## PAPER DETAILS

TITLE: Preparation of Poly(DL-Lactide/?-Caprolactone)-?-TCP Composites for Bone Tissue Repair

AUTHORS: Hasret Tolga SIRIN, Ebru AKDOGAN, Erhan BISKIN

PAGES: 133-141

ORIGINAL PDF URL: https://dergipark.org.tr/tr/download/article-file/699939



# Preparation of Poly(DL-Lactide/ε-Caprolactone)–β-TCP Composites for Bone Tissue Repair

### Kemik Dokusu Onarımı için Poli(DL-Laktat/ε-Kaprolakton)–β-TCP Kompozitlerin Hazırlanması

#### Hasret Tolga Sirin<sup>\*0</sup>, Ebru Akdogan<sup>0</sup>, Erhan Biskin<sup>0</sup>

Bioengineering Division, Institute for Pure and Applied Sciences, Hacettepe University, Ankara, Türkiye.

#### ABSTRACT

Poly-D,L-Lactide/ε-caprolactone (PDLLA-ε-CL) and β-tri-calcium phosphate (β-TCP) composites were prepared as an alternative for injectable filling material in bone tissue repair. β-TCP was synthesized by a wet precipitation method and characterised using Fourier Transform Infrared Spectroscopy (FTIR), X-Ray Diffractometer (XRD), Zeta-SIZER and Brunauer–Emmett–Teller (BET) analyser. PDLLA-ε-CL copolymer was synthesized under nitrogen (N2) atmosphere using ring-opening polymerization in the presence of stannous octoate as a catalyst. The chemical structures of copolymers were determined by FTIR and Proton Nuclear Magnetic Resonance (1H-NMR) analysis. The average molecular weights of copolymers were identified by Gel Permeation Chromatography (GPC). (PDLLA-ε-CL)-β-TCP composites were then prepared by loading differing amounts of β-TCP to the copolymer phase. The resulting composites were easily shaped by hand. The degradation profiles of the composites were determined by monitoring the changes in the molecular weight of the co-polymers over a period of 42 days. Degradation rate of the composites decreased as the percentage of β-TCP in the structure increased.

#### Keywords

Bio-ceramic, tricalciumphosphate, hard tissue repair, bone filler, Poly(D,L-lactide/ ε -caprolactone).

öz

Poly-D,L-Laktat/ε-kaprolakton (PDLLA-ε-CL) ve β-tri-kalsiyum fosfat (β-TCP) kompozitleri, alternatif bir biyobozunur enjekte edilebilir dolgu malzemesi olarak geliştirilmiştir. Bu amaçla β-TCP, çöktürme yöntemi ile sentezlenmiştir ve karekterizasyonu Fourier Dönüşüm Kızılötesi Spektroskopi (FTIR), X-Işını Defraktometresi (XRD), Zeta-SIZER ve Brunauer-Emmett-Teller (BET) kullanılarak yapılmıştır. PDLLA-ε-CL kopolimeri, katalizör olarak kalay oktoat kullanılarak halka açılım polimerizasyonu ile azot (N2) atmosferi altında sentezlenmiştir. Kopolimerlerin karakterizasyonu FTIR ve Proton Nükleer Manyetik Rezonans (H-NMR) analizi ile yapılmıştır. Ortalama moleküler ağırlığı, Jel Geçirgenlik Kromatografisi (GPC) ile hesaplanmıştır. (PDLLA-ε-CL)-β-TCP kompozitleri, kopolimere farklı miktarlarda β-TCP yüklenerek hazırlanmıştır. Elde edilen kompozitler kolayca elle şekillendirilebilmektedir. Kompozitlerin bozunma profilleri, 42 gün boyunca kopolimerlerin moleküler ağırlığındaki değişimlerin izlenmesi ile belirlenmiştir. β-TCP oranı arttıkça kompozitlerin bozunma oranının azaldığı gözlenmiştir.

#### Anahtar Kelimeler

Biyo-seramik, trikosifosfat, sert doku onarımı, kemik dolgusu, Poli (D, L-laktid / ɛ-kaprolakton).

