Synthesis and characterization of injectable composites of poly[D,L-lactide-co-(ε-caprolactone)] reinforced with b-TCP and CaCO₃ for intervertebral disk augmentation

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Abstract: Degeneration of the intervertebral disk constitutes one of the major causes of low back pain in adults aged 20–50 years old. In this study, injectable, in situ setting, degradable composites aimed for intervertebral disk replacement were prepared. b-TCP and calcium carbonate particles were mixed into acrylic-terminated oligo[D,L-lactide-co-(ε-caprolactone)], which were crosslinked at room temperature. The structure of the oligomers was confirmed by ¹H-NMR spectroscopy. The composites were examined via SEM, and the mechanical properties of the crosslinked networks were determined. The porous b-TCP particles showed good mechanical anchorage to the matrix due to polymer penetration into the pores. In vitro degradation tests showed that the composites containing b-TCP slowly degraded, whereas the composites containing CaCO₃ exhibited apatite formation capacity. It was concluded that the surface area, morphology, and solubility of the fillers might be used to control the degradation properties. The incorporation of fillers also increased both the elastic modulus and the maximum compression strength of the composites, properties that were similar to those of the physiological disk. These materials have potential for long-term intervertebral disk replacement and regenerative scaffolds because of their low degradation rates, bioactivity, and mechanical properties.

Key Words: intervertebral disk, degradable polymer(s), calcium phosphate(s), calcium carbonate(s), injectable composite(s)

INTRODUCTION

Between 70 and 85% of all people suffer back pain some time in life.¹ Degeneration of the intervertebral disk (IVD) constitutes one of the major causes of low back pain in adults between 20 and 50 years old.² The degeneration of the IVD may be attributed to factors such as aging, birth deformities, genetics, mechanical injuries, and tumors.^{2,3} Current treatment methods for the degenerated IVD mainly consist of three types: interbody fusion, total disk replacement, and nucleus pulposus replacement.⁴ Although interbody fusion stabilization methods have been found to alleviate the symptoms of back pain, they present disadvantages such as a reduced mobility of the joint and further degeneration due to increased stresses at the junction.^{2,5} Similarly to interbody stabilization, total disk replacement is intended for patients with an advanced state of IVD degeneration. However, it has a high number of contraindications such as osteoporosis, herniated nucleus pulposus with neural compression, scoliosis, and recess stenosis.⁶ Nucleus pulposus replacement represents a less invasive procedure than fusion and total disk replacement, but problems related to the extrusion of the implants and deformities of the endplates have been reported.⁷ Improved reliability and a minimum of postoperative complications are the current aims for the development of new methods and materials for treating degenerated intervertebral disks. Bioactive and/or degradable composites may be an alternative to initially stabilize and relieve the pain, followed by regeneration of the IVD. In addition, an injectable scaffold would reduce the operation time and permit minimally

invasive surgery. Injectable, in situ crosslinking, degradable polymers reinforced with ceramic particles constitute a promising approach for this purpose. The introduction of ceramic particles into degradable polymers may provide multiple advantages such as improvement of the mechanical properties, increased osteoconductivity, control of the implant degradation rates, and intrinsic radiopacity. However, previous composite systems have been focused mainly on hard tissue replacement. For example, Mikos and coworkers⁸ studied injectable poly(propylene fumarate) (PPF)-based composites. These polymers were blended with b-tricalcium phosphate (b-TCP) to form an injectable paste that cured in situ. These composites exhibited appropriate handling features and mechanical properties for trabecular bone substitution. Calcium carbonate has also been blended with poly(D,L-lactide-co-glycolide) to control the degradation rate of PLGA⁹ by neutralizing the acid degradation products, giving materials that showed potential for use in orthopedic fixation devices. Few attempts have been made in the literature to manufacture injectable materials intended for IVD replacement. Larraz et al.¹⁰ studied a nondegradable acrylic formulation containing poly(methyl methacrylate) (PMMA), an amphiphilic macromonomer, 2-hydroxyethyl methacrylate (HEMA), and acrylic acid (AA) for IVD replacement and drug delivery. This formulation showed an appropriate release of chondroitin sulphate (CS), absence of cytotoxicity in vitro, and normal tissue response in vivo. However, it cannot be used as a regenerative scaffold because of its nonbiodegradable nature.

