Development of a Bioactive Glass Fiber Reinforced Starch–Polycaprolactone Composite

H. Jukola,[1] L. Nikkola,[1] M. E. Gomes,[2,3] F. Chiellini,[4] M. Tukiainen,[1] M. Kellomäki,[1] E. Chiellini,[4] R. L. Reis,[2,3] N. Ashammakhi[1,5]

[1] Institute of Biomaterials, Tampere University of Technology, Tampere, Finland

[2] 3B's Research Group, University of Minho, 4710 Braga, Portugal

[3] Department of Polymer Engineering, Campus de Azurém, U. Minho, 4800 Guimarães, Portugal

[4] Laboratory of Bioactive Polymeric Materials for Biomedical and Environmental Applications, UdR-INSTM, Department of Chemistry and Industrial Chemistry, University of Pisa, Pisa, Italy

[5] Institute of Science and Technology in Medicine, Keele University, Robert Jones and Agnes Hunt Orthopaedic Hospital, Oswestry, Shropshire SY10 7AG, UK

Received 16 January 2007; revised 24 January 2008; accepted 4 February 2008
Published online 3 April 2008 in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jbm.b.31093

Abstract: For bone regeneration and repair, combinations of different materials are often needed. Biodegradable polymers are often combined with osteoconductive materials, such as bioactive glass (BaG), which can also improve the mechanical properties of the composite. The aim of this work was to develop and characterize BaG fiber reinforced starch–poly-ε-caprolactone (SPCL) composite. Sheets of SPCL (30/70 wt %) were produced using single-screw extrusion. They were then cut and compression-molded in layers with BaG fibers to form composite structures with different combinations. Mechanical and degradation properties of the composites were studied. The actual amount of BaG in the composites was determined using combustion tests. Initial mechanical properties of the reinforced composites were at least 50% better than the properties of the nonreinforced specimens. However, the mechanical properties of the composites after 2 weeks of hydrolysis were comparable to those of the nonreinforced samples. During the 6 weeks hydrolysis the mass of the composites had decreased only by about 5%. The amount of glass in the composites remained as initial for the 6-week period of hydrolysis. In conclusion, it is possible to enhance initial mechanical properties of SPCL by reinforcing it with BaG fibers. However, mechanical properties of the composites are typical for bone fillers and strength properties need to be further improved for allowing more demanding bone applications. © 2008 Wiley Periodicals, Inc. J Biomed Mater Res Part B: Appl Biomater 87B: 197–203, 2008

Keywords: starch; poly-ε-caprolactone; bioactive glass fiber; composite; bone applications

INTRODUCTION

Starch is used in blends with other biodegradable polymers as it is known to be biodegradable, it is economically feasible, it is easy to convert into a thermoplastic polymer, and it has good availability.[1–3] So far, there are several starch-based blends which have been studied. Starch has been blended at least with ethylene vinyl alcohol,[1–2,4–11] cellulose acetate,[2,4–7,12] polylactide,[5,13] and poly-ε-caprolactone (SPCL).[1,5–7,14,15] Starch-based polymers are nowadays manufactured for various applications, for example to agriculture applications and packaging segments.[16] In addition they have shown to have good potential to be used in different biomedical applications, especially in bone applications such as tissue engineering scaffolds, bone cements, drug-delivery applications, or bone fixation devices.[1,3,4,6–8,14,15]

SPCL is a potential new biocompatible and biodegradable blend which is studied to be used in bone applications. It has shown relatively good biocompatibility.[3,6,7,14]

Correspondence to: N. Ashammakhi (e-mail: n.ashammakhi@orth.keele.ac.uk)

Contract grant sponsor: Technology Development Center in Finland (TEKES); contract grant number: 90220.

Contract grant sponsor: European Commission (European Union Project EXPERTISSUES); contract grant number: NMP3-CT-2004-500328.

Contract grant sponsor: Academy of Finland; contract grant number: 37726.

