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Poly(ε-caprolactone) Composites Containing Gentamicin-Loaded β-Tricalcium Phosphate/Gelatin Microspheres as Bone Tissue Supports

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ABSTRACT: In this work, novel antibacterial composites were prepared by using poly(ε-caprolactone) (PCL) as the main matrix material, and gentamicin-loaded microspheres composed of β-tricalcium phosphate (β-TCP) and gelatin. The purpose is to use this biodegradable material as a support for bone tissue. This composite system is expected to enhance bone regeneration by the presence of β-TCP and prevent a possible infection that might occur around the defected bone region by the release of gentamicin. The effects of the ratio of the β-TCP/gelatin microspheres on the morphological, mechanical, and degradation properties of composite films as well as *in vitro* antibiotic release and antibacterial activities against *Escherichia coli* and *Staphylococcus aureus* were investigated. The results showed that the composites of PCL and β-TCP/gelatin microspheres had antibacterial activities for both bacteria. © 2012 Wiley Periodicals, Inc. J. Appl. Polym. Sci. 000: 000–000, 2012

KEYWORDS: poly(ε-caprolactone); β-tricalcium phosphate; gelatin; fillers; biodegradable; controlled release; antibacterial activity

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INTRODUCTION

Composite systems are becoming more popular in biomedical applications because of the effective combination of the desired properties of their constituents. From this perspective, inorganic bioceramic fillers and biocompatible polymer matrices are one of the mostly developed systems for orthopedic and dental applications. These systems have osteoconductive and osteoinductive properties as well as good mechanical strength of bioceramics and high biocompatibility and processability of polymers.[1] One of the application areas of such composites as two-dimensional film forms is guided bone regeneration (GBR), which is a promising therapy to repair mandible and alveolar bone defects suffered by periodontal diseases and applied at dental implant sites.[2] The commercially available GBR membranes are made of polymers, including nondegradable polytetrafluoroethylene (PTFE) and biodegradable polylactide, polyglycolide, polycarbonate, and collagen.[3] Although PTFE membranes have been indicated satisfactory clinical results, biodegradable polymer-based GBR membranes have been studied increasingly in the recent years because of the nonrequirement of second surgical procedure to remove the membranes.[3–5] Among many biodegradable polymers, poly(ε-caprolactone) (PCL) is a well-established one in tissue engineering studies owing to its biocompatible, biodegradable, and mechanical properties. Recent studies on PCL and its composites with bioactive inorganics have shown desired osteoblastic responses in *in vitro* experiments and good tissue interactions in *in vivo* conditions.[6,7]

Local application of antibiotic release systems is important for hard tissue engineering because of both poor vascularity in bone tissue for oral or intravascular therapy and easiness of microbial attack in dental sites where it is open area to environment.[8,9] Various microsphere systems prepared from biodegradable synthetic and natural polymers have been studied as antibiotic-carrying vehicles by many researchers, and it has been proposed that both the ability of controllable release and the

effect on cell proliferation are more effective for regular shaped microspheres compared with the irregular shaped ones.[10] Gelatin is one of the most studied natural polymers for drug delivery systems because of its nontoxic, biocompatible, biodegradable, and relatively inexpensive properties.[11–13] Its use for controlled release of gentamicin, rifampicin, and bone morphogenetic protein-2 (BMP-2) was successfully reported.[14–16]

Hard tissues are mainly composed of organic-based collagen and inorganic-based calcium phosphate ceramics. Therefore, various bioceramics are used as bone repairing and replacing materials. β-Tricalcium phosphate (β-TCP) has a special place among all because of its biocompatibility, bioactivity, mechanical strength, nontoxicity, and osteoconductivity.[17–19] It is reported that in clinical practices, β-TCP has demonstrated higher resorption rate and can more rapidly replace with the newly formed bone compared with hydroxyapatite.[20] Thus, composite systems prepared from β-TCP and a suitable polymer such as PCL can be good supporting material for bone tissue engineering applications. However, the direct incorporation of ceramic powder into PCL matrix can lead rapid spreading of bioceramics to the surrounding tissue because of poor interaction with matrix.[21] Preparation of composite microspheres with the combination of bioceramic with a polymer can prevent this phenomenon by increasing the compatibility of the constituents, in addition to providing controlled release of antibiotics. On the other hand, it was reported that direct addition of antibiotic in polymer matrix increased in burst release.[22–25] Therefore, it is preferable and more effective to have a controlled release system such as a crosslinked matrix within the biomaterial to decrease burst effect.

In view of the mentioned above, the aim of this study was to develop a composite material from biodegradable PCL and gentamicin-loaded β-TCP/gelatin microspheres. These materials can be good candidate for hard tissue applications. The mechanical properties, hydrophilicity, morphological characteristics, antibacterial properties, antibiotic release, and degradation behavior of the prepared systems were investigated.