An ideal IVD replacement material should have initial mechanical properties that are similar to those of the native tissues. For instance, the annulus fibrosus and the nucleus pulposus have a compressive modulus of 0.6 and 1.0 MPa, respectively.⁶ The relatively high modulus of bone grafts and metallic cages that are used in interbody fusion have presented problems such as degeneration of the adjacent vertebrae¹¹ and improper load distribution leading to failure of the fusion.⁶ Hence, a lower modulus material would be more suitable to replace the IVD. Furthermore, the material should have appropriate handling characteristics so that it can be injected within a clinically suitable period of time, followed by in situ crosslinking. Moreover, if the material is intended as a regenerative scaffold, it should be degradable and its degradation products noncytotoxic. In addition, its degradation kinetics would have to be in accordance with the tissue regeneration.

The aim of this study was to perform a preliminary study for the development of a degradable injectable material as an alternative for in situ treatment of degenerated intervertebral disks. The material is based on a degradable polymer matrix with ceramic reinforcements, and it has mechanical properties similar to those of the tissues found in the intervertebral disk.

MATERIALS AND METHODS

In this study, degradable polyester based composites containing particles of porous b-tricalcium phosphate and calcium carbonate were prepared. Acrylic-terminated oligo[D,L-lactide-co-(ε-caprolactone)] were first synthesized (Figure 1). After blending in the ceramic particles, the prepolymers were cured in the presence of benzoyl peroxide and N,N-dimethyl-p-toluidine (DMPT) as a peroxide decomposer allowing room temperature curing.

Oligomer synthesis: oligo[D,L-lactide-co-(ε-caprolactone)]
Appropriate amounts of vacuum-distilled 1,4 butanediol (BD), D,L-lactide (DLLA), ε-caprolactone (CL), and Sn(II)2-

ethylhexanoate ($\text{Sn}(\text{Oct})_2$) were added to a round-bottom flask in a glove box (MBRAUN, Germany). The molar ratio of DLLA, CL, and BD was 5:5:1, respectively. The mixture was stirred under nitrogen at 120 °C for 72 h.

For the synthesis of the acrylic-terminated oligomers, one mole of the hydroxyl-terminated oligomer reacted with two moles of acryloyl chloride in the presence of 2.02 moles of triethylamine (Et_3N). Toluene was chosen as the solvent and dried by azeotrope boiling for 30 min while stirring. A magnetic stirrer together with appropriate amounts of prepolymer, Et_3N , and toluene were put into a double-neck round-bottom flask. An addition funnel was connected to the flask and loaded with an appropriate amount of acryloyl chloride dissolved in toluene. The funnel was capped with a rubber septum with a nitrogen inlet and an outlet. The flask was immersed into an ice bath to carry out the reaction at 0 °C. After slowly dripping the contents of the funnel into the flask, the reaction was left overnight.

The crude mixture was washed twice with 500 mL of acetic acid (CH_3COOH) 1 vol %, twice with 500 mL sodium bicarbonate (NaHCO_3) 1 vol %, and finally distilled water. The resulting product was dried with magnesium sulfate anhydrous (MgSO_4), followed by filtration and solvent evaporation in a LABOROTA 4000 rotary evaporator (HEIDOLPH, Germany).

Preparation of the composites

Two pastes were used to prepare the in situ crosslinked degradable polymers. The first paste was prepared by adding 1 wt % of the radical initiator benzoyl peroxide and the ceramic filler to a certain amount of acrylic-terminated prepolymer.

The second paste was prepared by adding 1 wt % of the peroxide decomposer N,N-dimethyl-p-toluidine (DMPT) and the ceramic filler to a certain amount of acrylic-terminated prepolymer. The ceramic particles b-TCP (Curasan AB, Germany) with a porosity of 30% and calcium carbonate (Sigma-Aldrich, USA) were studied as fillers. Samples containing 5 and 30 wt % of b-TCP and 5 and 30 wt % of calcium carbonate were fabricated. The aim was to test relatively extreme compositions where 30 wt % is close to the maximum amount of particles that could be added to the liquid oligomer for it to remain injectable. A pure polymer sample was also produced as a control. To carry out the crosslinking, equal amounts of each paste were manually mixed together vigorously, for approximately 20 s, and then scooped into Teflon[®] moulds. For the water uptake study, and the microscopic analysis, cylindrical samples of 6 mm in diameter and 2.8 mm in height were prepared. For compression tests, five standard cylindrical samples of 6 mm in diameter and 12 mm in height, in accordance with ASTM F451-08, were prepared for each batch. The working and curing times were qualitatively determined as follows: the working time was considered to have come to an end when the blends became sticky. The curing time was that when the material had hardened and could be easily manipulated without generating permanent deformation.

