminary investigations for the development of degradable fiber reinforced polymer composite materials were performed. Phosphate glass fibers with diameters of around 125 µm were embedded into a polymer matrix based on methacrylate-modified oligolactide. The resulting specimens showed a good biocompatibility *in vitro*. 3-point bending tests showed bending strengths of around 115 MPa and elastic moduli of around 16 GPa, which are similar to those of cortical bone. Investigations of the fracture behavior showed the desired fibrous fracture mode but also delamination, indicating that fiber matrix bonding is not ideal. Further improvement of the mechanical properties of the fiber composites will be a subject of future studies.

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References

1. K. A. HING, *Philos. Trans. R. Soc. Lond A* **362** (2004) 2821
2. T. J. CORDEN, I. A. JONES, C. D. RUDD, P. CHRISTIAN and S. DOWNES, *Composites: Part A* **30** (1999) 737
3. L. CLAES, *J. Orthop. Res.* **7** (1989) 170
4. S. J. FERGUSON, U. P. WYSS and D. R. PICHORA, *Med. Eng. Phys.* **18** (1996) 241
5. P. TÖRMÄLÄ, *Adv. Mater. Deerfield* **4** (1992) 589
6. J. F. MANO, R. A. SOUSA, L. F. BOESEL, N. M. NEVES and R. L. REIS, *Compos. Sci. Technol.* **64** (2004) 789
7. D. A. RIKLI, R. CURTIS, C. SCHILLING and J. GOLDHAHN, *Injury, Int. J. Care Injured* **33** (2002) 77
8. A. SAIKKU-BÄCKSTRÖM, R. M. TULAMO, T. POHJONEN, P. TÖRMÄLÄ, J. E. RÄIHÄ and P. ROKKANEN, *J. Mater. Sci: Mater. Med.* **10** (1999) 1
9. P. TÖRMÄLÄ, *Clin. Mater.* **10** (1992) 29
10. V. MAQUET, A. R. BOCCACCINI, L. PRAVATA, I. NOTINGHER and R. JEROME, *Biomaterials* **25** (2004) 4185
11. K. G. MARRA, J. W. SZEM, P. N. KUMTA, P. A. DIMILLA and L. E. WEISS, *J Biomed. Mater. Res.* **47** (1999) 324
12. J. C. KNOWLES, G. W. HASTINGS, H. OHTA, S. NIWA and N. BOEREE, *Biomaterials* **13** (1992) 491
13. G. JIANG, M. E. EVANS, I. A. JONES, C. D. RUDD, C. A. SCOTCHFORD and G. S. WALKER, *Biomaterials* **26** (2005) 2281
14. E. URAL, K. KESENCI, L. FAMBRI, C. MIGLIARESI and E. PISKIN, *Biomaterials* **21** (2000) 2147
15. M. A. SLIVKA, C. C. CHU and I. A. ADISAPUTRO, *J. Biomed. Mater. Res.* **36** (1997) 469
16. D. S. BRAUER, C. RÜSSEL, W. LI and S. HABELITZ, *J. Biomed. Mater. Res. A* **77A** (2006) 213
17. A. J. PARSONS, M. EVANS, C. D. RUDD and C. A. SCOTCHFORD, *J. Biomed. Mater. Res. A* **71A** (2004) 283
18. J. C. KNOWLES, *J. Mater. Chem.* **13** (2003) 2395
19. M. UO, M. MIZUNO, Y. KUBOKI, A. MAKISHIMA and F. WATARI, *Biomaterials* **19** (1998) 2277
20. A. E. MARINO, S. R. ARRASMITH, L. L. GREGG, S. D. JACOBS, G. R. CHEN and Y. DUC, *J. Non-Cryst. Solids* **289** (2001) 37
21. J. VOGEL, P. WANGE, S. KNOCHE and C. RÜSSEL, *Glass Sci. Technol.* **77** (2004) 82
22. J. C. KNOWLES and G. W. HASTINGS, *J. Mater. Sci.: Mater. Med.* **4** (1993) 102
23. R. L. PRABHAKAR, S. BROCCHINI and J. C. KNOWLES, *Biomaterials* **26** (2005) 2209
24. D. S. BRAUER, C. RÜSSEL, S. VOGT, J. WEISSER and M. SCHNABELRAUCH, *J. Biomed. Mater. Res. A* **80A** (2007) 410
25. M. NAVARRO, M. P. GINEBRA, J. A. PLANELL, S. ZEPPETELLI and L. AMBROSIO, *J. Mater. Sci: Mater. Med.* **15** (2004) 419
26. K. P. ANDRIANO, A. U. DANIELS, W. P. SMUTZ, R. W. B. WYATT and J. HELLER, *J. Appl. Biomater.* **4** (1993) 1
27. K. P. ANDRIANO, A. U. DANIELS and J. HELLER, *J. Appl. Biomater.* **3** (1992) 197
28. P. CHRISTIAN, I. A. JONES, C. D. RUDD, R. I. CAMPBELL and T. J. CORDEN, *Composites: Part A* **32** (2001) 969
29. R. L. DUNN, R. A. CASPER and B. S. KELLEY, *Trans. Soc. Biomater.* **8** (1985) 213
30. T. C. LIN, *Trans. Soc. Biomater.* **9** (1986) 166
31. K. J. LOWRY, K. R. HAMSON, L. BEAR, Y. B. PENG, R. CALALUCE, M. L. EVANS, J. O. ANGLEN and W. C. ALLEN, *J Biomed. Mater. Res.* **36** (1997) 536
32. T. J. CORDEN, I. A. JONES, C. D. RUDD, P. CHRISTIAN, S. DOWNES and K. E. MCDOUGALL, *Biomaterials* **21** (2000) 713
33. M. SCHNABELRAUCH, S. VOGT, Y. LARCHER and I. WILKE, *Biomol. Eng.* **19** (2002) 295
34. S. VOGT, J. VOGEL and M. SCHNABELRAUCH, *Eur. J. Trauma* **2** (2002) 119
35. S. VOGT, Y. LARCHER, B. BEER, I. WILKE and M. SCHNABELRAUCH, *Eur. Cell Mater.* **4** (2002) 30
36. S. M. ZEBARJAD, *Mater. & Des.* **24** (2003) 531
37. M. C. ZIMMERMAN, H. ALEXANDER, J. R. PARSONS and P. K. BAJPAI, *ACS Symp. Ser.* **457** (1991) 132
38. P. K. VALLITTU, *J. Oral Rehabil.* **25** (1998) 100
39. S. VOGT, S. BERGER, I. WILKE, Y. LARCHER, J. WEISSER and M. SCHNABELRAUCH, *BioMed. Mater. Eng.* **15** (2005) 73
40. L. KROLL and W. HUFENBACH, *Mech. Compos. Mater.* **35** (1999) 277
41. Y. TOMITA, T. TAMAKI and K. MORIOKA, *Mater. Character.* **41** (1998) 123