EXPERIMENTAL

Materials

PCL was obtained from Sigma-Aldrich (M_w = 80,000, Steinheim, Germany). Gelatin was purchased from Sharlau (Barcelona, Spain). Vegetable oil was obtained from Kristal Corn Oil (Izmir, Turkey). Glutaraldehyde (50 wt %) was purchased from BDH Limited (Poole, England). Gentamicin (80 mg/mL) was obtained from I.E. Ulagay (Istanbul, Turkey). Phosphate-buffered solution (PBS, pH 7.4) was prepared by chemicals (K_2HPO_4 and KH_2PO_4) of Merck Chemicals (Darmstadt, Germany). Lipase from *Pseudomonas fluorescencens* (40.2 U/mg) was purchased from Sigma (Steinheim, Germany). Chloroform was obtained from Lab-Scan (Dublin, Ireland).

Preparation of Gentamicin-Loaded β-TCP/Gelatin Microspheres

β-TCP powder was synthesized with a multistep procedure starting from $CaCO_3$. Briefly, pyrophosphate ($Ca_2P_2O_7$) was combined with $CaCO_3$ in stoichiometric proportions, calcinated at 900°C for 1 h at open air conditions, and then sintered at 1150°C for 1.5 h. Gentamicin-loaded β-TCP/gelatin microspheres were prepared with water-in-oil emulsion method. Shortly, synthesized β-TCP powder was slowly added to the aqueous gelatin solution with the ratio of β-TCP/gelatin 0.5 by weight with continuous magnetical stirring. This suspension was added dropwise into 60 mL oil while mechanical stirring the whole solution and stirring maintained for extra 30 min. Two milliliters of glutaraldehyde solution (2%) was added dropwise to the medium as crosslinker agent to stabilize the microspheres. The mixture was cooled to 4°C, washed with acetone, and dried at 25°C where the relative humidity was 34%. To load gentamicin to microspheres, 0.5 mL of gentamicin solution containing 40 mg of gentamicin was added onto 100 mg of β-TCP/gelatin microspheres by applying vacuum-pressure cycle. As control group, gentamicin-loaded gelatin microspheres without the addition of β-TCP were also prepared. The gentamicin-loaded microspheres were dried at 25°C in vacuum oven for 48 h. The average particle sizes of the pure gelatin microspheres (used in the preparation of PCL-30G matrices) and the composite microspheres (used in the preparation of other matrices) were 5 $\pm$ 2 μm and 9 $\pm$ 7 μm, respectively [Figure 1(a-a′,b′)].

Preparation of PCL Matrices Containing Gentamicin-Loaded Microspheres

Gentamicin-loaded β-TCP/gelatin microspheres (10, 30, or 50% by weight) were added to the PCL solution (3%) prepared in chloroform and stirred magnetically for 6 h to obtain homogeneous dispersion. The mixtures were molded in glass Petri dishes and kept at 25°C for 48 h and then in vacuum oven for 24 h to remove all the solvent. Pure PCL and PCL matrices containing only gelatin microspheres or PCL matrices containing only β-TCP powder (30%) were also prepared separately for comparison. Table I summarizes the compositions of the prepared samples.

Characterization of Matrices

The morphology of the prepared samples was characterized by scanning electron microscope (SEM) analysis by using a JSM-6400 electron microscope (JEOL). The samples were sputter coated by Au-Pd thin film before SEM investigations.

The mechanical properties of the composite matrices prepared as films were investigated under both dry and wet conditions by using a mechanical testing machine (Lloyd Instrument, Fareham, UK), equipped with a 100 N load cell, with a cross-head speed of 10 mm/min. Samples were cut as rectangular strips, and the gauge length and width were 60 and 10 mm, respectively, for each sample. The thickness of each sample was determined by a micrometer having at least five measurements from different parts, and the average values were used in calculations. The wet samples were prepared by incubation of strips in PBS for 6 h to mimic the body environment. Ultimate tensile strength (UTS), Young's modulus (E), and percent elongation at break (EAB%) values were obtained. The load deformation curve was printed for each specimen. The UTS was obtained from the equation $\sigma = F/A$, where σ is the tensile strength (MPa), F is the maximum load applied (N) before rapture, and A is the initial area (mm^2) of the specimen. Young's modulus is