Materials characterization

The hydroxyl-terminated and the acrylic-terminated oligomers were characterized by ^1H -NMR spectroscopy. A JEOL ECP-400 NMR (JEOL, Japan) system was used for this purpose, and deuterated chloroform (CDCl_3) was used as a solvent.

The calcium carbonate powder was characterized by X-ray diffraction (XRD) with a Siemens D5000 diffractometer (SIEMENS, Germany). The surface area of both fillers was assessed by BET analysis by using an ASAP 2020 porosimeter (MICROMERITICS, USA) after keeping them at

150 °C for 10 h. The crosslinked composites and degraded specimens were examined by scanning electron microscopy (SEM). The SEM images were acquired on a Zeiss LEO 440 scanning electron microscope (ZEISS, Germany) operated at acceleration voltages of 3 and 10 kV with the secondary electron and backscattered electron detector, respectively. A working distance of 10 mm was used. Elemental analysis was performed by Energy Dispersive X-Ray Spectroscopy (EDX), using an EDAX detector and Genesis Software at an acceleration voltage of 20 kV.

Mechanical tests

Compression tests of the samples were performed using a Shimadzu AGS-H (SHIMADZU, Japan) strength tester with a crosshead speed of 1 mm/min and a 5-kN load cell. The corresponding stress-strain curves were nonlinear and J-shaped. The compressive elasticity modulus was calculated from the linear part after the toe-region, the maximum compressive strength and compressive strain at break were also determined. A batch of five samples (N = 5) for each material was tested. Statistical analysis of the results was performed in PASW Statistics 18 (SPSS, Chicago, IL) using analysis of variance (ANOVA) with Tamhane's post-hoc test since equal variance could not be ensured. A significance level of 0.05 was used.

Water uptake study

For the water uptake study, five samples containing 5 and 30 wt % porous b-TCP, 5 and 30 wt % calcium carbonate, and a control sample were used. The samples were immersed in phosphate-buffered saline (PBS) and placed into an oven (BINDER, Great River, NY) at 37 °C. The samples were removed from the medium at days 1, 2, 4, 8, 10, 12, 23, 27, 30, 34, 38, and 41, air-dried on the surface, and weighed five times. The mean value was assumed to be the sample weight, and the standard error was calculated for each set of measurements. The PBS media were changed frequently to maintain them at a pH of 7.40 to simulate an in vivo environment. The percentage of water uptake (H₂O%) was calculated from Eq. (1), where the initial dry weight of the samples corresponds to w_0 whereas w_i corresponds to the wet weight.

$$\text{H}_2\text{O}\% = \frac{w_i - w_0}{w_0} \cdot 100 \quad (1)$$

The samples were dried in a desiccator, their surfaces were examined via SEM, and elemental analysis was performed by EDX.

RESULTS

¹H-NMR spectroscopy

The chemical shift assignments for the hydroxyl-terminated oligomer are shown in Figure 2. The number average molecular weight (M_n) of the hydroxyl-terminated oligomers was determined to be 2644 g/mol via end group analysis. This was done through the integrated intensities of OCH methine proton signal (a), from the DLLA, OCH₂ methylene protons signal (b), and from the CL and the end group proton signals (c, d, e).

From one batch of hydroxyl-terminated oligomer, three batches of acrylic-terminated oligomer were prepared under the same conditions. The yields were calculated from the ratio of the final oligomer mass to that of the initial feed. The yields were 17, 72, and 82%. The differences are attributed to material loss during the filtration process. The chemical shift assignments for the second batch (72% yield) of

acrylic-terminated oligomer are shown in Figure 3. The number average molecular weight of the second batch was determined via end group analysis to be 2443 g/mol. This was done through the integrated intensities of OCH methine proton signal: (a) from the DLLA, OCH₂ methylene protons signal and (b) from the CL and the acrylic end group proton signals.