Article History: Received: Jan 31, 2019; Revised: Feb 30, 2019; Accepted: Feb 30, 2019; Available Online: Mar 01, 2019.
DOI: 10.15671/HJBC.2019.283
Correspondence to: H.T. Sirin, Bioengineering Division, Institute for Pure and Applied Sciences, Hacettepe University, Ankara, Türkiye.
E-Mail: stolga@hacettepe.edu.tr

#### INTRODUCTION

nioceramics have been widely used in hard  ${\sf D}$ tissue repair [1-5]. They can be classified into three main groups: (i) bioinert bioceramics such as alumina, zirconia etc.; (ii) bioactive bioceramics such as bioactive glasses, bioactive glass-ceramics etc.; and (iii) degradable ceramics such as calcium phosphate, tricalcium phosphate (TCP), aluminiumcalcium phosphate, zinc-calcium-phosphorus oxide etc. Among these, TCP and hydroxyapatite (HA) are the most attractive bioceramics due to having similar composition to the inorganic fraction of human bones [1,3,4,6-9]. Bioceramics are inherently stiff materials and it is hard to place an artificial graft made of only bioceramics into a bone defect without any void. Thus, the soft tissue cells can easily penetrate and grow into these voids and not allow proper bone tissue formation. In order to avoid the soft tissue cell migration and growth, biodegradable and injectable filling materials, such as polymeric composites containing TCP and HA, have been widely used [10-16]. In addition to allowing minimally invasive procedure, the injectable fillers can fill even the irregular defects by taking the shape of the cavity in which they are placed [13,16]. The rationale behind polymer/bioceramic composites is to combine the bioactive properties of the bioceramics with the elastic properties of the polymers. The degree of bioactivity, biodegradation rate, and mechanical properties of the composite can be adjusted by the volume ratio of the bioceramic to the polymer [17].

Synthetic biodegradable polymers, such as PLLA, PLGA, PCL and their copolymers are the most commonly used polymers for preparing composite matrices for bone repair [2,11,18-21]. For instance, Hong et al. reported the preparation of a poly(L-lactic acid) and sol-gel-derived bioactive glass-ceramic (BGC) composite for orthopedic application [23]. An injectable calcium phosphate/ poly(DL-lactide-co-glycolide) composite, in which each CP nanoparticle was coated with the copolymer, have been successfully prepared by Ignjatović et.al. [12]. A composite of poly-*ε*-caprolactone (PCL) loaded with gatifloxacine and  $\beta$ -TCP have been proposed as a matrix to prevent osteomyelitis and promote new bone formation [24]. Yang et al. have coated electrospun PCL scaffold with calcium phosphate (CaP) [25] while Guarino et al. fabricated PCL scaffolds using the combination of a filament winding technique and a phase inversion/salt leaching process where PLA fibers and CaP were used as reinforcement material [26].

The use of (PDLLA/ $\epsilon$ -CL)- $\beta$ -TCP composites in various forms were also reported in the literature. A (PLLA/ε-CL)/β-TCP phosphate membrane and gelatin sponge incorporated with basic fibroblastic growth factor (bFGF) have been found to be promising for bone regeneration in mandibular ridges [28]. Kikuchi et al. prepared biodegradable and thermoplastic membranes from the composites of  $\beta$ -TCP and (PLLA/ $\epsilon$ -CL) by a heat-kneading method [29]. López et al. fabricated injectable, in situ setting, degradable composites for intervertebral disk replacement by mixing β-TCP and calcium carbonate into acrylic-terminated oligo (DLLA/ε-CL) which were crosslinked at room temperature [15]. In a previous study conducted in our group, we reported the synthesis and characterisation of (PLLA/ε-CL)/hydroxyapatite composites as novel bone-filling materials. However, there is no report on (PDLLA/ $\epsilon$ -CL)- $\beta$ -TCP composites in the form of a degradable injectable filler material.

The aim of the study was to prepare a degradable injectable filler material from a  $\beta\text{-TCP}$  and biodegradable PDLLA/ɛ-CL co-polymer, which are flexible and can be easily shaped by hand, to use in bone tissue defects. For this purpose,  $\beta$ -CP was synthesized by precipitation method using calcium hydroxide and phosphoric acid. Dried and sintered TCP was characterised using FTIR, XRD, Zeta-SIZER and BET analyser. PDLLA/ɛ-CL copolymer was synthesized by ring opening polymerization and subsequently characterised using FTIR and <sup>1</sup>H-NMR analysis. The average molecular weights of copolymers were identified with GPC. (PDLLA/ $\epsilon$ -CL)- $\beta$ -TCP composites were prepared using varying amounts of  $\beta$ -TCP and their degradation profile were determined by monitoring the changes in the molecular weight of the co-polymers for 42 days.