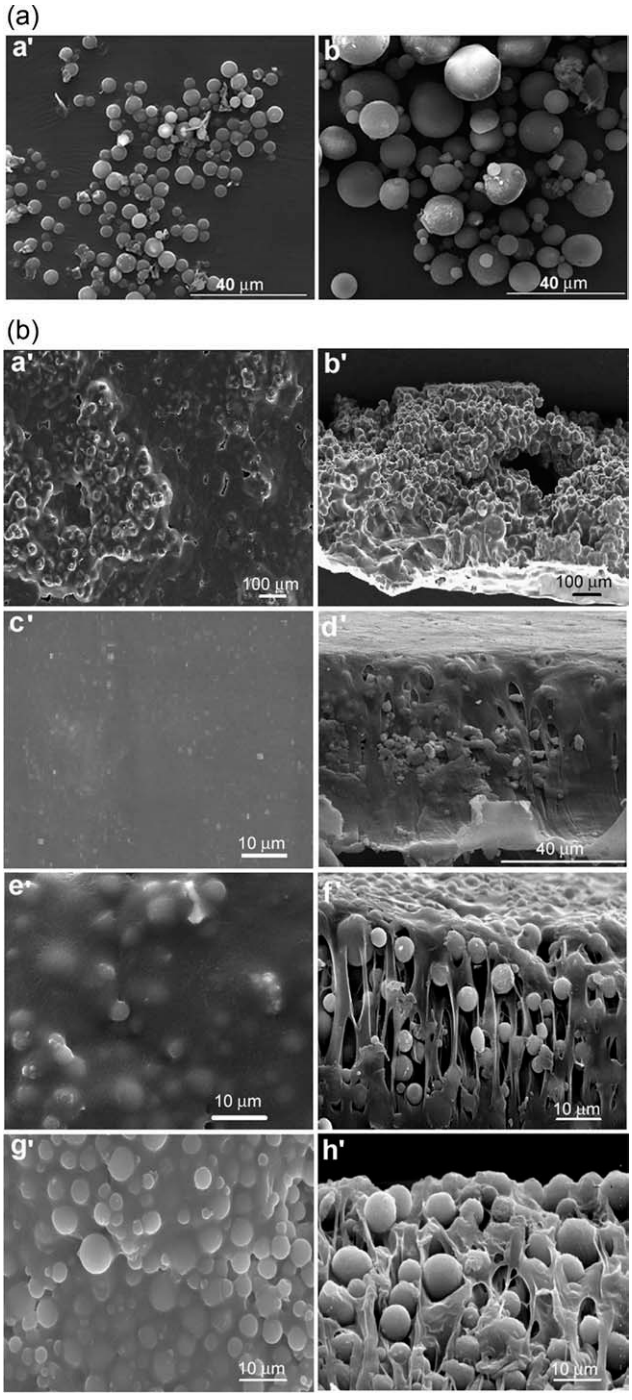

Figure 1. SEM images of microspheres (a) and composite matrices (b): (a-a′) gelatin microspheres, (a-b′) gelatin/β-TCP microspheres, (b-a′) PCL-30G (surface), (b-b′) PCL-30G (cross section), (b-c′) PCL-30β (surface), (b-d′) PCL-30β (cross section), (b-e′) PCL-30β/G (surface), (b-f′) PCL-30β/G (cross section), (b-g′) PCL-50β/G (surface), and (b-h′) PCL-50β/G (cross section).

calculated from the initial slope of σ versus ε where ε is the fraction of deformation per unit length. EAB is calculated by dividing extension at break to gauge length. For each type of sample, at least five experiments were achieved, and the average values of Young's modulus, tensile strength, and percent EAB were calculated.[26]

The surface hydrophilicity of the composite matrices was determined by a goniometer (CAM 200, Finland) at room temperature both in dry and wet conditions. Wet samples were prepared with incubation in PBS for 6 h to mimic body environment. Five microliters of deionized water was dropped on the samples, and the contact angles of at least 10 drops were measured and averaged.

In Vitro Gentamicin Release from Composite Matrices

In vitro gentamicin release from composite matrices was carried out in PBS (0.1 M, pH 7.4) at 37°C. The samples were cut as rectangles (1 cm × 2 cm), two samples were placed in vials, and 5 mL of PBS solution was added. The vials were placed in shaker bath at 100 rpm at 37°C. The solution was collected at predetermined time intervals and replaced with fresh PBS solution (5 mL) at each time interval. The amount of gentamicin was determined with UV–vis spectrometer at 256 nm. The experiments were triplicated.

Degradation Studies of Composite Matrices

The hydrolytic and enzymatic degradation studies were carried out for the selected PCL-30β/G samples. The samples were cut into pieces (1cm × 2 cm). The hydrolytic degradation studies of matrices were achieved at 37°C in PBS (0.1 M, pH 7.4, containing 0.02% sodium azide). For enzymatic degradation studies, 0.1 mg/mL of lipase was added into PBS. The solution of each sample was changed and replaced with fresh ones in every 24 h. For each case, the solutions were drawn out, the samples were washed with distilled water, freeze dried at −80°C, and weighed. The loss in weight was determined gravimetrically by comparing the dried and initial weights of the samples. The morphological characteristics of degraded samples were also investigated by SEM.