X-ray diffraction

The X-ray diffraction pattern of the calcium carbonate powder used as a filler for the preparation of the injectable composites is shown in Figure 4. The aim was to determine which phase of calcium carbonate corresponded to that of the commercial powder. The X-ray diffractogram revealed that the powder was principally pure calcite, which exhibited a characteristic reflection at 29.4°.

BET surface area

The surface area of the ceramic fillers was determined by BET analysis of the adsorption curves at five different pressures. The b-TCP particles resulted to have a smaller surface area of 0.25 m²/g, whereas the CaCO₃ presented a surface area of 0.41 m²/g.

Handling

Table I shows the working and setting time values of the composites. The samples containing 30% calcium carbonate exhibited the lowest setting time of 11 min. The rest of the samples, including the control, exhibited similar setting times of approximately 20 min. Because of technical inconveniences, the handling data for poly[D,L-lactide-co-(ε-caprolactone)] . 5 wt % b-TCP could not be determined.

Scanning electron microscopy

The backscattered electron detector was used to obtain images with compositional contrast. In these images, the elements with a higher atomic number (Z) correspond to the brighter regions.

Figure 5 depicts a low magnification view of the cured composites cut in transverse sections. The presence of macropores, due to air trapped during the blending, can be considered negligible and the particles appear to be evenly distributed throughout the matrix. Figure 6 shows a high magnification view that illustrates the ceramic/polymer physical interaction at the microlevel and the particle morphology. Figure 6(a) shows the porous b-TCP embedded into the polymeric matrix. The particle size is approximately 100 μm (a particle size distribution of 50–150 μm is given by the manufacturer), and the particles are irregularly shaped. It can be noted that the polymer has penetrated the porous structure of the b-TCP particle [Figure 6(a)]. These pores range from 2 to 10 μm. Figure 6(b) depicts the calcium carbonate particles in a polymeric matrix. The particles are rhombohedral with an approximate maximum dimension of 20 μm and therefore much smaller than the b-TCP particles. No signs of porosity were detected on their surfaces. Detachment can be appreciated in the micrograph indicated by a darker area at the interface between one of the crystals and the matrix.

After 21 days in PBS solution, samples containing b-TCP did not show any sign of surface degradation nor apatite formation, whereas the samples containing CaCO₃ grew a layer with a flake-like morphology. An example is shown in Figure 7, which is a micrograph from the surface of the specimen with 5 wt % of CaCO₃. The surface seems to be unevenly covered by this layer. The corresponding EDX acquired spectrum showed characteristic peaks for Ca, P, C,

and O (Figure 7).

Mechanical tests

The compression test resulted in ductile failure of all samples. In fact, most of the samples regardless of the filler content conserved their original shape after the test, indicating an elastic behavior of the polymer matrix. The compressive modulus, maximum compressive strength, and the compressive strain at break were determined from the stress-strain curves. The mean values are reported in Table II. From Table II, it is observed that the addition of ceramic fillers increased both the compressive modulus and the compressive strength. The control sample showed the lowest stiffness, which was significantly different from the specimen containing 30 wt % CaCO_3 ($p < 0.04$). The control sample also exhibited the lowest strength. The compressive strain at break was found to decrease with an increase in the filler loading. On the other hand, at a constant filler loading, materials containing CaCO_3 exhibited a higher strain at break compared to the ones containing b-TCP.

In vitro water uptake

The water uptake of the composites was measured as a function of time. Figure 8 shows the water absorption percentage. It is observed that all the samples containing ceramic fillers absorbed a small percentage of water after 1 day in PBS solution, whereas the control sample maintained its initial mass. After day 4, only the samples containing calcium carbonate fillers exhibited a prominent water uptake with a maximum at day 27. The same maximum was observed for the control sample, which initially presented weight loss followed by water absorption after day 8. On the other hand, the samples containing porous b-TCP did not exhibit mass gain beyond day 1, but a nearly linear mass loss up to day 41. By the end of day 41, all the specimens containing fillers conserved their structural integrity and original shape. However, the control specimen showed clear signs of degradation on its surface while also became sticky and crumbly.

