

Supporting Information

Effect of the Reinforcement Phase on the Mechanical and Biocompatibility Properties of PLA Matrix Nano Composites

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Abstract: In this study, it was aimed to obtain nanocomposite structure with poly (lactic acid) (PLA) matrix and to improve the mechanical and morphological properties of the PLA matrix nano-reinforced composites. Titanium dioxide (TiO₂) and hydroxyapatite (HA) nanoparticles were used as reinforcing elements. Solvent casting particle leaching method was used for the production of the composites. Thermal characterization and compression test of the composite samples were carried out. Microstructural properties and the phases formed on the surface of in vitro tested samples were examined. As a result of the study, it was observed that nano-reinforced composites with PLA matrix are biocompatible and apatite formation increases gradually every week. Also the highest compressive strength was obtained in PLA/HA samples, while the lowest was obtained in PLA sample. It was concluded that composites prepared within the scope of the study can be used as artificial tissue scaffolds in biomedical and medical applications.

Keywords: poly (lactic acid); hydroxiapatite; titanium dioxide; biocompatibility; nano composite; tissue scaffold

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1. Material

In this study, Poly (lactic acid) (PLA) was used as the matrix material. The PLA is an injection moulding grade with a molecular weight of 2×10^4 gmol⁻¹ and melting temperature of 165°C. The HA (with 60 nm grain size and % 99.9 purity) and TiO₂ (with 17 nm grain size and %99.995 purity) nanoparticles used were purchased from Nanography, Turkey. TiO₂ that used for composite producing was anatase structure. Dichloromethane was purchased from Balmumcu Chemistery, Turkey.

2. Method

Production of composites by using Solvent Casting Particle Leaching

In this study, sample production was carried out by solvent casting particle leaching technique. The method is shown schematically in Figure S1.

Figure S1. Schematic representation of the solvent casting particle leaching technique

For solvent casting-particle leaching, PLA was dissolved in methylene chloride (Dichloromethane-DCM) at room temperature. Then, adding different rates of HA and TiO₂, mixing was done in a magnetic stirrer at 150 rpm for 4 hours. The amounts of materials used in composite preparation are given in Table S1. If the pore-forming agent (NaCl) is poured directly into the mixture, the NaCl grains accumulate and porogen does not mix in the polymer solution. As a result, the desired porosity cannot be achieved. Therefore, an infiltration unit was used in this study and homogeneous distribution of the pores on the sample was provided. The obtained mixture was infiltrated into a mould containing 600-1054 µm of porogen salt. Infiltration was performed at room temperature, with 5 bar pressure value and 3 minutes pressure time. Argon, an inert gas, is used to prevent the gas that will be released during the infiltration process from reacting with the composite structure in the mould. After the infiltration process in Argon environment, the samples were dried in order to evaporate the DCM used to dissolve the PLA material. Since DCM is a volatile material, boiling occurs when the drying temperature rises above 30°C, resulting with closed air gaps. Since the cell cannot hold onto the scaffold in closed spaces, closed pores are undesirable in tissue scaffolding studies. Therefore, drying was carried out at 30°C for 15 days. After the solvent was completely evaporated, the remaining solid PLA composite-NaCl mixture was kept in water to remove NaCl. At room temperature, the magnetic stirrer is kept at 200 rpm for 2 days and the salts inside the structure are dissolved and a hollow structure is obtained. The samples were then re-dried for 2 days at 50 ° C.

Thus, composites samples with approximately 500 ~ 600 μm pore size were obtained. Then, bioactivity, mechanical, thermal and morphological analyses were applied to the obtained porous samples.

Table S1. Mixing amounts of the prepared solution.

Bioactivity Tests

All equipment used in the preparation of simulated body fluid (SBF) solution are sterilized with pure water. SBF solution was prepared according to Kukubo's formula.¹ The pH of the fluid was adjusted close to the pH of the human blood plasma (pH 7.4). The pH of the solution was adjusted using 1M HCl.