Contract grant sponsors: The Ministry of Italian University (MIUR)-Rome, through the PRIN'06 prot. n. 2006038548 project; InterUniversity Consortium of Materials Science and Technology (INSTM), Florence, through the PRISMA'04 project.

Studies have shown that degradation of SPCL is enzymatic.[1] α-Amylase is mainly responsible for the degradation of starch, but also glucoamylase and some other enzymes can affect to the rate of degradation. PCL also degrades by enzymatic oxidation and/or hydrolysis after implantation. Enzymes that affect to the degradation of PCL are lipase, cholesterol esterase, and carboxyl esterase. These enzymes are present in the human body, and they catalyze the hydrolysis of the ester bonds present in polyesters.

Bioactive glass (BaG) is a group of inorganic materials which is well known for its bioactive properties. When implanted into a bone, BaG remodels to form hydroxy carbonate apatite, which is the chemical and structural equivalent of bone mineral. This leads to the formation of a bond with the bone. BaG has been compounded with synthetic and natural polymers or their combinations not only because of its bioactivity, but also for reinforcing purposes.[17,18–20] In this study we used BaG 1-98. The mechanical properties of BaG 1-98 has been studied and compared to other bioactive glasses.[21] The diameter of the fibers studied was 20–35 μm. It was found that the tensile strength of the glass fibers 1-98 was about 2200 MPa, and it was the highest obtained in this study for bioactive glasses. However, the mechanical strength is lost fast, so that after immersion in Na-PBS for 2 h the mechanical strength of the fibers is only about 310 MPa. Bioactive glasses 1-98 and 13-93 have much in common in their compositions; however, the strength properties of BaG 1-98 are remarkably higher than for BaG 13-93 fiber (about 1350 MPa). Pirhonen et al. studied the tensile strength properties of BaG 9-93, and they found that the tensile strength glass fibers (diameter 20–40 μm) was 1625 MPa.[22] Average tensile strength of BaG 45S5 fibers (diameter 79 μm) has been reported to be about 340 MPa.[23]

The problem with many current biodegradable screws and plates is the lack of osteoconductive properties. Our attempt in the current study was to develop a material that can be osteoconductive and can be used to develop devices for reparative medicine (e.g., plates, screws, filling materials) and potentially for regenerative medicine (scaffolds). Practically, the aim of this study was to develop and characterize BaG fiber-reinforced SPCL composite. BaG 1-98 fibers were chosen to be used as reinforcing material because they have high initial mechanical strength and they also add bioactivity to the implant.[24] The properties of the composite were studied during the 6-week hydrolysis.

MATERIALS AND METHODS

Materials

SPCL (30/70 wt %) polymer (Novamont, Novara, Italy) was kindly donated by University of Minho, 3B's Research Group. It was a blend material in the form of small granules containing 70 wt % PCL and 30 wt % starch. Bioactive glass 1-98 (BaG) fibers (Tampere University of Technology, Institute of Biomaterials) were used for reinforcement of the polymer (diameter, 31–43 μm). The composition and manufacturing of the BaG fibers is presented in previous studies.[24]

Processing

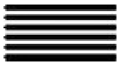
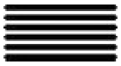
The polymer was extruded into a thick sheet using a single-screw extruder Gimac Microextruder TR 12/24 B.V.O (Gimac di Maccagnan, Castronno, Italy) and a rectangular nozzle die (40 mm × 0.5 mm). The polymer was fed into the extruder using a feeding unit with nitrogen atmosphere. Processing temperatures in heating elements were 110–150°C. The thickness of the processed billet was 0.6 mm and the width was 30 mm. The billet was further cut to 10 mm × 50 mm × 0.6 mm pieces for the compression molding.