Antibacterial Activity

The antibacterial activities of composites were studied by disc diffusion method. For this purpose, two bacterium Gram-negative *Escherichia coli* and Gram-positive *Staphylococcus aureus* were chosen. Either *E. coli* or *S. aureus* was spread onto agar plates with cotton swabs from bacterial suspension. Pure PCL, which was used as control group, and composite samples were assembled on planar round substrates (10 mm in diameter) and were placed on top of the inoculated agar. The prepared samples were adhered to the bacterial strains carefully. The agar plates were then inverted and incubated at 37°C for 24 h. The

Table I. Composition of PCL Matrices

Sample	Type and amount of filler in PCL matrices
PCL	Pure PCL with no filler
PCL-30G	30 wt % gelatin microsphere
PCL-30β	30 wt % β-TCP
PCL-10β/G	10 wt % β-TCP–gelatin microsphere
PCL-30β/G	30 wt % β-TCP–gelatin microsphere
PCL-50β/G	50 wt % β-TCP–gelatin microsphere

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zone of inhibition, where no visible bacterial colonies formed, was measured using a caliper.[27]

RESULTS AND DISCUSSION

This study aimed to develop multifunctional composites that can be good candidates for hard tissue treatments. The osteoconductivity of β-TCP and antibacterial activity of gentamicin were combined within gelatin microsphere by forming a coat on the particles so that these stable composite microspheres can be used as multifunctional fillers. The hydrophilic filler was expected to alter the hydrophobicity of PCL, which restricts the use of PCL in tissue engineering applications because of the low degradation rate and poor cell adhesion. To make a comparison, composites of only gelatin microspheres or only β-TCP powder within the PCL matrix and PCL matrix free of filler were investigated.

SEM micrographs of the composite matrices containing gelatin microspheres, β-TCP powder, and the ones containing β-TCP/gelatin microspheres are given in Figure 1(b). The surface and the cross section of composites are shown in Figure 1(b-a′,c′,e′,g′) and Figure 1(b-b′,d′,f′,h′), respectively. PCL-30β samples formed composites with homogeneous distribution of β-TCP particles [Figure 1(b-c′,d′)]. However, in PCL-30G samples, gelatin microspheres were accumulated on the surface of the matrices and did not have homogeneous distribution [Figure 1(b-a′)]. Gelatin microspheres on top of the matrix and PCL layer at the bottom can be seen in Figure 1(b-b′). Results showed insufficient homogeneity for PCL-30G, which can be attributed to the poor interaction between the highly hydrophilic gelatin and highly hydrophobic PCL. On the other hand, composite matrices containing β-TCP/gelatin microspheres showed homogeneous dispersion. As seen from Figure 1(b-e′–h′), the β-TCP/gelatin microspheres are embedded into PCL matrix, but still the compatibility of PCL and microspherical fillers was not proper (PCL-10β/G also demonstrated similar homogeneous dispersion without proper interaction; the image is not shown here). For all samples, an exclusion interface of the microparticles and the matrix was observed. That is also expected because of the differences of the polar characters of the microparticles and the matrix. The roughness of the surfaces of composite matrices increased with the amount of the microspheres [Figure 1(b-g′,h′)]. The cross-sectional SEM images of composites [Figure 1(b-f′,h′)] clearly show homogeneous distribution in the structure. As it can be seen, PCL-30β/G had more homogeneous dispersion than that of PCL-50β/G. This can be resulted from the high concentration of microspheres so that PCL matrix did not completely cover the fillers [Figure 1(b-h′)].

The surface hydrophilicities of composite matrices were assessed through water contact angle measurements. PCL is a hydrophobic polymer and the additives that composed of gelatin and β-TCP are hydrophilic materials. However, the water contact angle of the samples increases in dry state with the addition of composite microspheres, contrary to the expectation. Water contact angle of pure PCL matrices was found as 71°, and this value increased to 75°, 88°, and 92° (with the deviation of ±3°) for PCL-10β/G, PCL-30β/G, and PCL-50β/G, respectively. As seen from the Figure 1(b-e′,g′) images, the composites have rougher topography and the trapped air in the grooves of the surface as well as the presence of the hydrophobic PCL coating (proved by ATR-FTIR analysis, data not shown) increased the water contact angle, similar to lotus effect.[28–30]