#### **MATERIALS and METHODS**

#### Preparation and characterisation of TCP powders

β-TCP was prepared using calcium hydroxide  $(Ca(OH)_2, Sigma, US)$  and phosphoric acid  $(H_3PO_4, BDH, UK)$ . To reach a Ca/P atomic ratio of 1.5.; 2.3 g of Ca(OH)<sub>2</sub> (Sigma, USA) was suspended in 200 mL of ultrapure water. The suspension was pre-heated to 40°C in a hot plate (IKA C MAG HS 7) with continuous stirring. 250 ml of 0.08 M phosphoric acid  $(H_3PO_4, BDH, UK)$  was applied dropwise into this suspension with the aid of a dropping funnel. The pH was monitored throughout the reaction, and 1:1 ratio ammonia (Aklar Chemistry, Turkey) - water mixture was used to maintain the pH constant. It is known that the pH

value of the reaction effects  $\beta$ -TCP formation. Thus, the reaction was conducted at pH 8.00 according to literature reports [1,9,30-33]. After the phosphoric acid solution was completely depleted, the temperature was increased to 70 °C. The mixture was kept under continuous stirring for 2 hours at 70°C. After 2 hours, the heater was turned off and the mixture was kept in continuous stirring for an additional 18 hours. In order to easily remove the remaining solute, the resulting white precipitate was allowed to stand for 24 hours to settle down to the bottom of the beaker. The precipitate was centrifuged for 15 min at 2000 rpm (Herhaus Biofuge Stratus, Germany). Centrifugation was repeated 4 times and the resulting white gel was collected to a petri dish and dried in an oven at 70°C for 24 hours. Resulting dried powders were made into pellets of 1 cm in diameter and 3 mm in thickness under 1700 kg/cm<sup>2</sup> pressure. Finally, the pellets were sintered at 1000°C.

The chemical structure of  $\beta$ -TCP was investigated using Fourier Transform Infrared Spectroscopy (FTIR, Shimadzu, DR 8101, Japan) at a resolution of 4 cm<sup>-1</sup> between 400-4000 cm<sup>-1</sup>. 40 scans were performed for each sample.

XRD measurements were conducted at 50 kV x 150 mA. Measurements were performed for 60 minutes between 5-100° TTH. Each measurement was repeated 6 times. Results were compared to the  $\beta$ -TCP (Whitlockite, PDF No:9-0169) and hydroxyapatite (HA, PDF No:09-0432) WRD patterns in the ICDD literature.

Zeta-SIZER (Malvern-3000) was used to measure the particle size of the  $\beta$ -TCP gel. Samples were diluted with distilled water prior to the measurement. The measurements were repeated three times.

The specific surface areas of  $\beta$ -TCP powders were determined using a BET analyzer (Quantachrome NOVA 2000) using nitrogen gas at 99.99% purity. Gas removal from the sample was carried out in a vacuum environment for 5 hours.

## Preparation and Characterization of PDLLA/ $\epsilon$ -CL copolymer

A PDLLA/ $\epsilon$ -CL co-polymer synthetized previously by our group was used in this study [19]. Briefly; D,L-lactic acid (Purac, pharmaceutical grade, The Netherlands) was recrystallized twice under reduced pressure at 45°C before use while  $\epsilon$ -caprolactone (Acros, Belgium) was dried on molecular sieve for about 24h. Stannous octoate (Sigma, USA) were use as catalyst. Polymerization system consisted of a glass polymerization reactor and a nitrogen flow-through inlet with a vacuum connection. Temperature was adjusted by placing the reacor in a via silicone oil bath. Polymerization was carried out under nitrogen atmosphere for 24h. The lactide/caprolactone ratio, the dimer/catalyst ratio, and the polymerization temperature were the same in both polymerization, and were 50/50 (w/w), 0.003/1 (w/w) and 145°C.

The polymerisation method is described elsewhere in detail [19]. The polymerisation conditions are given in Table 1.