DISCUSSION

From the ^1H -NMR spectrum of the hydroxyl-terminated oligomer (Figure 2), it is observed that the signal for unreacted hydroxyl groups from 1,4-butanediol (BD) at 3.46 ppm is absent, indicating a complete consumption of the coinitiator.

¹² This also suggests that the BD initiates the chain growth of the linear oligomers from both ends.¹³ Furthermore, the presence of a weak resonance (d) at 3.61 ppm, corresponding to the terminal CL methylene protons (CH_2OH), shows that some chains are actually CL terminated.

Figure 2 also shows that most of the chains are DLLA terminated as indicated by the signal of the methine protons (c) next to the terminal hydroxyl group (CHOH) at 4.33 ppm and the signal of the hydroxyl proton (e) at 2.85 ppm.¹⁴ Given the low concentration of co-initiator with respect to the monomer, it is assumed that each BD molecule may initiate two polymer chains.¹⁵ Therefore, terminal CL methylene and DLLA methine protons are observed. In any case, the oligomers will be hydroxyl-terminated. Furthermore, no unreacted monomers were detected indicating that the reaction was completed successfully.

In the ^1H -NMR spectrum for the acrylic-terminated copolymer (Figure 3), the appearance of signals at 7.17, 7.24, and 2.36 ppm reveals the presence of residual toluene.¹⁶ The resonances corresponding to the terminal hydroxyl groups have almost completely vanished. At the same time,

the signals for the acrylic end groups appear clearly at 5.89, 6.17, and 6.44 ppm indicating a successful preparation of the acrylic-terminated oligomers.¹² However, small protrusions corresponding to the terminal DLLA methine and methyl protons can still be observed at 4.24 and 4.36 ppm indicating the existence of some unreacted hydroxyl end groups from the DLLA.

The copolymer structure confirmed via ¹H-NMR along with the number average molecular weight of 2443 g/mol assessed via end group analysis indicates a successful preparation of crosslinkable oligomers. The relatively low molecular weight of these acrylic-terminated oligomers will give them the necessary consistency for injectability. In addition, the acrylic end groups will permit in situ crosslinking via radical initiation.

When comparing both the working and setting times for the specimens containing CaCO₃ (Table I), it can be noted that the values are lower for the higher concentration of filler. This suggests that a higher presence of calcium carbonate accelerates the crosslinking of the polymer. However, this may be due to the lower amount of acrylic end groups that are available to react due to a lower concentration of the copolymer. Since the concentration of BPO and DMPT is maintained (1 wt %) for all the specimens the reaction may take place at faster rates. More importantly, these setting times are similar to those of acrylic bone cements in current clinical use (5.5–28.3 min).^{17,18} It may however be necessary to increase the working times. Decreasing the concentrations of radical initiator and chemical activator, to slow down the crosslinking reaction, could be an option. Qualitatively, the composite pastes including the fillers exhibited a proper viscosity for injectability. However, further studies are required to measure this property in a quantitative manner.

In Figure 5, it was observed that the particles appeared to be evenly spread throughout the polymer matrix in all cases. This indicates that the manual mixing technique that was used to prepare the composites was adequate for this purpose.

From Figure 6(a), it was observed that the morphology of the porous b-TCP particles appeared to promote a mechanical interlocking at the microscopic level giving a good particle/matrix adhesion. This may delay possible particle detachment from the composite's surface. This may also limit the dissolution of the b-TCP mainly to chemical dissolution and therefore to reduce the degradation rate of the whole composite. On the other hand, Figure 6(b) shows that geometrically shaped calcite crystals may cause its detachment from the surface easier than the porous b-TCP particles due to a reduced or lack of mechanical interlocking.

After soaking the composites in PBS for 21 days, an apatite layer was formed on top of the specimens containing CaCO₃ as confirmed by EDX (Figure 7). Previous studies have shown that negatively charged surfaces such as those containing carboxylic groups (ACOOH), strongly promote the apatite nucleation after Ca²⁺ adsorption.¹⁹ Carboxylates are one of the degradation products of poly[D,L-lactide-co-(ε-caprolactone)] and provide a substrate for apatite nucleation.