BaG fibers (diameter 31–43 μm, length 250 mm) were bound together using SPCL-acetic acid solution before compression molding for ease of handling. First, the polymer was dissolved in to acetic acid to obtain a solution of 6 wt % of SPCL. Then about 3 g of the BaG fibers were laid unidirectionally into a Teflon mold (10 cm × 19 cm) and moistened with about 25 mL of polymer–solvent solution that was spread all over the fibers. The mold was left in the fume chamber until the next day. The larger membrane was removed from the mold and cut to pieces (10 mm × 50 mm). BaG membranes and SPCL sheets were compression molded to composite structures (NIKE Hydraulics ZB110, NIKE Hydraulics Ab, Eskilstuna, Sweden). Parameters used in processing the samples were as follows: temperature 90°C, pressure 10 MPa, and a heating time of 1.5 min after reaching the maximum temperature. Data about the manufactured specimens is presented in Table I.

Characterization *In Vitro*

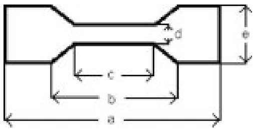
In vitro tests were performed using a phosphate buffered solution, containing NaOH (0.04 mol/L) and KH_2PO_4 (0.05 mol/L). The pH of the solution was 7.4 ± 0.05 and the incubation temperature was 37°C. *In vitro* studies were carried out in static conditions. The amount of buffer solution was about 30 times the weight of the sample, according to the standard ISO 15814:1999(E), and it was changed weekly.[25] For the samples tested for bending and shear strength, the amount of the buffer solution was 60 mL, and for the samples tested for tensile strength, the amount of the buffer solution was 30 mL. When changed, pH of the *in vitro* solution was measured. Measurements were done using Mettler Toledo MP125 pH meter (Mettler Toledo). There were six parallel samples at each time point, and the total incubation period was 6 weeks. Material characteristics were measured at follow-up points of 0, 2, 4, and 6 weeks.

TABLE I. Names, Structures, Sizes, BaG Contents, Amount of Tested Samples, and Test Procedures Performed for Different Kinds of Samples Made from Extruded SPCL Sheets and BaG Membranes by Using Compression Molding As a Method

Name	Structure	Size of the sample (mm) ($w \times l \times t$)	BaG content (wt %)	Amount of tested samples	Mechanical test procedure
6xSPCL		10 × 50 × 3	0	3	Bending
6xSPCL+5xBaG		10 × 50 × 3.7	13 ± 2	3	Bending
6xSPCL		8 × 50 × 3	0	6	Shear
6xSPCL+5xBaG		8 × 50 × 3.3	13 ± 1	6	Shear
3xSPCL		*Thickness, 1.4	0	6	Tensile
3xSPCL+2xBaG		*Thickness, 1.6	10 ± 1	6	Tensile
3xSPCL+4xBaG		*Thickness, 1.8	17 ± 2	6	Tensile
3xSPCL+6xBaG		*Thickness, 1.9	21 ± 3	6	Tensile

Black lines indicate SPCL sheet and gray lines BaG membrane.

* The test specimen was as follows: a = 45 mm, b = 25 mm, c = 20 mm, d = 6 mm, e = 10 mm.



Mass Changes. Changes in mass of the 6xSPCL and 6xSPCL+5xBaG samples were followed during the hydrolysis. The measurements were made using Mettler Toledo AT261 DeltaRange® precision scales (Mettler Toledo). The initial mass of the samples was measured prior to incubation. Immediately upon removal from the incubation solution, the wet mass of the specimens was measured. The specimens were then dried in a fume chamber for at least 72 h and then in the vacuum chamber at room temperature for 24 h. Subsequently, the dry mass of the samples was measured. Three parallel samples from both series (6xSPCL and 6xSPCL+5xBaG) were measured at each time point.

Mechanical Properties. The mechanical properties of the specimens were followed during the hydrolysis by means of shear, bending, and tensile tests. Tests were performed using Instron 4411 material testing machine (Instron, High Wycombe, England). Sample types, sizes, glass contents in the samples, and the amounts of tested pieces are shown in Table I.