The average molecular weights and heterogeneity indexes (HI) of the co-polymer were determined using gel permeation chromatography (GPC). The GPC system consisted of an HPLC pump (Spectra-Physics, P1500, USA), a column for polymers up to 400,000 molecular weight (Shodex, K-804, Japan), a refractive index detector with Tungsten lamp (Shodex, RI-71, Japan) and data processing system (Shodex, Showa Denko, Japan). Chloroform (Riedel de Haen, Germany) was used through the column at a rate of 1 ml/min as the solvent and the carrier phase. Commercially available polystyrene (PS) standards (Shodex, Japan) with narrow molecular weight ranging from 17500 to 105000 was used for calibration.

The chemical structure of PDLLA/ $\epsilon$ -CL co-polymers was investigated using FTIR at a resolution of 4 cm<sup>-1</sup> between 400-4000 cm<sup>-1</sup>. For each sample 40 scans were performed [19].

Table 1.	Pol	ymerisation	conditions.
----------	-----	-------------	-------------

Polymerisation duration	Polymerisation Temperature	Monomer Ratio	Amount of DLLA	Amount of ε-CL	Amount of Stannous oktoat	Atmosphere
20 hours	145°C	1:1	30 g	30 g	0.62 g	N <sub>2</sub>

The structure of the co-polymer was determined using Proton Nuclear Magnetic Resonance (H-NMR) analysis (Varian XL-300, USA). The working frequency was 399.96 MHz. Sample concentration was 4 mg/ml copolymer/chloroform. Measurements were made at a constant temperature of 18°C [19].

#### Preparation of PDLLA/ε-CL /TCP composites

(PDLLA/ $\epsilon$ -CL)-  $\beta$ -TCP composites containing 20%, 40% and 60% of  $\beta$ -TCP were prepared. Firstly, the copolymer was dissolved in chloroform at the concentration of 50% (w/v). This viscous copolymer solution was stirred at room temperature in a magnetic stirrer and the certain amounts of  $\beta$ -TCP were added to the solution to obtain the different ratios of  $\beta$ -TCP content. The mixture was stirred using an ultrasonic bath until the composite reached dough-like consistency. The solvent was then evaporated from the composite under vacuum at 50°C for 5 days.

In vitro degradation profiles of the composites were determined in phosphate buffered saline (PBS) solution (pH=7.4) at 37°C. The solution was refreshed every 3 days in order to keep the pH constant and to prevent microbial contamination. Samples were formed into cylinders with a diameter of 0.5 cm and a length of 1 cm and were placed in sachets of silk filter with a pore diameter of 88  $\mu$ m. The sachets were soaked into the plastic tubes containing PBS and the tubes were placed in an oven at 37°C. The samples were removed at predefined time intervals and then dried under vacuum at 35°C for 5 days. The average molecular

weights and heterogeneity indexes (HI) of the samples were determined GPC as described above.

#### **RESULTS and DISCUSSION**

#### **TCP Powders**

The FTIR spectrum of  $\beta$ -TCP powder is presented in Figure 1. The characteristic peaks of  $\beta$ -TCP are in accordance with the literature [38]. -PO<sub>4</sub> bending vibrations were observed at 562, 601, 1094 cm<sup>-1</sup> [34, 35]. Characteristic peaks for -PO<sub>4</sub> stretching vibrations were observed at 961, 1031 cm<sup>-1</sup> [34, 35]. P-OH stretching vibrations were observed at 873 cm<sup>-1</sup>. Bending mode and -OH symmetrical stretching mode vibrations of -OH groups were observed at 631 cm<sup>-1</sup> [34-36]. The peak at 3426 cm<sup>-1</sup> was attributed to free water molecules [34, 37].

XRD spectra of  $\beta$ -TCP powders dried at 75°C and sintered at 1000°C are given in Figure 2. From Figure 2a, it can be seen that crystallization was completed for the samples dried at 75°C. The peaks with the highest intensity at 26°C and 32°C TTH indicate the formation of calcium phosphate. Those peaks are more pronounced and separated from each other in the spectra of sintered powder, which suggests that the crystallization was increased. It is previously reported in the literature that the crystallization of TCP is completed at 1000°C [39]. The XRD spectra of sintered  $\beta$ -TCP was compared to the spectra of  $\beta$ -TCP and HA in the ICDD literature for further confirmation of  $\beta$ -TCP formation (Figure 3) [38,39]. While



Figure 1. FTIR spectra of β-TCP powder.



Figure 2. XRD spectra of  $\beta$ -TCP powders a) dried at 75°C, b) sintered at 1000°C.