For these apatite nuclei to grow, a supersaturated solution with respect to the apatite is required.²⁰ The Ca²⁺ ions supplied from the rapid dissolution of CaCO₃ fillers may provide this supersaturation. In fact, it has been shown that apatite is able to grow as fast as in 3 h on poly(L-lactic acid)/CaCO₃ composites in SBF through this mechanism.^{21,22} These studies used vaterite because of its higher solubility when compared to other CaCO₃ phases. Pure calcite (Figure 4) was found to be the phase corresponding to the CaCO₃

used here.²³ These results show that the highly stable calcite can promote the apatite formation on poly[D,L-lactide-co-(ε-caprolactone)]. Furthermore, an apatite layer did not grow on the specimens containing b-TCP indicating an absence of apatite supersaturation and therefore a lower solubility of b-TCP when compared to CaCO₃ (calcite).

The mechanical properties of particulate-polymer composites are significantly affected by the particle size, particle/matrix adhesion, and particle loading.²⁴ In this study, the particle size is expressed in terms of the surface area, which is higher for smaller particles at a certain particle loading. All the specimens showed a general increase in both stiffness and compressive strength because of the addition of fillers (Table II). However, only the specimens containing a load of 30 wt % CaCO₃ were significantly stiffer than the control. The specimens containing 30 wt % CaCO₃ generally showed a higher stiffness than those containing 5 wt % CaCO₃. This correlates with the fact that above a critical particle size, the modulus of a particulate-polymer composite is significantly affected by the particle loading.²⁵ This is because below the elastic limit, stiffer fillers increase the rigidity of the matrix regardless of the particle/matrix interfacial adhesion. Nonetheless, an opposite effect was observed for the specimens containing b-TCP particles, which exhibited a higher stiffness for a lower particle loading. It is possible that for such high particle sizes, porous fillers may have a detrimental effect on the stiffness. The Young's modulus of tricalcium phosphates is known to decrease with an increase in the porosity,²⁶ and therefore an increase of fillers may decrease the overall stiffness of the whole composite.

b-TCP composites showed a decrease in compressive strength with an increase in the particle loading, whereas the CaCO₃ composites showed the opposite behavior (Table II). On the other hand, the compressive strength decreased by increasing the fillers' surface area for a low particle loading, whereas the opposite was observed for a high particle loading. In general, a stronger particle/matrix interface will increase the strength by improving the loads transfer between the matrix and the filler. However, the variable results are not surprising since the strength is known to depend on the combined effects of particle size, particle loading, and particle/matrix adhesion.^{25,27} Therefore, further tests are necessary to improve the statistical power and confirm the effect of the fillers on the mechanical properties.

Previous studies have suggested that brittleness of many materials is inversely proportional to the strain at break.^{28,29} From Table II, it is observed that increasing the amount of fillers may enhance the brittleness of these composites. Moreover, the composites containing CaCO₃ showed higher strains at break indicating a possibly lower brittleness compared to the composites containing b-TCP. These results are in accordance with the literature suggesting a general increase of brittleness for polymer-ceramic composites because of the relatively high brittleness of the ceramic component,³⁰ but data on the storage modulus may be necessary to further confirm these trends.

The advantage of using a polymeric matrix is its mechanical similarity to that of the physiological intervertebral disk. In fact, the mean values for the compressive modulus of the composites studied here, which range from 1.8 to 4.9 MPa (Table II), are similar to those of the tissues in the disk.⁶ However, the nucleus pulposus has to withstand pressures between 0.1 and 0.2 MPa at rest to 1.0 and 3.0 MPa when standing or lifting,⁶ suggesting an IVD replacement would need a somewhat higher strength than

that found in this study. This may be provided by an increase in the degree of crosslinking in the matrix and through the incorporation of the ceramic fillers. Furthermore, this resemblance shows that these materials have potential to be used as injectable replacements for the IVD. However, the variation of the mechanical properties with the degradation time also constitutes a topic of interest for further studies.