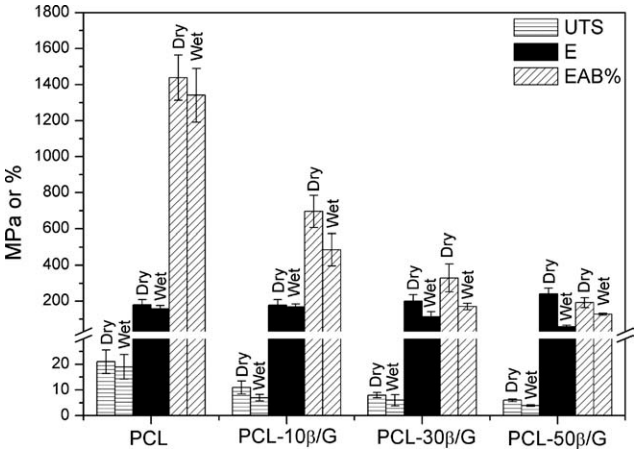

Figure 2. Tensile properties of the composite matrices in dry and wet conditions.

On the other hand, the contact angles for all wet composite samples (after immersion in PBS for 6 h) showed a decrease, whereas the pure PCL sample did not have any significant change (70°). The water contact angles of wet samples of PCL-10β/G, PCL-30β/G, and PCL-50β/G were 68°, 64°, and 60° (with the deviation of ±4°), respectively. Surface hydrophilicities increased with the amount of the hydrophilic filler. PCL-50β/G sample indicated the most lowering of the water contact angle by changing from 92° to 60° (35% decrease). This phenomenon can be resulted by the more water absorption because of the higher content of hydrophilic filler. Gogolewski and Galletti reported that contact angle value of 60°–80° is a sufficient range for the cell attachment and growth.[31] All composites prepared in this study indicated 68°, 64°, and 60° contact angle values and, therefore, can promote good results in body environment for the tissue engineering applications.

The UTS, Young's modulus (E), and EAB% of dry and wet composites are illustrated in Figure 2. UTS, which is the value of maximum force to break the sample, decreased with increasing microsphere ratio. The value of UTS is 21 MPa for pure PCL matrix and dropped down to 11, 8, and 6 MPa; for the PCL-10β/G, PCL-30β/G, and PCL-50β/G composites, having 48, 62, and 71% decrease, respectively. PCL sample showed the E value of 180 MPa that is very similar to the PCL-10β/G sample. Increasing microsphere ratio in dry composites increased the E values up to 240 MPa for PCL-50β/G. This increase indicated that β-TCP/gelatin microspheres behaved as reinforcing fillers. The microspheres in composite PCL matrices improved the elasticity in dry conditions most probably because of the presence of hard ceramic structure in the matrix, which resists the applied forces preventing deformation. It is known that PCL has high elongation, and EAB% value was obtained as 1439% for the pure PCL matrix. EAB% values of the composite

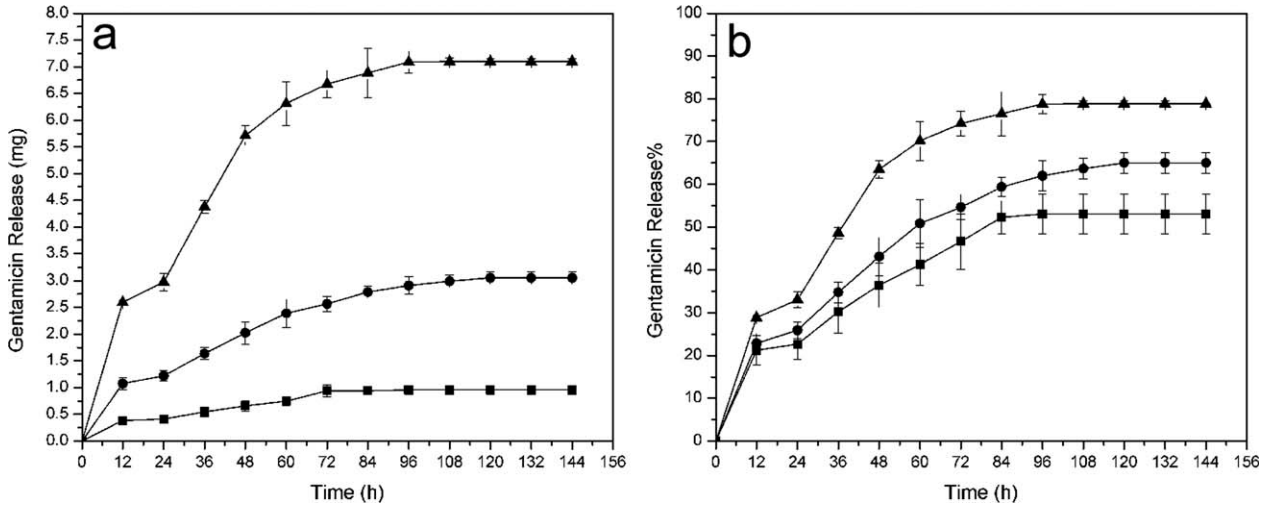

Figure 3. Gentamicin release profiles of the composite samples: (a) as mg and (b) as %; PCL-10β/G (■), PCL-30β/G (●), and PCL-50β/G (▲).