Figure 3. Comparison of sintered  $\beta$ -TCP powder with the ICDD literature.

the dominant phase is  $\beta$ -TCP (shown in green peaks), the peaks at around 32° TTH (red peaks) indicates the presence of HA [38]. The Ca/P ratio was found to be 1.55.

The particle size of the  $\beta$ -TCP in the gel form was measured as 300 nm, where the specific surface area and the crystal size of the dried powder were 82 nm and 0.90 m<sup>2</sup>/g respectively.

#### Preparation and Characterization of PDLLA/ɛ-CL co-Polymer

The average molecular weights in terms of number (Mn) and weight (Mw) and the heterogeneity indices (H.I.) showing the molecular weight distribution of the copolymer were determined as 53430 Da, 73541 Da and

1.38 respectively. These values were in good agreement with the literature [19,40].

FTIR spectrum of the PDLLA/ $\varepsilon$ -CL is presented in Figure 4. The characteristic bands assigned to the copolymer were as follows; the peak at 2943 cm<sup>-1</sup> is attributed to -CH<sub>3</sub> stretch, peak at 2866 cm<sup>-1</sup> is attributed to -C-H stretch, peak at 1736 cm<sup>-1</sup> is attributed to the -C=O stretch [19]. The peaks between 1454-1380 cm<sup>-1</sup> were attributed to -CH<sub>3</sub> bending, while the peaks between 1188-1047 cm<sup>-1</sup> were attributed to the -C-O stretching vibrations [19].

<sup>1</sup>H-NMR spectrum of the co-polymer is given in Figure 5. The most specific signal belonging the DLLA group can be observed in the region between 5.0-5.3



Figure 4. FTIR spectra of the PDLLA/ε-CL co-polymer.



Figure 5. H-NMR spectrum of the PDLLA/ $\epsilon$ -CL co-polymer.

ppm and represents the only proton in -CH [19]. The signal representing the  $\epsilon$ -caprolactone ( $\epsilon$ -CL) group in the copolymer can be observed in the region between 4.0-4.2 ppm and corresponds to the 2 protons of -OCH<sub>2</sub>- [19]. The ratio of DLLA/ $\epsilon$ -CL was calculated as 60/40 from the areas under these peak groups even though the initial ratio for DLLA/ $\epsilon$ -CL was 50/50. This result was expected because the reactivity of PDLLA is higher than the reactivity of  $\epsilon$ -CL [40-43]. Therefore, the results were consistent with the results of FT-IR and confirmed the successful synthesis of PDLLA/ $\epsilon$ -CL copolymer.

#### **Biodegradation of (PDLLA/ε-CL)-β-TCP Composite**

Biodegradable polymeric matrices consisting of a bioactive and biodegradable ceramic was obtained by loading  $\beta$ -TCP into the PDLLA/ $\epsilon$ -CL copolymer. The

small particle size allowed easy and well dispersion of β-TCP particles into the polymeric phase. The resulting matrices were easy to handle and shaped by hand at room temperature. The degradation profile of the PDLLA/ $\epsilon$ -CL copolymer and (PDLLA/ $\epsilon$ -CL)-  $\beta$ -TCP composites are presented in Figure 6. The change in the molecular weight of the copolymer increased after day 21 [40,42,43] while it was after day 35 for composites as a result of  $\beta$ -TCP incorporation to the structure. The degradation rate of the composites decreased as the percentage of  $\beta$ -TCP in the structure increased. The alteration of the degradation behaviour of the polymer is explained by the rapid exchange of protons in water for alkali in the ceramic [17]. This mechanism provides a pH buffering effect at the polymer surface that modifies the acidic polymer degradation [17]. The

**Table 2.** Changes in the molecular weight of PDLLA/ $\epsilon$ -CL co-polymer and (PDLLA/ $\epsilon$ -CL)- $\beta$ -TCP composites.