Newly prepared SBF was used in bioactivity tests. 3 samples from each of porous composite groups (PLA, PLA / HA, PLA / HA / 0.5% TiO₂, PLA / HA / 1% TiO₂, PLA / HA / 1.5% TiO₂) were kept in SBF between 1 ~ 4 weeks. Bioactivity tests were applied to a total of 60 samples. These samples were immersed in 33 cc glass containers inside the SBF, which was kept constant at 36.5 °C. Due to the change of ion concentration in SBF, SBF was replaced every two days with a freshly prepared simulated body fluid. Thus, the ion concentration of the medium was returned to its original values. Weights of the test samples were determined before the SBF experiment. When the waiting time of each parameter in SBF expires, it is taken from SBF and washed with pure water and left to dry at 30 °C and then dry weights determined again. Thus, weight increase has been determined according to the holding time in SBF. The surface morphologies of the samples immersed in simulated body fluid were examined before and after bioactivity test by using SEM, EDS (point and MAPPING) and the results were supported by XRD analysis.

Image Analysis with SEM / EDS (Point and Mapping)

Image analysis of the samples was carried out by Carl Zeiss Ultra Plus Gemini Fesem brand emission scanning electron microscope. In the images, apatite layer and pores formed on the surface of the samples as a result of the bioactivity experiment were examined. Image analysis was performed on PLA, PLA / HA, PLA / HA / 0.5% TiO₂, PLA / HA / 1% TiO₂, PLA / HA / 1.5% TiO₂ composite samples that subjected to in vitro test.

X-Ray Diffraction (XRD) Analysis

X-ray diffraction (XRD) analysis was performed with the X-ray diffractometer, the Ultima IV model of the RIGAKU brand. Analysed at 2 θ (10 ° -90 °) angular range and 2°/min.

Thermal Analysis with DTA/Tg/DSC

Thermal analysis of the samples were made with the Hitachi STA 7300 Model device. In this context, TG, DTA analysis and DSC calculation were made. Thermal degradation and mass losses of pure PLA and nanocomposite samples were investigated in the nitrogen environment at a temperature range of 25-785 °C at a heating rate of 15 °C / min. Thermal analyses were applied to PLA, PLA / HA, PLA / HA / 0.5% TiO₂, PLA / HA / 1% TiO₂, PLA / HA / 1.5% TiO₂ samples that were not subjected to biocompatibility testing. DTA / TG / DSC analysis was performed on a total of 5 samples.

As a result of thermal analysis, temperature difference between samples and reference, and weight change in samples were measured simultaneously. Temperature differences occur as a result of endothermic or exothermic reactions caused by the change of temperature in the sample. Melting, glass transition and decomposition temperatures, enthalpy and crystallinity values of samples were determined with TGA-DTA device.

Compression Test

Compressive strength measurements of all composites prepared in this study were made with Zwick / Roell Z600 brand compression test device at 50% deformation with 1.5 mm / min compression speed. Compression test was carried out at room temperature according to TS EN ISO 844 test standard. For all composites whose compression stress is determined, 3 samples are tested and the average compression value is determined.

The compression test was applied to PLA, PLA / HA, PLA / HA / 0.5% TiO₂, PLA / HA / 1% TiO₂, PLA / HA / 1.5% TiO₂ samples that were not subjected to biocompatibility testing. Compression tests were applied to a total of 15 samples.

3. Results and discussion

3.4. Compression Test Results.

The maximum stress used in the compression test was calculated using the values such as the height and surface area of the sample, while the compression stress and elastic modulus were calculated using Equations 1, 2 and 3.²

$$\sigma_m = \frac{F_m}{A_0} \quad (1)$$

$$E = \sigma_e \times \left(\frac{h_0}{X_e} \right) \quad (2)$$

$$\sigma_e = \frac{F_e}{h_0} \quad (3)$$

Here;

σ_m : Compression Stress

F_m : Maximum Compression Force

A_0 : Initial surface area of the sample,

E : Elasticity module,

σ_e : Compression stress in Fe,

h_0 : Initial height of the sample,

X_e : Displacement in Fe,

F_e : Force corresponding to X_e (conventional proportional limit) expressed.

As a result of the compression test, the average compression stress of pure PLA samples was determined as 0.16 MPa and the modulus of elasticity as 1.35 MPa. With the addition of HA to pure PLA, the compression stress increased by 275% to 0.44 MPa, while the modulus of elasticity decreased by 6.67% to 0.09 MPa (Table S2). In the sample prepared by fixing the PLA ratio in the composite structure by reducing the HA ratio by the amount of TiO₂ added, compression stress increases with parallel to the increase in the amount of TiO₂. However, lower compression stress was observed than PLA / HA samples. The compression stresses of 0.5% TiO₂, 1% TiO₂ and 1.5% TiO₂ reinforced samples are 0.25, 0.29, 0.33 MPa and elastic modulus are 0.02, 0.08, 0.13 MPa, respectively.