matrices decreased about 66, 77, and 87% for the PCL-10β/G, PCL-30β/G, and PCL-50β/G composites, respectively, where the lowest value was observed as 192% for PCL-50β/G. The reduction in UTS and EAB% values most probably resulted from the microspheres that created phase segregation in polymer matrix.

During the application of stress, composite matrices have higher resistant to the applied load by showing less deformation compared with the pure PCL sample. PCL is more ductile compared with other polylactides, and this lower elastic modulus can limit the usage of PCL in orthopedic tissue engineering applications.[32] Many researchers suggested copolymers or blends of PCL with polylactides[33,34] or addition of fillers[35,36] to achieve this obstacle. In literature, many composites composed of PCL and bioceramic fillers show reduction in tensile modulus when exceeding the filler composition of 30%.[13,37] However, in our dry samples, the modulus was maintained an increase up to the addition of 50% composite microspheres, most probably enhanced interaction between PCL and composite fillers.

When the wet samples were examined, it was observed that addition of composite microspheres reduced elastic modulus, tensile strength, and elongation ratio considerably (Figure 2). The tensile properties of PCL did not change significantly because of the hydrophobic character of PCL. However, wet composite matrices exhibited noticeable differences in their UTS, *E*, and EAB% values compared with dry forms. Tests in wet conditions resulted in a decrease in UTS 27, 32, and 38%, in *E* 5, 43, and 75%, and in EAB 30, 33, and 42% for PCL-10β/G, PCL-30β/G, and PCL-50β/G, respectively. As a result, PCL-50β/G samples having the highest β-TCP/gelatin microsphere ratio indicate the lowest values in tensile properties. The lower values obtained in the wet state can be explained by the swelling of the gelatin part of the β-TCP/gelatin microspheres, the weakness of the interface, and the plasticization effect because of the presence of water. Also, this high reduction in tensile properties can be attributed to the porosity caused by the inhomogeneity of composite microspheres and PCL composite samples.[37] Fujihara et al. reported that the tensile strength of PCL/calcium carbonate composite GBR film is 3.6 MPa and 200% for the EAB.[2] Other studies with the natural polymers focused on the GBR films were reported lower UTS and EAB values.[4,37] With respect to these results, the tensile properties of the composite matrices prepared in this study have the required mechanical properties for GBR applications.

Drug release studies were performed *in vitro* for the gentamicin-containing composite matrices. PCL-10β/G, PCL-30β/G, and PCL-50β/G samples were loaded with β-TCP/gelatin microspheres containing 1.8, 4.7, and 9.0 mg of gentamicin, respectively. The amounts of released gentamicin by time either as milligram [Figure 3(a)] or as percent [Figure 3(b)] values are shown. As seen from the figure, all samples indicated controlled release and the release kinetics reached to balance after 72, 96, and 120 h, having the calculated percentages of the released gentamicin as 53, 64, and 78% for PCL-10β/G, PCL-30β/G, and PCL-50β/G composite matrices, respectively. All samples prepared in this study indicated sustained gentamicin release in *in vitro* conditions. The results showed that some of the loaded gentamicin was trapped in the matrix and could not be detected in the release medium. This can be explained by the complete coverage and encapsulation of β-TCP/gelatin microspheres with PCL, especially for the samples that contain low amount of microspheres. As the amount of microspheres increases, the heterogeneity of the matrix and therefore porosity increase, leading more of the drug diffuse out from the matrix.

Gelatin is a fast-degradable polymer because of its high hydrophilicity, and it has the ability of swelling in aqueous medium. Vacuum-pressure cycle was applied after addition of gentamicin aqueous solution onto the microspheres. Aqueous medium provides swelling of gelatin and, therefore, gentamicin can diffuse to the inner parts of the microspheres with pressure application. It is expected that some amount of gentamicin would be adsorbed onto the microspheres, and this amount would release with a burst effect during the early stage. This is the case observed in release studies. The composite microspheres degraded partially in the later periods of the gentamicin release [Figure 5(d)]. As a result, the initial release is based on diffusion, whereas the later release is caused by both, diffusion of