Time (Days)		(PDLLA/ε-CL)-β-TCP containing 20% β-TCP	Molecuar weight (Da)		
	PDLLA/e-CL		PDLLA/ε-CL)-β-TCP containing 40% β-TCP	(PDLLA/ε-CL)-β-TCP containing 60% β-TCP	
3	67531	70713	71256	72546	
7	64167	69289	70815	71573	
14	61284	67468	69348	70546	
21	57649	63397	65972	67413	
28	49761	59716	60583	63413	
35	39118	54074	57346	59871	
42	27811	44842	46273	48245	



Figure 6. Degradation profile of the PDLLA/ $\epsilon$ -CL copolymer and the (PDLLA/ $\epsilon$ -CL)- $\beta$ -TCP composites.

molecular weight of the copolymer decreased to 27811 Da at the end of day 42, while the molecular weights of the composites decreased to 44842, 46273, 48245 Da for composites containing 20, 40 and 60% of  $\beta$ -TCP respectively. It is also possible that the presence of HA in the structure slowed down the degradation rate as reported for the degradation of HA containing  $\beta$ -TCP [19]. It is usually undesirable for the  $\beta$ -TCP and the polymer to completely decompose before the bone healing process begins. Considering that HA slows down the degradation, its presence might provide the time required for the bioceramic part of composites to show their osteogenic properties.

#### CONCLUSION

The synthesis and biodegradion profiles of (PDLLA/  $\epsilon$ -CL)-  $\beta$ -TCP composites in the form of a degradable injectable filler material are reported for the first time.  $\beta$ -TCP was synthesized using chemical precipitation method. FTIR and XRD spectra showed characteristic bands belonging to calcium phosphates. XRD analysis revealed that although  $\beta$ -TCP is the dominant phase, the powders also contained HA.

D,L-Lactate and  $\epsilon$ -caprolactone were copolymerized into a PDLLA/ε-CL copolymer with a Mw of 53430 Da, Mw of 73541 Da and H.I. of 1.38 via ring-opening polymerization using stannous octoate as a catalyst. FTIR spectrum of the copolymer revealed the characteristic peaks of PDLLA/ε-CL co-polymers. DLLA/ε-CL ratio was 60/40 as calculated from <sup>1</sup>H-NMR. Then, both components were used to prepare (PDLLA/ $\epsilon$ -CL)- $\beta$ -TCP composites with different ceramic ratios. The composites were soft, flexible, easy to handle and hand shaped at room temperature. Degradation rate of the composites inversely proportional  $\beta$ -TCP amount of the composites.  $\beta$ -TCP was successfully provide an osteoconductive property for bone ingrowth, and may produce interconnected structure vascularization and full thickness bone tissue ingrowth after the copolymer degradation throughout the material. The synthesized composite structure could be suitable as bone substitute for bone tissue engineering applications. Along with the osteogenic effect of  $\beta$ -TCP, these findings make the (PDLLA/ $\epsilon$ -CL)-  $\beta$ -TCP composites a good option for use as a bone filling material.