Figure S2. Compression stress results of samples

As seen in Table S2. and Figure S2, the highest compressive strength was obtained in PLA / HA samples. The lowest compressive strength was obtained in PLA samples. When HA and TiO₂ are added to the PLA matrix as a reinforcing phase, the strength of the composite structure increases and the elasticity decreases. Compared to PLA samples, increase in an average of 175% compression stress was observed with HA reinforcement and increase in an average of 90% compression stress with TiO₂ reinforcement. Compression stress of TiO₂ reinforced structures is 34% less than PLA / HA structure. As a result of the researches, it has been supported by the literature that HA and TiO₂ reinforced to the PLA polymer increase the strength, and the elasticity decreases due to the reinforcement's deterioration of the crystal structure of the PLA polymer.^{3,4,5,6}

Table S2. Thermal and mechanical properties of PLA and composite samples.

As can be seen in Table S2, the crystallinity percentage of pure PLA is 15%, 0.5% TiO₂, 1% TiO₂ and 1.5% TiO₂ reinforced samples crystallinity percentage are, respectively, 16%, 13%, 12%. From here; when a low amount of TiO₂ supplement is added to the PLA matrix, it is seen that the crystallization rate increases and the degree of crystallization decreases again with the increase of TiO₂ supplement amount. This situation is supported by literature.⁷ It is seen in Table S2 that the elasticity of the composite decreases with a low amount of TiO₂ reinforcement in reverse proportion and the elasticity increases again as the amount of TiO₂ reinforcement increases. This situation can be clearly seen from the elasticity and compression stress values obtained from the compression test results. In Figure S2, stress strain curve of compression test is given.

The degree of crystallization and glass transition temperature in a polymer have a significant effect on mechanical properties. This is because crystallization affects intermolecular bonds.⁸ In polymer nanocomposites, the interfaces formed by the nano additives with the matrix form new bonds between the matrix material and the reinforcement phase by the introduction of polymer chains between the layers. These bonds disrupt the crystal structure of the polymer, causing it to differentiate. While the nano-additives that are optimally reinforced with the polymer matrix affect the properties of the materials, it is in the literature that it has a negative effect when this limit is exceeded.²

Crystallinity generally adds rigidity and strength to the polymer structure. When the crystallinity is 5-10%, the polymer remains flexible. 20-60% crystallinity brings the material to leather consistency. Makes 70-90% crystalline material hard and durable.⁹ The crystallinity rates obtained in the study are between 10-20%. The degree of crystallinity decreased with the increase in the amount of TiO₂. This is an indication that TiO₂ gives flexibility to the structure.

In a study conducted by Rewzan et al. PLA stated that the composite material produced by reinforcing TiO₂ in increasing amounts to the matrix decreased the elasticity with the TiO₂ reinforcement to the PLA matrix.

However, he observed that as the amount of TiO₂ reinforcement increased, the elasticity increased again and TiO₂ nanoparticles added flexibility to the structure.¹⁰

Bone is morphologically present in two forms, cortical (tight) 20% of the total skeleton and cancellous (spongy) bone, 80%. Cancellous bone has 50-90% porosity and is commonly found in the middle of long bones.¹¹

Density and porosity properties of cancellous and cortical bone are different from each other. This is because the cancellous bone has a porous structure. Small changes in density cause large changes on the modulus of strength and elasticity. In addition, the elasticity modulus of the cortical bone depends on the porosity, and the elasticity modulus of the cancellous bone depends on the degree of mineralization.¹² Table S3 shows the compression, tensile stress and elastic modulus of cortical and cancellous bone.

Table S3. Mechanical properties of cortical and cancellous bone. ^{13, 14}

The mechanical data (Table S2) obtained within the scope of the study is lower compared to the data available in the literature (Table S3). As a result, it is thought that the pore sizes in the samples are larger than the ideal pore size and as a result the strength is less than 1.9 MPa. Another reason is that the PLA polymer is affected by environmental conditions. PLA absorbs moisture as the contact time with oxygen increases. Therefore, a decrease in strength values occurs ¹⁵.