Degradation of poly[D,L-lactide-co-(ε-caprolactone)] is known to occur by hydrolysis of the ester bonds, which is autocatalyzed by the carboxylic acid degradation products.¹⁴ It is also well known that CL degrades at lower rates than DLLA, and therefore their relative amounts will control the copolymer degradation rate.¹⁴ Depending on the relative crystallinity and hydrophilic properties of the polymers, the degradation of the matrix can occur through two mechanisms: surface erosion and bulk degradation.³¹ It is recognized that the amorphous nature of PDLLA facilitates the water absorption and consequently bulk degradation takes place. This can occur via random scission of the ester bonds.³² Furthermore, surface erosion is more likely to take place on PCL, which is semicrystalline and hydrophobic giving relatively slow degradation rates due to less exposed area for hydrolytic attack.¹² This can occur via stepwise scission of the crystalline zones.³² Therefore, it is expected that a combination of the two mechanisms initiated by bulk erosion of the DLLA followed by surface erosion of the CL may arise in a case like this. Since the composition of the matrix was maintained, hydrolytic degradation of specimens with different fillers was studied to investigate the effect of the ceramic particles on the matrix degradation mechanism in vitro. All the samples containing ceramic fillers retained their initial shape and appearance throughout the study, whereas the sample without fillers became soft and sticky after 2 days in PBS solution. This indicates that the presence of the particles increases the mechanical stability and reduces the polymer matrix degradation rate. In fact, it has been shown that the presence of CaCO₃ fillers within copolymers of poly(L-lactide) and poly(glycolic acid) is effective to avoid a considerable reduction in the local pH of such implants.³³ In addition, another study concluded that TCP fillers into poly[lactide-co-glycolic acid-co-(ε-caprolactone)] inhibited the hydrolytic process by buffering the pH of the medium.³⁴ Moreover, the incorporation of b-TCP fillers into poly(propylene fumarate) degradable networks was confirmed to delay the in vitro degradation of these materials.³⁵ Therefore, both fillers may have a pH buffering effect, which can lower the degradation rates. If the composite is intended as a regenerative scaffold for IVD including an active pharmaceutical component and/or a cell component, the buffer effect may be used to raise the pH which otherwise may affect the cell proliferation.³⁶ Another factor of significance is that the calcium ions from either b-TCP or CaCO₃ can bind to the carboxylic end groups from the degrading polymer matrix and form ionic bonds as proposed by Xie et al.,³⁷ stabilizing the composite and slowing down its degradation. This may be beneficial since the scaffold material should be able to initially provide the necessary mechanical support until the new tissue has grown enough to overtake the loading.³⁸ This means that the initial mechanical properties could be controlled via stabilization of degrading carboxylic end groups due to the calcium ions from the ceramic fillers. In fact, an increase of the mechanical properties at the initial stages of degradation has been observed in similar studies on PPF-based composites.³⁵ Even though it was attributed to further crosslinking of the polymer matrix, it is speculated that calcium ions from b-

TCP might also have an influence on this phenomenon. Given the higher chemical solubility of calcium carbonate than that of the apatite formed for most calcium phosphates, it is believed that calcium carbonate particles will easier dissociate into the solution. Furthermore, the higher surface area of the calcium carbonate fillers when compared to b-TCP as determined from BET analysis may enhance this effect. Moreover, as evidenced by SEM, porous b-TCP particles will be mechanically attached to the polymer matrix to a higher degree, and therefore reduce the probability of physical detachment from the surface. If a ceramic particle detaches from the surface of the polymer, a void is left behind that may facilitate the water absorption by capillary effect. Another consequence of particle detachment is a higher exposed surface area, which is susceptible to hydrolysis. This suggests that materials containing rhombohedral calcite will absorb more water than those containing porous b-TCP particles. In fact, Figure 8 shows that the samples containing a higher concentration of calcite exhibited a higher percentage of water absorption. Unreacted oligomers (that are soluble in water) might also contribute to mass loss. These oligomers would be more likely to move throughout the composites containing smaller, less mechanically attached, more soluble CaCO_3 particles. Consequently, more space is available for water uptake and hence the water absorption would increase compared to the specimens containing b-TCP, which exhibited mass loss. The water absorption is especially interesting for the IVD because of the naturally occurring swelling of the nucleus pulposus, which is important to the compressive resistance, and for the transport of nutrients.¹⁰ Furthermore, injectable in situ crosslinking degradable composites with bioactive capabilities may also be of interest as endplates substitutes given their ability for bone bonding.