#### References

- 1. M. Jarcho, R.L. Salsbury, M.B. Thomas, R.H. Doremus, Synthesis and fabrication of  $\beta$ -tricalcium phosphate (whitlockite) ceramics for potential prosthetic applications, J. Mater. Sci., 14 (1979) 142-150.
- 2. J. Enderle, J. Bronzino, Introduction to biomedical engineering, Elsevier, Burlington, USA, 2012
- S. Higashi, T. Yamamuro, T. Nakamura, Y. Ikada, S.-H. Hyon, K. Jamshidi, Polymer-hydroxyapatite composites for biodegradable bone fillers, Biomaterials, 7 (1986) 183-187.
- S.M. Zakaria, S.H.S. Zein, M.R. Othman, F. Yang, J.A. Jansen, Nanophase hydroxyapatite as a biomaterial in advanced hard tissue engineering: a review, Tissue Eng. Part B: Rev., 19 (2013) 431-441.
- J. Park, R. Lakes, Biomaterials: an Introduction, Springer Science Business Media, LLC, New York, USA, 2007
- C. Doyle, E. Tanner, W. Bonfield, In vitro and in vivo evaluation of polyhydroxybutyrate and of polyhydroxybutyrate reinforced with hydroxyapatite, Biomaterials, 12 (1991) 841-847.
- Z. Huang, J. Tian, B. Yu, Y. Xu, Q. Feng, A bone-like nanohydroxyapatite/collagen loaded injectable scaffold., Biomed. Mater., 4 (2009) 055005.
- W. Thein-Han, R. Misra, Biomimetic chitosan– nanohydroxyapatite composite scaffolds for bone tissue engineering, Acta Biomater., 5 (2009) 1182-1197.
- 9. H. Zhou, J. Lee, Nanoscale hydroxyapatite particles for bone tissue engineering, Acta Biomater., 7 (2011) 2769-2781.
- D. Apelt, F. Theiss, A.O. El-Warrak, K. Zlinszky, R. Bettschart-Wolfisberger, M. Bohner, S. Matter, J.A. Auer, B. von Rechenberg, In vivo behavior of three different injectable hydraulic calcium phosphate cements, Biomaterials, 25 (2004) 1439-1451.
- J.E. Dumas, K. Zienkiewicz, S.A. Tanner, E.M. Prieto, S. Bhattacharyya, S.A. Guelcher, Synthesis and characterization of an injectable allograft bone/polymer composite bone void filler with tunable mechanical properties, Tissue Eng. Part A, 16 (2010) 2505-2518.
- N.L. Ignjatović, C.Z. Liub, J.T. Czernuszka, D.P. Uskokovića, Micro-and nano-injectable composite biomaterials containing calcium phosphate coated with poly (DL-lactideco-glycolide), Acta Biomater., 3 (2007) 927-935.
- J.D. Kretlow, L. Klouda, A.G. Mikos, Injectable matrices and scaffolds for drug delivery in tissue engineering, Adv. Drug Deliver. Rev., 59 (2007) 263-273.
- A. La Gatta, A. De Rosa, P. Laurienzo, M. Malinconico, A novel injectable poly (ε-caprolactone)/calcium sulfate system for bone regeneration: Synthesis and characterization, Macromol Biosci., 5 (2005) 1108-1117.
- 15. A. López, C. Persson, J. Hilborn, H. Engqvist, Synthesis and characterization of injectable composites of poly [D, L-lactide-co-(ε-caprolactone)] reinforced with β-TCP and CaCO<sub>3</sub> for intervertebral disk augmentation, J. Biomed. Mater. Res. B: Appl. Biomater., 95 (2010) 75-83.
- K.L. Low, S.H. Tan, S.H.S. Zein, J.A. Roether, V. Mouriño, A.R. Boccaccini, Calcium phosphate-based composites as injectable bone substitute materials, J. Biomed. Mater. Res. Part B: Appl. Biomater., 94 (2010) 273-286.

- K. Rezwan, Q.Z. Chen, J.J. Blaker, A.R. Boccaccini, Biodegradable and bioactive porous polymer/inorganic composite scaffolds for bone tissue engineering, Biomaterials, 27 (2006) 3413-3431.
- A. Senköylü, E. Ural, K. Kesenci, A. Simşek, S. Ruacan, L. Fambri, C. Migliaresi, E. Pişkin, Poly (D, L-lactide/εcaprolactone)/hydroxyapatite composites as bone filler: an in vivo study in rats, Int. J. Artif. Organs, 25 (2002) 1174-1179.
- E. Ural, K. Kesenci, L. Fambri, C. Migliaresi, E. Piskin, Poly (D, L-cactide/ε-caprolactone)/hydroxyapatite composites, Biomaterials, 21 (2000) 2147-2154.
- R.Y. Basha, M. Doble, Design of biocomposite materials for bone tissue regeneration, Mater. Sci., 57 (2015) 452-463.
- B. Torabinejad, J. Mohammadi-Rovshandeh, S.M. Davachi, A. Zamaniana, Synthesis and characterization of nanocomposite scaffolds based on triblock copolymer of L-lactide, ε-caprolactone and nano-hydroxyapatite for bone tissue engineering, Mater. Sci. and Eng. C., 42 (2014) 199-210.
- H. Nie, C.H. Wang, Fabrication and characterization of PLGA/ HAp composite scaffolds for delivery of BMP-2 plasmid DNA, J. Control. Release, 120 (2007) 111-121.
- Z. Hong, R.L. Reis, J.F. Mano, Preparation and in vitro characterization of novel bioactive glass ceramic nanoparticles, J. Biomed. Mater. Res. A, 88 (2009) 304-313.
- T. Miyai, A. Ito, G. Tamazawa, T. Matsuno, Y. Sogo, C. Nakamura, A. Yamazaki, T. Satoh, Antibiotic-loaded poly-εcaprolactone and porous β-tricalcium phosphate composite for treating osteomyelitis, Biomaterials, 29 (2008) 350-358.
- F. Yang, J. Wolke, J. Jansen, Biomimetic calcium phosphate coating on electrospun poly (ε-caprolactone) scaffolds for bone tissue engineering, Chem. Eng. J., 137 (2008) 154-161.
- V. Guarino, L. Ambrosio, The synergic effect of polylactide fiber and calcium phosphate particle reinforcement in poly ε-caprolactone-based composite scaffolds, Acta Biomater., 4 (2008) 1778-1787.
- C.C.P.M. Verheyen, J.R. De Wijn, C.A. Van Blitterswijk, K. DeGroot, Evaluation of hydroxylapatite/poly (l-lactide) composites: Mechanical behavior, J. Biomed. Mater. Res., 26 (1992) 1277-1296.
- Y. Kinoshita, M. Matsuo, K. Todoki, S. Ozono, S. Fukuoka, H. Tsuzuki, M. Nakamura, K. Tomihata, T. Shimamoto, Y. Ikada, Alveolar bone regeneration using absorbable poly (L-lactideco-ε-caprolactone)/β-tricalcium phosphate membrane and gelatin sponge incorporating basic fibroblast growth factor, Int. J. Oral and Maxillofac. Surg., 37 (2008) 275-281.
- M. Kikuchi, Y. Koyama, T. Yamada, Y. Imamura, T. Okada, N. Shirahama, K. Akita, K. Takakuda, J. Tanaka, Development of guided bone regeneration membrane composed of β-tricalcium phosphate and poly (L-lactide-co-glycolide-coε-caprolactone) composites, Biomaterials, 25 (2004) 5979-5986.