Tables

Table S1. Mixing amounts of the prepared solution.

	DCM (g)	PLA (g)	HA (g)	TiO ₂ (g)	NaCl (g)
PLA	100	21	0	0	120
PLA/HA	100	21	2,33	0	120
PLA/HA/0,5% TiO ₂	100	21	2,21	0,12	120
PLA/HA/1% TiO ₂	100	21	2,10	0,23	120
PLA/HA/1,5% TiO ₂	100	21	1,98	0,35	120

Table S2. Thermal and mechanical properties of PLA and composite samples.

Sample Name	Compression Stress (σ_m)(MPa)	Elasticity Module (E) (MPa)	Tg (°C)	ΔH (mJ/mg)	Xc (% Crystallinity of PLA)
PLA	0.16	1.35	71.67	13.78	14.71
PLA/HA	0.44	0.09	77.20	10.08	10.76
PLA/HA/0,5% TiO ₂	0.25	0.02	79.08	15.10	16.12
PLA/HA/1% TiO ₂	0.29	0.08	75.13	12.47	13.31
PLA/HA/1,5% TiO ₂	0.33	0.13	75.88	10.81	11.54

Table S3. Mechanical properties of cortical and cancellous bone ^{13, 14}.

Material	Elasticity Module (MPa)	Tensile Strength (MPa)	Compression Strength (MPa)
Cortical	17,2	121	150
Cancellous	0,09	1,2	1,9

Schemes and Figures

Figure S1. Schematic representation of the solvent casting particle leaching technique

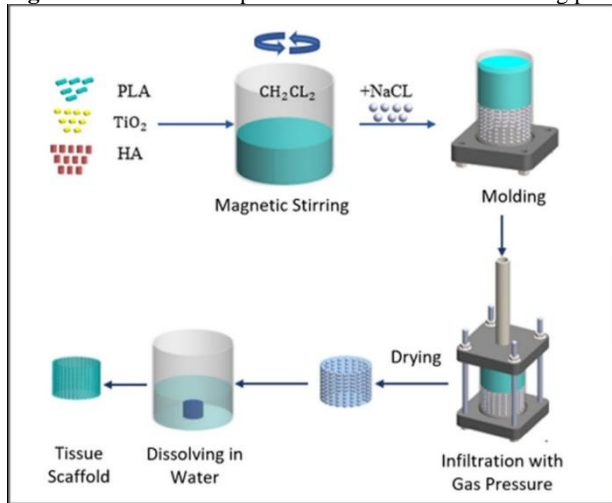
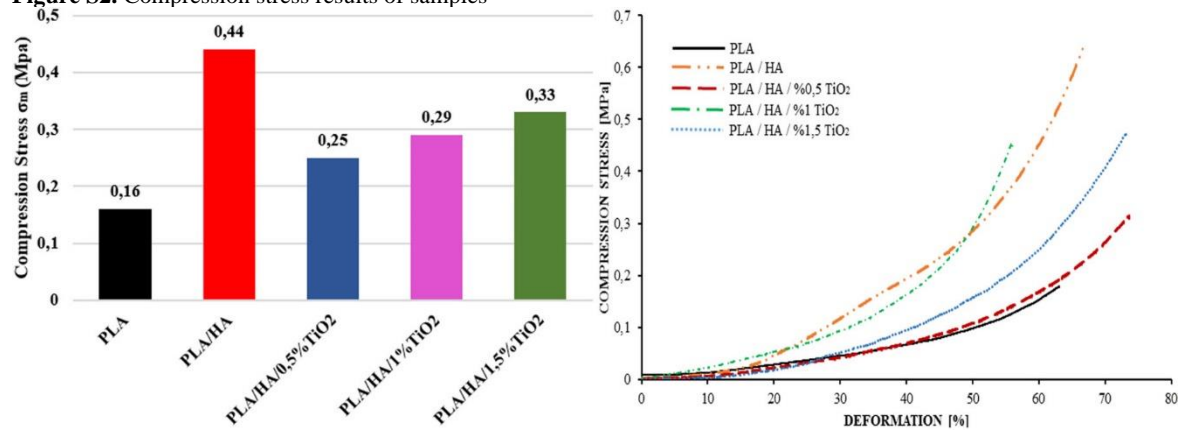