Shear strength was determined by modifying the standard BS 2782,[26] the size of the tested samples was different than in the standard. The shear strength of each piece was calculated as follows:

$$S = \frac{F}{BT} \tag{1}$$

where S is the shear strength (in MPa), F is the force (in N) at fracture, B is the mean width (in mm) of the test piece, and T is the mean thickness (in mm) of the test piece. Shear tests were performed using 5 kN cell. The crosshead speed was 10 mm/min. Shear strength was tested perpendicular to the glass fibers.

Bending strength and strain at maximum load were determined by modifying the standard SFS-EN ISO 178.[27] The size of the tested samples was not as it is recommended in the standard, and that is why the test set up was modified also by means of the radius of the testing parts loading nose and the radius of the edges of the supports and the distance between the edges of the supports. In addi-

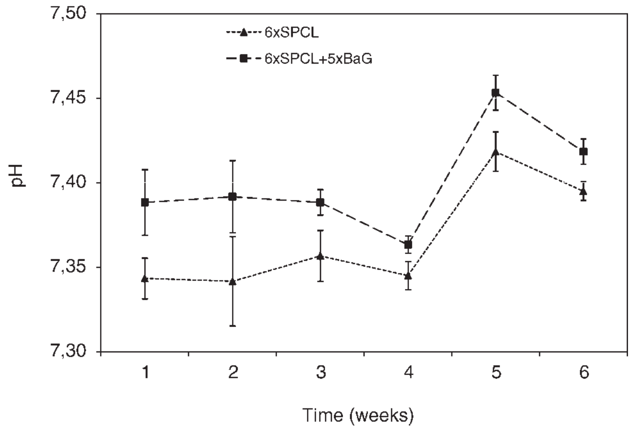


Figure 1. Changes in pH during 6 weeks hydrolysis in samples with and without BaG.

tion, there were only three parallel samples. The radius of the testing parts loading nose was 5 mm, and the radius of the edges of the supports was 1.5 mm. The distance between the edges of the supports was 32 mm. Bending tests were performed using 500 *N* cell. Speed of testing was 10 mm/min. Bending strength was tested perpendicular to the glass fibers.

The test for determining the ultimate tensile strength and the strain at maximum load was done according the standard SFS-EN ISO 527-1.[28] Tensile strength was measured by drawing the sample at a constant speed parallel to the glass fibers and measuring the strength resisting the strain until the sample broke or elongated for 30 mm. The cell used in tensile tests was 5 kN, and the crosshead speed was 20 mm/min. The gauge length was 20 mm and the grip distance 25 mm.

BaG Amount in the Composites. The actual amount of glass fiber in the composites was measured by utilizing the combustion test modified from standard ISO 1172.[29] The actual amount of glass in the composites can be calculated using following Eq. (2)

$$P = \frac{m_3 - m_1}{m_2 - m_1} \times 100 \qquad (2)$$

where m_1 is the weight of the crucible, m_2 is the weight of the crucible and specimen, and m_3 is the weight of the crucible and inorganic fraction. Standard ISO 1172 was modified because the initial mass of the samples was not enough (standard requires at least 2 g) and because there were only three parallel samples when studying bending tested samples (the standard requires at least four samples). The glass amount was determined for all mechanically tested samples (contents given as averages).

RESULTS

Processing

It was possible to utilize extrusion as processing method for SPCL to produce sheet. Solvent casting using acetic acid as a solvent for SPCL was feasible when manufacturing the BaG membranes. By using compression molding, it was easy to manufacture the final composites, although the process was quite slow.

Changes in pH

The change in the pH of the buffer solution during the degradation test indicates the effect of BaG when compared to pure SPCL. The results are presented in Figure 1. In the samples containing bioactive glass, the pH remained at 7.39 for the first 3 weeks, while the pH of the samples without BaG was about 7.35. There was a small drop for both samples at the 4 weeks *in vitro* but at 5 weeks the pH increased about 0.1 U. Then at the 6 weeks, the pH of both samples again decreased about 0.05 U. It seems that the pH of the samples containing BaG was about 0.05 U higher after the 6 weeks in hydrolysis than the pH of the samples containing only SPCL. However, the difference was not remarkable.