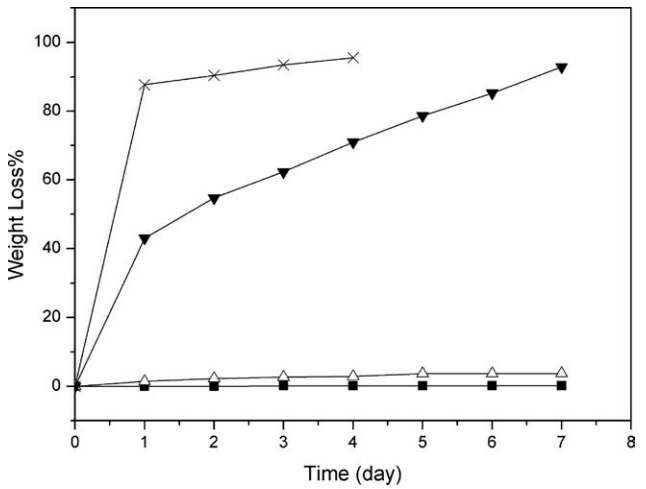

Figure 4. Degradation profiles: (×) PCL in lipase, (▲) PCL-30β/G in lipase, (■) PCL in PBS sloution, (△) PCL-30β/G in PBS.

gentamicin through PCL and degradation of TCP microspheres. Previously, complete release of gentamicin from β-TCP/gelatin microspheres was observed in about 60 h (data not shown). However, in this study, incorporation of the gentamicin-containing β-TCP/gelatin microspheres into the PCL matrix extended the release period, which also increase the period of the antibacterial effect.

Degradation studies gave information about the quantity and morphology of the samples. Degradation of matrices was studied for PCL-30β/G composites, PCL-30β, and pure PCL matrices. Weight losses of hydrolytic and enzymatic degradation of these samples are given in Figure 4. No significant difference was observed in weight loss for PCL and for PCL-30β/G composite after 1 week in PBS. The small decrease in weight for hydrolytic degradation of composites might be resulted from dissolution of gelatin existing in β-TCP/gelatin microspheres. On the other hand, in enzymatic degradation, 87.7% of PCL and 43.0% of PCL-30β/G composite were degraded after 24 h of incubation. After 1 week, these values reached to almost 100% for PCL and 95.6% for PCL-30β/G composites. SEM images of the samples after degradation are given in Figure 5. Interestingly, fibrous structure was observed for the PCL-30β/G matrices. To detect the influence of β-TCP in this fibrous structure, PCL-30β sample was degraded enzymatically. PCL-30G samples were not used in degradation studies because they did not produce homogeneous matrices. For the PCL-30β matrix, destroyed and leached regions were observed after enzymatic degradation period (1 week). The morphological characteristics are similar to the PCL matrix that demonstrated bulky destroyed regions.

PCL is a hydrophobic semicrystalline polymer and degrades slowly in the absence of enzymes both in *in vitro* and *in vivo* conditions.[38] Several studies have reported that PCL hydrolysis can be catalyzed by lipase.[39–41] Therefore, enzymatic degradation of the composites was achieved in lipase-containing PBS medium. In enzymatic degradation, because of the specific ability of lipase to degrade polyesters, more rapid degradation and higher value of weight loss were observed for composite matrices. The morphological characteristics of degraded composite samples showed fibrous structure. In the case of PCL-30β composite matrix, there is no such fibrous structure observed after same enzymatic degradation period. It can be stated that the