Figure S2. Compression stress results of samples



References

- (1) Kokubo, T.; Takadama, H. How useful is SBF in predicting in vivo bone bioactivity. *Biomater.*, 2006, 27, 2907–2915.
- (2) Salerno, A.; Fernández-Gutiérrez, M.; San Román, J.; Domingo, C. R. Macroporous and nanometre scale fibrous PLA and PLA-HA composite scaffolds fabricated by a bio safe strategy. *Royal Soc. of Chem.*, 2014, 4, 61491–61502.
- (3) Kothapalli, C. R.; Shaw, M. T.; Wei, M. Biodegradable HA-PLA 3-D porous scaffolds: effect of nano-sized filler content on scaffold properties. *Acta Biomat.*, 2005, 1, 653–662.
- (4) Nazhat, S. N.; Kellomaki, M.; Törmälä, P.; Tanner, K. E.; Bonfield, W.; Dynamic mechanical characterization of biodegradable composites of hydroxyapatite and polylactides. *J. of Bio. Mater. Res.*, 2001, 58, 335–343.
- (5) International Standardization Organisation, ISO 844-2014, Rigid Cellular Plastics-Determination of Compression Properties. <https://www.iso.org/standard/55473.html>. (accessed July 16, 2020).
- (6) Fonseca, C.; Ochoa, A.; Ulloa, M. T.; Alvarez, E.; Canales, D.; Zapata, P. A. Poly (lactic acid)/TiO₂ nanocomposites as alternative biocidal and antifungal materials. *Mater. Sci. and Eng.*, 2015, 57, 314–320.
- (7) Farhoodi, M.; Dadashi, S.; Mousavi, S. M. A.; Sotudeh-Gharebagh, R.; Emam-Djomeh, Z.; Oromiehie, A.; Hemmati, F. Influence of TiO₂ Nanoparticle Filler on the Properties of PET and PLA Nanocomposites. *Poly. (Korea)*, 2012, 36, 745-755.
- (8) Kodai, M.; Sirin, H.; Ozkoc, G. Effects of Reactive and Nonreactive POSS Types on The Mechanical, Thermal and Morphological Properties of Plasticized Poly (lactic acid). *Polym. Eng. and Sci.*, 2014, 264-275.
- (9) Fompak Automotive Plastic and Interiors, Plastik enjeksiyon prosesinde oluşan yüzey hatalarının önlenmesi ve çevrim süresinin düşürülmesi için variotherm yöntemi uygulanan kalip geliştirilmesi. https://www.academia.edu/32530676/FOMPAK_AUTOMOT%C4%B0VE_PLAST%C4%B0CS_AND_INTER%C4%B0ORS. (accessed July 16, 2020).
- (10) Rezwani, K.; Chen, Q. Z.; Blaker, J. J.; Boccaccini, A. R. Biodegradable and bioactive porous polymer/inorganic compositescaffolds for bone tissue engineering. *Biomater.*, 2006, 27, 3413–3431.
- (11) Salgado, A. J.; Coutinho, O. P.; Reis, R. L. Bone tissue engineering: state of the art and future trends. *Macromol. Biosci.*, 2004, 4, 743–765.
- (12) Dong, X. N.; Guo, X.E. The dependence of transversely isotropic elasticity of human femoral cortical bone on porosity. *J. of Biomech.*, 2004, 37, 1281-1287.
- (13) Agarwal, S.; Wendorff, J. H.; Greiner, A. Use of electrospinning technique for biomedical applications. *A. Poly.*, 2008, 49, 5603–5621.
- (14) Ravichandran, R.; Sundarrajan, S.; Venugopal, J. R.; Mukherjee, S.; Ramakrishna, S. Advances in polymeric systems for tissue engineering and biomedical applications. *Macromol. Biosci.*, 2012, 12, 286–311.
- (15) Evlen, H.; Özdemir, M. A.; Çalışkan, A. Doluluk Oranlarının PLA ve PET Malzemelerin Mekanik Özellikleri Üzerine Etkileri. *J. of Politech.*, 2019, 22.

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