Mass Change

There were no major differences between the plain samples or composites. After 2 weeks hydrolysis, the wet mass was about 10% higher than the dry mass of the samples and this remained same for the 6 weeks of hydrolysis. The dry mass of the samples decreased about 5% from the initial after 2 weeks in hydrolysis and remained the same for the rest of the hydrolysis.

Changes in Mechanical Properties

Results from shear strength tests are presented in Figure 2. The initial shear strength of the BaG fiber reinforced composites (25 MPa) was about 56% higher than the shear strength of the samples without the BaG fibers (16 MPa). However, after 2 weeks of hydrolysis, the shear strength of both types of sample was about the same (10 MPa) and this continued for the 6 weeks hydrolysis.

Changes in bending strengths are presented in Figure 3. The initial bending strength of the BaG fiber reinforced composites (50 MPa) was about 66% higher than that of

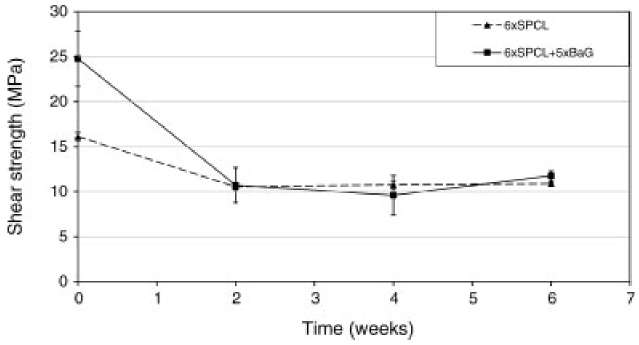


Figure 2. Changes in shear strength *in vitro* for 6xSPCL+5xBaG and 6xSPCL series.

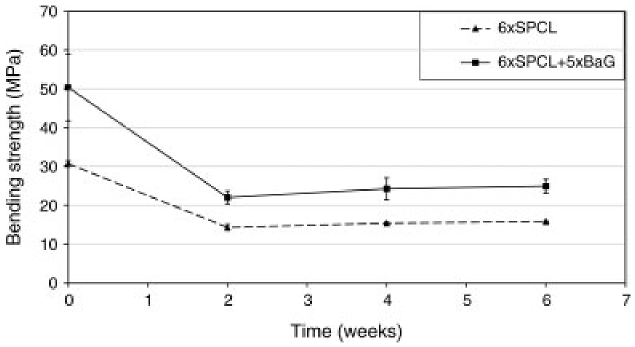


Figure 3. Changes in bending strength *in vitro* for 6xSPCL+5xBaG and 6xSPCL series.

the polymer blend samples (30 MPa). After 2 weeks of hydrolysis, the bending strength of both types of samples had decreased being 15 MPa for 6xSPCL and 24 MPa for 6xSPCL+5xBaG. Strain at maximum load increased slightly during hydrolysis for both series; more with the composite series. For the series 6xSPCL, the strain was first at 0.1 mm/mm and it increased to 0.14 mm/mm. The initial strain for the series 6xSPCL+5xBaG was 0.05 mm/mm and increased to 0.15 mm/mm.

Results from tensile strength tests are presented in Figure 4. Initial tensile strength was highest for the series that contained the smallest amount of BaG fibers (26 MPa) and lowest for the series without reinforcement (17 MPa). After 2 weeks of hydrolysis of the series reinforced with BaG fibers, tensile strengths were almost equal (10–14 MPa) and this behavior remained the same for the rest of the hydrolysis.

BaG Content

Initial amounts of BaG in different composites are presented in Table I. Within the hydrolysis, there were some changes in the amount of BaG in the samples, which, however, remained within variation.