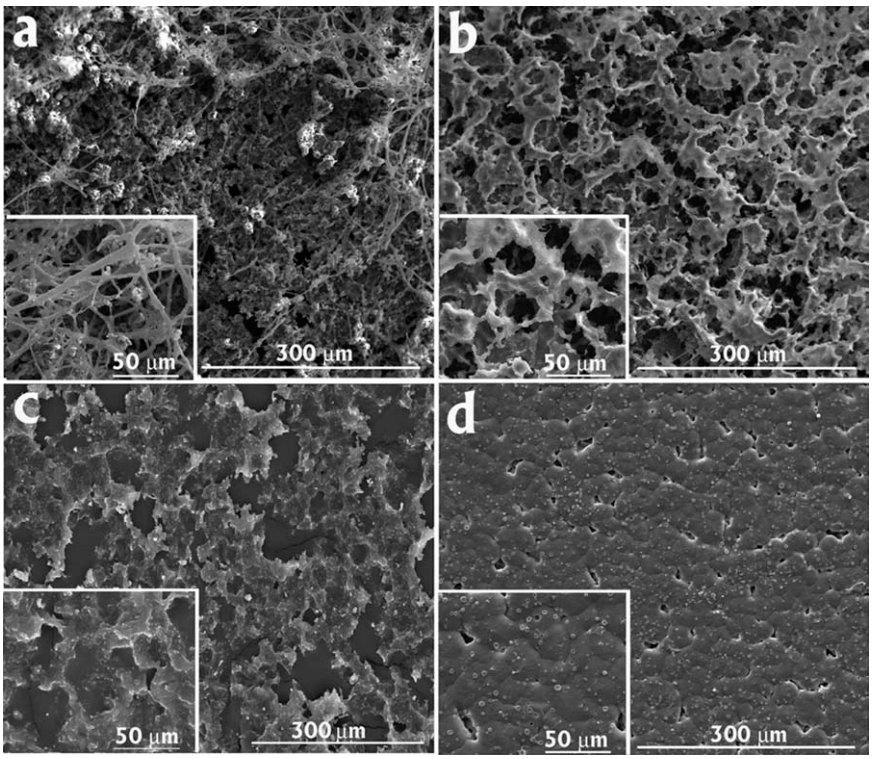

Figure 5. SEM images of samples after 1 week of degradation: (a) PCL-30β/G in lipase, (b) PCL in lipase, (c) PCL-30β in lipase, and (d) PCL-30β/G in PBS.

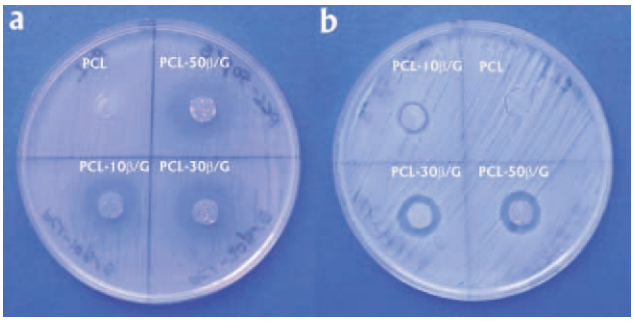

Figure 6. Disc diffusion test results of composite matrices: (a) *E. coli* and (b) *S. aureus.* [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

presence of both gelatin and β-TCP has roles in the formation of fibrous structure. Gomes et al. studied degradation of fiber mesh PCL–starch composite scaffold. They reported no significant changes in morphology for the hydrolytic degradation, but they observed increased surface roughness in the presence of lipase.[42]

In the image of PCL-30β/G composite matrix after 1 week of hydrolytic degradation, destroyed microspheres and created pores on the surface were observed, where there was no significant morphological change in the bulk of the matrix [Figure 5(d)]. In literature, it has been given that porosity in the matrices is essential for transformation of body fluid for nutrient supply and surface cell adaptation, and there are different types of TCPs synthesized for these purposes.[43,44]

Therefore, it can be concluded that the incorporation of β-TCP/gelatin microspheres in PCL matrix has an effect on the formation of pores enhancing biocompatibility of the matrices. Especially, the result of fibrous structure during degradation can be effective on cell attachment and proliferation in *in vitro* conditions as it was reported previously.[45]

Antibacterial assays of Gram-negative *E. coli* and Gram-positive *S. aureus* were carried out to examine the bacterial growth over 24 h by disc diffusion method. The results are given in Figure 6. The pure PCL sample did not indicate any antibacterial activity against both *E. coli* and *S. aureus* under the test conditions as seen from the absence of zone inhibition. The composites containing gentamicin-loaded microspheres exhibited distinctive microbial inhibition zones, and in the disc diffusion method which are measured as 21, 23, and 25 mm against *E. coli* and 12, 15, and 16 mm against *S. aureus* for PCL-10β/G, PCL-30β/G, and PCL-50β/G, respectively. Enhanced antibacterial activities were observed for composite matrices containing higher amount of fillers because of the presence of more gentamicin content within the fillers. As it is known that gentamicin is more effective against Gram-negative bacterium, the inhibition zones are larger for *E. coli* than the ones obtained for *S. aureus.*

CONCLUSION

Homogeneous composites from hydrophobic matrix of PCL and hydrophilic microspheres of β-TCP/gelatin loaded with gentamicin were prepared. Tensile tests showed an increase in *E* and decrease in UTS and EAB values compared with PCL when the samples were dry, whereas all these values decreased when the samples were hydrated. The morphology of the partially degraded samples of composites states that fillers have effect on the constitution of the fibrous structure, which can enhance the cell attachment and proliferation in the matrix in tissue engineering applications. The release profiles demonstrated extended release of gentamicin. Effective antibacterial activities of all composite samples against *E. coli* and *S. aureus* were observed. Further *in vivo* studies are required to show the samples efficiency in biomedical applications. However, the results obtained from this study, with the comparison with literature, suggest that β-TCP/gelatin microspheres are good fillers for PCL matrix, and these composite matrices can be good candidates as GBR film in dental applications or as bone tissue supports.

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