CONCLUSIONS

Injectable in situ crosslinking degradable composites with mechanical properties similar to those of the tissues found in the intervertebral disk have been produced. In terms of the mechanical properties, the introduction of ceramic particles into poly[D,L-lactide-co-(ε-caprolactone)] appeared to increase both the compressive modulus and the maximum compressive strength. Furthermore, it was shown that both b-TCP and CaCO_3 reduce the degradation rates of poly[D,L-lactide-co-(ε-caprolactone)], which is attributed to carboxylic groups stabilization and a buffering effect. This study also shows that poly[D,L-lactide-co-(ε-caprolactone)]/ CaCO_3 composites promote the formation of an apatite layer on its surface, whereas poly[D,L-lactide-co-(ε-caprolactone)]/b-TCP does not exhibit this property. The solubility rate of calcite is enough to supersaturate the PBS solution with a Ca^{2+} supply for the apatite nuclei to grow when compared to less soluble b-TCP. These materials constitute alternative potential substitutes for different parts of the intervertebral disk and further research is underway to improve them.

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FIGURE 1. Synthesis scheme of acrylic-terminated oligomers.

FIGURE 2. ¹H-NMR spectrum of the hydroxyl-terminated oligo[D,L-lactide-co-(ε-caprolactone)] in CDCl₃ showing the peak assignments.

FIGURE 3. ¹H-NMR spectrum of the acrylic-terminated oligo[D,L-lactide-co-(ε-caprolactone)] in CDCl₃ showing the peak assignments.

FIGURE 4. XRD pattern of the calcium carbonate powder used as a filler. The pattern exhibits a characteristic peak at 29.4° corresponding to the calcite phase.

TABLE I. The Working and Setting Time 6 Standard Deviation for Some of the Composites

Sample	Working Time (min)	Setting Time (min)
P(DLLA-co-CL) . 5% b-TCP	N/A	N/A
P(DLLA-co-CL) . 5% CaCO ₃	1.6	6 0.3 20 6 3
P(DLLA-co-CL) . 30% b-TCP	1.7	6 0.7 22 6 6
P(DLLA-co-CL) . 30% CaCO ₃	1.0	6 0.1 11 6 1
P(DLLA-co-CL)	1.0	6 0.3 20 6 8

All the pastes were mixed during 20 s before the specimens were transferred to the moulds.

FIGURE 5. Backscattered electron micrographs of crosslinked composites cut in transverse sections acquired at a magnification of 100_x: (a) P(DLLA-co-CL) . 5 wt % porous b-TCP; (b) P(DLLA-co-CL) . 5 wt % calcium carbonate; (c) P(DLLA-co-CL) . 30 wt % porous b-TCP; (d) P(DLLA-co-CL) . 30 wt % calcium carbonate.

FIGURE 6. High magnification, backscattered electron micrographs of ceramic particles embedded in the polymeric matrix: (a) porous b-TCP at a magnification of 600_x; (b) rhombohedral calcite (calcium carbonate) at a magnification of 3000_x.

FIGURE 7. Micrograph (left) of the specimen containing 5 wt % CaCO₃ after 21 days in PBS at 37 °C showing an apatite layer on the surface. EDX spectrum (right) showing the characteristic peaks for Ca and P.

TABLE II. Mean Modulus (E_c), Maximum Compressive Strength (t_{max}), and Strain at Break (ε_b) 6 Standard Deviation for the Five Composite Formulations

Sample	E _c (MPa)	t _{max} (MPa)	ε _b
P(DLLA-co-CL) . 5% b-TCP	4.0	6 2.3 0.7 6 0.4 0.23 6 0.02	
P(DLLA-co-CL) . 5% CaCO ₃	2.9	6 0.9 0.5 6 0.2 0.25 6 0.01	
P(DLLA-co-CL) . 30% b-TCP	3.0	6 1.1 0.4 6 0.2 0.15 6 0.04	

P(DLLA-co-CL) . 30% CaCO₃ 4.9 6 1.4 0.8 6 0.2 0.19 6 0.00

P(DLLA-co-CL) 1.8 6 0.8 0.3 6 0.2 0.25 6 0.04

All the properties were measured in compression and 5 samples were tested for each batch.

FIGURE 8. Water absorption curves for specimens containing different filler compositions immersed in 7.5 mL of PBS at 37_C during 41 days.