DISCUSSION

Polymers blended with starch have been studied for different bone applications [2,4,8,12–15,30]. Blending with starch may have an effect on degradation time and on mechanical properties of the resulting blends. Starch is a polysaccharide and due to its heteropolymeric nature (macromolecular backbone containing oxygen atoms), it is expected that biodegradation mediated by enzymes may occur as in the case of chitosan.[31] However, more studies are needed to investigate the degradation of starch-based polymers *in vivo.*

pH changes during the hydrolysis were quite small for both kinds of samples, and pH varied between 7.35 and 7.45 during the 6 weeks hydrolysis. But, still through the 6 weeks period of hydrolysis the samples with BaG had higher pH. This behavior has also been seen in other studies where BaG and biodegradable polymer has been compounded,[19] and it is possibly due to the surface reactions of BaG, which occur from the contact with buffer solution.[32] The pH of the 6xSPCL series was slightly lower than the initial pH of the buffer solution for the first 4 weeks. This is due to the degradation of PCL, which usually begins with random hydrolytic break of the ester linkages autocatalyzed by the acidic carboxyl end groups, decreasing the pH of the solution.[33] However, the changes during the hydrolysis were small and the pH was near the physiological pH (7.4). BaG seemed to neutralize the acidic effect of PCL degradation products.

There were no major mass changes of the samples during the hydrolysis. Weight loss was similar for both kinds (6xSPCL, 6xSPCL+5xBaG) of samples. Usually, the degradation of PCL starts with the continuous decrease in the molar mass, but there is no weight loss during the first stage of the degradation.[33] Because of quite short hydrolysis study, it may be that the degradation had not yet proceeded to the second stage, where the decrease of mass is faster. Molar mass was not followed up during this study. Water absorbing ability of both kinds of samples was quite similar.

It was possible to obtain at least 50% higher initial mechanical properties for the compression-molded SPCL samples by using BaG fiber reinforcement. However, after 2 weeks in hydrolysis, the strengths were quite close to nonreinforced controls and they were lower than the strength of bone (e.g., the shear strength of cancellous bone is 1–21.4 MPa[34]). It can be assumed that the matrix carries most of the load and BaG did not reinforce after 2 weeks in hydrolysis. When BaG amount was determined using burning test, the results showed that it did not change during the 6-week hydrolysis. It should be noted that some amount of BaG might have been remodeled to hydroxyapatite (HA) precipitation, which was not determined during this study. However, it can be assumed that the mechanical properties of the fibers have changed, so that they do not have enough mechanical strength to carry almost any load. Probably, the amount of BaG (or HA precipitation) remained the same during the hydrolysis, because it was between the polymer layers where it was difficult to escape. Mechanical properties of the composites were not

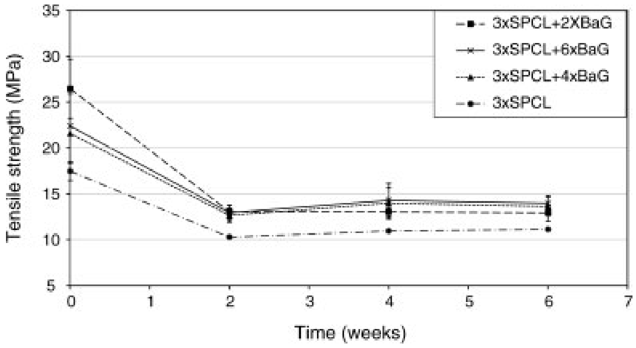


Figure 4. Changes in tensile strength *in vitro* for 3xSPCL, 3xSPCL+2xBaG, 3xSPCL+4xBaG, and for 3xSPCL+6xBaG series.

found to be adequate for bone fracture fixation applications. Although the initial strength of the composites was higher than the strength of bone, for bone fixation applications, longer lasting and higher mechanical properties are needed. Higher strengths have also been achieved for rods made from polyglycolide or poly-L-lactide, which are commonly used materials for bone fixation implants.[34,35]

CONCLUSIONS

BaG fiber-reinforced composites were manufactured by using compression molding from extruded SPCL billet and BaG fiber sheet. Nonreinforced samples were used as reference. It was possible to obtain at least 50% higher initial mechanical properties by reinforcing the samples with BaG fibers. However, mechanical properties decreased to same level as non-reinforced controls after 2 weeks in hydrolysis. During the 6-week hydrolysis, the mass of both kinds of samples, reinforced with BaG and non-reinforced, decreased only about 5%. The amount of BaG in the composites remained the same for the 6-week period in hydrolysis, thus mechanical properties of the BaG fibers had changed dramatically.