- P. N. Kumta, C. Sfeir, D.H. Lee, D. Olton, D. Cho, Nanostructured calcium phosphates for biomedical applications: novel synthesis and characterization, Acta Biomater., 1 (2005) 65-83.
- S.V. Dorozhkin, Bioceramics of calcium orthophosphates, Biomaterials, 31 (2010) 1465-1485.
- L.L. Hench, Bioceramics: from concept to clinic, J. Am. Ceram. Soc., 74 (1991) 1487-1510.
- S. Raynaud, E. Champion, D. Bernache-Assollant, P. Thomas, Calcium phosphate apatites with variable Ca/P atomic ratio I. Synthesis, characterisation and thermal stability of powders, Biomaterials, 23 (2002) 1065-1072.
- B. Schrader, Infrared and Rarnan spectroscopy: Methods and applications VCH Publishers. Inc., New York, USA, 1995.
- 35. B. Stuart, Infrared spectroscopy, Kirk-Othmer Encyclopedia of Chemical Technology, (2000) 1-18.
- E. Akdogan, M. Demirbilek, Y. Sen, M.A. Onur, O.K. Azap, E. Sonmez, H.T. Sirin, M. Mutlu, In-vitro and in-vivo bacteria anti-fouling properties of phosphite plasma treated silicone, surface innovations, 7 (2018) 122-132.
- B. Stuart, Infrared spectroscopy: Fundamentals and applications, John Wiley & Sons, Ltd, Weinheim Germany, 2005
- K. Ishikawa, P. Ducheyne, S. Radin, Determination of the Ca/P ratio in calcium-deficient hydroxyapatite using X-ray diffraction analysis, J. Mater. Sci. Mater. Med., 4 (1993) 165-168.
- M. Akao, H. Aoki, K. Kato, A. Sato, Dense polycrystalline β-tricalcium phosphate for prosthetic applications, J. Mater. Sci. Mater. Med., 17 (1982) 343-346.
- Y. Lemmouchi, E. Schacht, C. Lootens, In vitro release of trypanocidal drugs from biodegradable implants based on poly (ε-caprolactone) and poly(D,L-lactide), J. Control. Release, 55 (1998) 79-85.
- 41. H. Kricheldorf, Syntheses and application of polylactides, Chemosphere, 43 (2001) 49-54.
- L. Wu, J. Ding, In vitro degradation of three-dimensional porous poly (D, L-lactide-co-glycolide) scaffolds for tissue engineering, Biomaterials, 25 (2004) 5821-5830.
- H. Pistner, D.R. Bendi, J. Muhling, J.F. Reuther, Poly (I-lactide): a long-term degradation study in vivo: Part III. Analytical characterization, Biomaterials, 14 (1993) 291-298.
- Y. Ikada, S.H. Hyon, K. Jamshidi, S. Higashi, T. Yamamuro, Y. Katutani, T. Kitsugi, Release of antibiotic from composites of hydroxyapatite and poly(lactic acid), J. Control. Release, 2 (1985) 179-186.