The valuable advices of Ville Ellä, MSc and Mikko Huttunen, MSc, are greatly appreciated. Thanks are due to Ms. Hilkka Losoi for administrative assistance. Ms. Maria Viola is kindly thanked for having retyped the amendments during the finalization of the ultimate version of the manuscript.

REFERENCES

1. Azevedo HS, Gama FM, Reis RL. In vitro assessment of the enzymatic degradation of several starch based biomaterials. Biomacromolecules 2003;4:1703–1712.
2. Gomes ME, Ribeiro AS, Malafaya PB, Reis RL, Cunha AM. A new approach based on injection moulding to produce biodegradable starch-based scaffolds: Morphology, mechanical and degradation behaviour. Biomaterials 2001;22:883–889.
3. Marques AP, Cruz HR, Coutinho OP, Reis RL. Effect of starch based biomaterials on the in vitro proliferation and viability of osteoblast-like cells. J Mater Sci: Mater Med 2005;16: 833–842.
4. Gomes ME, Godinho JS, Tchalamov D, Cunha AM, Reis RL. Alternative tissue engineering scaffolds based on starch: Processing methologies, morphology, degradation and mechanical properties. Mater Sci Eng C 2002;20:19–26.
5. Mano JF, Koniarove D, Reis RL. Thermal properties of thermoplastic starch/synthetic polymer blends with potential biomedical applicability. J Mater Sci: Mater Med 2003;14:127–135.
6. Marques AP, Reis RL, Hunt JA. Evaluation of the potential of starch-based biodegradable polymers in the activation of human inflammatory cells. J Mater Sci: Mater Med 2003; 14:167–173.
7. Marques AP, Reis RL, Hunt JA. An in vivo study of the host response to starch-based polymers and composites subcutaneously implanted in rats. Macromol Biosci 2005;5:775–785.
8. Salgado AJ, Figueiredo JE, Coutinho OP, Reis RL. Biological response to pre-mineralized starch based scaffolds for bone tissue engineering. J Mater Sci: Mater Med 2005;16:267–275.
9. Vaz CM, Reis RL, Cunha AM. Use of coupling agents to enhance the interfacial interactions in starch-EVOH/hydroxyapatite composites. Biomaterials 2002;23:629–635.
10. Elvira C, Mano JF, San Román J, Reis RL. Starch-based biodegradable hydrogels with potential biomedical applications as drug delivery systems. Biomaterials 2002;23:1955–1966.
11. Sousa RA, Mano JF, Reis RL, Cunha AM, Bevis MJ. Mechanical performance of starch based bioactive composite biomaterials molded with preferred orientation. Polym Eng Sci 2002;42:1032–1045.
12. Pereira CS, Cunha AM, Reis RL, Vázques B, San Román J. New starch-based thermoplastic hydrogels for use as bone cements or drug-delivery carriers. J Mater Sci: Mater Med 1998;9:825–833.
13. Silva GA, Costa FJ, Coutinho OP, Radin S, Ducheyne P, Reis RL. Synthesis and evaluation of novel bioactive composite starch/bioactive glass microparticles. J Biomed Mater Res A 2004;70:442–449.
14. Mendes SC, Bezemer J, Claase MB, Grijpma DW, Bellia G, Degli-Innocenti F, Reis RL, de Groot K, van Blitterswijk CA, de Bruijn JD. Evaluation of two biodegradable polymeric systems as substrates for bone tissue engineering. Tissue Eng 2003;9:91–101.
15. Tuzlakoglu K, Bolgen N, Salgado AJ, Gomes ME, Piskin E, Reis RL. Nano- and micro-fiber combined scaffolds: A new architecture for bone tissue engineering. J Mater Sci: Mater Med 2005;16:1099–1104.
16. Day M, Cooney JD, Shaw K, Watts J. Thermal analysis of some environmentally degradable polymers. J Therm Anal 1998;52:261–274.
17. Kellomäki M, Niiranen H, Puumanen K, Ashammakhi N, Waris T, Törmälä P. Bioabsorbable scaffolds for guided bone regeneration and generation. Biomaterials 2000;21:2495–2505.
18. Niemelä T, Niiranen H, Kellomäki M, Törmälä P. Self-reinforced composites of bioabsorbable polymer and bioactive glass with different bioactive glass contents. Part I: Initial mechanical properties and bioactivity. Acta Biomaterialia 2005; 1:235–242.
19. Niiranen H, Pyhältä T, Rokkanen P, Kellomäki M, Törmälä P. In vitro and in vivo behavior of self-reinforced bioabsorbable polymer and self-reinforced bioabsorbable polymer/bioactive glass composites. J Biomed Mater Res A 2004;4:699–708.
20. Thompson ID, Hench LL. Mechanical properties of bioactive glasses, glass-ceramics and composites. Proceedings of the institution of mechanical engineers. Part H. J Eng Med 1998; 212:127–136.
21. Hietikko L. Bioactive glass fibers and their biodegradable composites *in vitro*. MSc Thesis. Institute of Biomaterials, Tampere University of Technology, Tampere, 2007. 87 p.
22. Pirhonen E, Moimas L, Brink M. Mechanical properties of bioactive glass 9-93 fibres. Acta Biomaterialia 2006;2:103–107.
23. Clupper D, Gough J, Embanga P, Notingher I, Hench L. Bioactive evaluation of 45S5 Bioactive glass fibres and preliminary study of human osteoblast attachment. J Mater Sci: Mater Med 2004;15:803–808.
24. Arstila H, Tukiainen M, Hupa L, Ylänen H, Kellomäki M, Hupa M. In vitro reactivity of bioactive glass fibers. Adv Sci Technol 2006;49:246–251.
25. ISO 15814. Implants for surgery—Copolymers and blends based on polylactide—In vitro degradation testing. International Standard Organization, 1999, 9 p.
26. BS 2782: 340B. Determination of shear strength of sheet material. British Standard Methods for Testing, 1978, 3 p.
27. SFS-EN ISO 178. Plastics. Determination of flexural properties. Suomen standardoimisliitto SFS, 1997, 16 p.

28. SFS-EN ISO 527-1. Plastics. Determination of tensile properties. Part 1: General principles. Suomen standardisoimisliitto SFS, 1996, 17 p.
29. ISO 1172. Textile-glass-reinforced plastics—Determination of loss on ignition. International Standard Organization, 1975, 3 p.
30. Leonor IB, Sousa RA, Cunha AM, Reis RL, Zfong ZP, Greenspan D. Novel starch thermoplastic/Bioglass® composites: Mechanical properties, degradation behaviour and in vitro bioactivity. J Mater Sci: Mater Med 2002;13:939–945.
31. Vårum KM, Ottoy MH, Smidsrod O. Acid hydrolysis of chitosans. Carbohyd Polym 2001;46:89–98.
32. Hench LL, Anderson Ö. Bioactive glasses. In: Hench LL, Wilson J, editors. An Introduction to Bioceramics. Singapore: World Scientific; 1993. Chapter 3, pp 41–62.
33. Li S, Vert M. Biodegradable polymers: Polyorthoesters. In: Mathiowitz E, editor. Encyclopedia of Controlled Drug Delivery. United States of America: Wiley; 1999. pp 71–93.
34. Manninen MJ. Self-Reinforced Polyglycolide and Poly-L-Lactide Devices in Fixation of Osteotomies of Weight-Bearing Bones. Doctoral Thesis. Finland, Helsinki: Helsinki University Central Hospital; 1993.
35. Törmälä P. Biodegradable self-reinforced composite materials; manufacturing structure and mechanical properties. Clinical Materials 1992;10:29–34.